Halothane

Health-based recommended occupational exposure limit

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Minister van Sociale Zaken en Werkgelegenheid
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Onderwerp	: Aanbieding adviezen 'Halothane' en 'Hydrogencyanide, sodium cyanide, and potassium cyanide'
Uw kenmerk	: DGV/MBO/U-932542
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Mijnheer de Minister,

Bij Brief van 3 december, nr DGV/BMO-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 u hierbij een advies aan over Halothaan en Cyanides. Het is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb deze adviezen vandaag aangeboden aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid en ter kennisname gezonden aan de Minister van Volksgezondheid, Welzijn en Sport en de Staatssecretaris van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

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Health-based recommended occupational exposure limit

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. 2002/14OSH, The Hague, 29 October 2002

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

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Samenvatting en advieswaarde

1 Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid beveelt de Gezondheidsraad gezondheidskundige advieswaarden aan voor beroepsmatige blootstelling aan toxische stoffen in 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 drietraps-procedure die moet leiden tot de wettelijke grenswaarden (MAC-waarden).

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan halothaan en presenteert zij, indien mogelijk, een gezondheidskundige advieswaarde voor deze stof. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór juni 2002 zijn verschenen.

2 Fysische en chemische eigenschappen

Halothaan (CAS nr 151-67-7) is een onbrandbare zeer vluchtige vloeistof met een zoete aangename geur. Halothaan ($CF_3CHBrCl$) is opgebouwd uit ethaan met daaraan verschillende halogenen (fluor (F), broom (Br) en chloor (Cl)) gekoppeld. Voor zover bekend komt halothaan van nature niet voor.

Het kookpunt van halothaan is 50°C. Bij verhitting ontleedt het in zeer giftige dampen van fluoride, chloride en bromide. Het mengt goed met petroleumether en andere vetoplosmiddelen.

Halothaan wordt uitsluitend gebruikt als narcosegas (82,1-164,2 g/m^3), meestal in combinatie met lachgas.

3 Monitoring

In de Verenigde Staten adviseert OSHA (Occupational Safety and Health Administration) om luchtmonsters van halothaan te nemen m.b.v. kokos-koolstof of m.b.v. Anasorb® 747 adsorptiebuisjes. De hoeveelheid bemonsterde halothaan wordt gaschromatografisch bepaald m.b.v. een vlam-ionisatiedetector (FID) of bij lage concentraties m.b.v. een 'electron capture' detector (ECD).

In Duitsland wordt inwendige blootstelling aan halothaan indirect gemeten door in het bloed trifluorazijnzuur (TFA) gehaltes te bepalen. TFA is een belangrijk afbraakproduct van halothaan. Bloedafname vindt plaats aan het einde van de werkdag of over verschillende tijdsperiodes als men meer wil weten over chronische blootstelling aan halothaan.

4 Huidige Grenswaarden

In Nederland geldt een bestuurlijke MAC-waarde van 40 mg/m^3, gemiddeld over een 8-urige werkdag. In Duitsland wordt dezelfde norm geadviseerd. In de VS wordt een TLV van 404 mg/m^3 (ACGIH) en een REL van 16,4 mg/m^3 (TGG 1 uur; NIOSH) aanbevolen.

In 2000 heeft de Commissie Reproductietoxische stoffen van de Gezondheidsraad geadviseerd halothaan in categorie 3 te classificeren en te kenmerken met R63. Onder categorie 3 vallen stoffen die in verband met hun mogelijke voor de ontwikkeling schadelijke effecten reden geven tot bezorgdheid voor de mens. Onder R63 wordt bedoeld mogelijk gevaar voor beschadiging van het ongeboren kind. Verder classificeert Duitsland halothaan in groep B. Dat wil zeggen dat de huidige beschikbare kennis wijst op een mogelijk risico voor schade aan de ontwikkelende embryo of foetus.

Duitsland heeft als enig land een biologische grenswaarde gesteld van 2,5 mg TFA per liter bloed.

5 Kinetiek

Uit patiëntenonderzoek blijkt dat direct na de narcose en afhankelijk van de toegediende dosis halothaan nog 50 tot 70% aanwezig is in de longen (alveoli). Een vergelijkbaar percentage is ook gevonden in een dieronderzoek met ratten die lage doses halothaan kregen toegediend.

Na opname hoopt halothaan zich gemakkelijk op in lichaamsvet. Daarbij kunnen volgens *in vitro* onderzoek de concentraties halothaan in vet 110 tot 220 maal hoger zijn dan in de lucht. Halothaan passeert verder de placenta; zo zijn in navelstrengaders van foetussen concentraties halothaan gemeten die 44-71% zijn van wat in het bloed van de moeders is gevonden.

Halothaan wordt in het lichaam enzymatisch afgebroken, waarbij onder andere trifluorazijnzuur (TFA) wordt gevormd en een bromide ion vrijkomt. Tussen de 5 en 55% van de in het lichaam opgenomen halothaan wordt daadwerkelijk enzymatisch afgebroken. Die variatie wordt verklaard doordat de mate van afbraak omgekeerd evenredig is met de blootstellingsconcentratie en onder andere genetisch is bepaald.

De afbraakproducten van halothaan worden uitgescheiden in de urine. Halothaan zelf wordt voornamelijk weer uitgeademd. Zowel dier- als mensgegevens tonen aan dat halothaan nog dagen na de blootstelling meetbaar is in het bloed.

6 Effecten

Humane gegevens

De concentratie halothaan die nodig is om de helft van de patiënten onder narcose te krijgen (= volledige sedatie) wordt aangegeven als de Minimale alveolaire Concentratie (MalvC) en die ligt tussen de 57 en 65 g/m^3. Het is niet bekend bij welke concentratie de patiënten geen sedatie meer ondervinden. Wel leidde een 30 minuten durende blootstelling aan 3,3 g/m^3 tot verminderde saccadische oogbewegingen, wat wijst op een lichte vorm van sedatie. In het algemeen is de sedatieve werking volledig verdwenen binnen 5 tot 30 minuten na het stoppen van de blootstelling.

In een enkel geval van beroepsmatige blootstelling is een halothaan-geassocieerde hepatitis gerapporteerd. De oorzaak van deze snelle en vaak dodelijke leverziekte is waarschijnlijk een verhoogde immuunrespons tegen TFA-eiwit complexen, omdat in het bloedserum van patiënten antilichamen tegen deze TFA-eiwitten zijn aangetroffen.

In verschillende epidemiologische onderzoeken zijn een toename van spontane abortussen en misvormingen bij de pasgeboren beschreven bij vrouwen die beroepsmatig zijn blootgesteld aan narcosemiddelen. Deze onderzoeken zijn echter, volgens de commissie, geen van allen volgens de wetenschappelijk objectieve standaarden uitgevoerd en daarom ongeschikt voor de risico evaluatie.

De commissie heeft geen aanwijzingen gevonden dat de werking van halothaan versterkt of verzwakt in combinatie met andere narcosegassen. Wel draagt lachgas additief bij aan de narcotische werking van halothaan.

Dierengegevens

De MalvC voor dieren (ratten, muizen, cavia's, etc.) varieert tussen de 73,9 en 114,9 g/m^3 halothaan, hoger dus dan voor mensen. Lagere concentraties halothaan veroorzaakten gedragsveranderingen in muizen; muizen blootgesteld aan 8,2 tot 16,4 g/m^3 vertoonden geen gedragsveranderingen meer.

Net als bij mensen zijn in verschillende diersoorten (ratten, muizen, konijnen en cavia's), die zijn blootgesteld aan halothaan (4 uur, 82,1 g/m^3) TFA-eiwitten in de lever aangetroffen. Daarnaast zijn leverbeschadigingen geconstateerd in muizen - en niet in ratten en cavia's - die 35 dagen lang continue zijn blootgesteld aan 123 mg/m^3.

De commissie heeft geen dierexperimentele aanwijzingen gevonden dat langdurige blootstelling aan halothaan tot kanker kan leiden.

In verschillende dieronderzoeken met ratten en muizen zijn effecten van halothaan op het nageslacht beschreven. Zo zijn in het nageslacht van vrouwelijke ratten die vlak voor en tijdens de zwangerschap zijn blootgesteld aan slechts 82 mg/m^3 levervetveranderingen, weefselveranderingen in nieren en hersenen en gedragsveranderingen waargenomen.

Ook zijn effecten op de vruchtbaarheid van zowel de vrouwtjes als de mannetjes beschreven, uiteenlopend van resorptie van de vrucht (ratten), embryosterfte tijdens de zwangerschap (muizen 8,2 g/m^3; ratten 65,7 g/m^3), effecten op eierstokken en baarmoeder (ratten 82 mg/m^3) en bij mannelijke ratten dosis-gerelateerde toename van chromosomale afwijkingen in beenmerg en spermatogonia (8,2 en 82 mg/m^3). Daarbij moet worden opgemerkt dat de effecten op de eierstokken en baarmoeder en op het mannetje zijn gevonden na een combinatieblootstelling met lachgas (90 of 900 mg/m^3). Vanwege de combinatieblootstelling acht de commissie deze studie niet geschikt om als uitgangspunt te dienen voor de berekening van een gezondheidskundige advieswaarde. De commissie is zich er echter wel van bewust dat de effecten kunnen zijn veroorzaakt door halothaan, omdat de gebruikte doses lachgas ruimschoots onder de geen-waargenomen-nadelig-effect-niveau (NOAEL) van 1520 mg/m^3 lachgas liggen en vindt daarom dat er reden tot zorg is.

Er zijn nog een aantal onderzoeken gepubliceerd, waarin dieren zijn blootgesteld aan mengsels van halothaan en andere narcosemiddelen, zoals lachgas en fluranen. Daarbij zijn geen synergistische of antagonistische effecten van deze mengsels gevonden op een aantal biochemische parameters, op de pathologie van verschillende organen, op het aantal tumoren en op de vruchtbaarheid.

7 Evaluatie en advieswaarde

Omdat halothaan regelmatig wordt gebruikt in combinatie met lachgas zouden zij elkaars werking kunnen beïnvloeden. De commissie heeft hiervoor echter geen duidelijke aanwijzingen gevonden.

De humane gegevens geven onvoldoende informatie voor een goede risico evaluatie. Uit de diergegevens maakt de commissie op dat de effecten op de ontwikkeling van het nageslacht de meest gevoelige effecten zijn als gevolg van halothaan blootstelling.

Het laagste-waargenomen-nadelig-effect-niveau (LOAEL) op het nageslacht van ratten is 82 mg/m^3 halothaan. Voor de berekening van een gezondheidskundige advieswaarde is deze LOAEL gecorrigeerd met een onzekerheidsfactor van 200. Deze factor is samengesteld uit een factor van 10 voor verschillen tussen soorten en individuen, en uit een factor van 20 voor de omzetting van de LOAEL naar de geen-waargenomen-nadelig-effect-niveau (NOAEL). In deze factor van 20 zit verdisconteerd het feit dat de commissie er rekening mee houdt dat de effecten op de vruchtbaarheid van zowel vrouwelijke als mannelijke ratten, die zijn waargenomen bij een mengselblootstelling van 8,2 mg/m^3 halothaan plus 90 mg/m^3 lachgas, kunnen zijn veroorzaakt door halothaan.

Met inachtneming van de onzekerheidsfactor van 200 beveelt de commissie een gezondheidskundige advieswaarde voor halothaan aan van 0,41 mg/m^3 (0,05 ppm), gemiddeld over een achturige werkdag.

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 workplace air. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS). It constitutes the first step in a three-stage procedure that results in legally binding exposure limits (MAC-values).

In the present report the committee discusses the effects of halothane and recommends a health-based occupational exposure limit. The committee's conclusions are based on scientific publications obtained from data retrieval systems from prior to June 2002.

2 Physical and chemical properties

Halothane (CAS no. 151-67-7) is a non-flammable highly volatile liquid with a sweetish pleasant odour. Halothane ($CF_3CHBrCl$) is composed of ethane to which various halogens (Fluor (F), Bromide (Br) and Chlorine (Cl)) are coupled. So far known, halothane does not exist naturally.

The boiling point of halothane is 50°C. When heated, it decomposes in very toxic fumes of chloride, fluoride and bromide. Halothane is miscible with petroleum ether and other fat solvents.

The compound is exclusively used as an anaesthetic gas and mostly in combination with nitrous oxide (N_2O), a general anaesthetic. The dose of halothane given for anaesthesia ranges between 82.1 and 164.2 g/m^3 (1-2%).

3 Monitoring

The OSHA (Occupational Safety and Health Administration) of the USA requires that air samples of halothane be taken with coconut-charcoal or with Anasorb® 747 sample tubes. The amount of halothane sampled is analysed by gas liquid chromatography using a flame ionisation detector (FID) or, when lower concentrations are expected, using an electrochemical detector (ECD).

In Germany, halothane is biologically monitored by measuring blood levels of trifluoroacetic acid (TFA), a breakdown product of halothane. Blood is preferentially taken at the end of the working day or, by prolonged exposure, at the end of different working days.

4 Current limit values

In the Netherlands, the administrative MAC-value for halothane is 40 mg/m^3, averaged on an eight-hour working day. In Germany, a same MAK-value is recommended. In the USA, a TLV of 404 mg/m^3 (ACGIH) and a REL of 16.4 mg/m^3 (ceiling 1-hour TWA; NIOSH) is advised.

In 2000, the Health Council's Committee for Compounds Toxic to Reproduction recommended classifying halothane in category 3 and to label it with R63. Category 3 substances are defined as causing concern for humans owing to possible developmental toxic effects. R63 substances have possible risk of harm to the unborn child. Furthermore, Germany classified halothane in pregnancy group B, meaning that currently available information indicates that a risk of damage to the developing embryo or foetus should be considered to be probable.

Germany has set a Biological Exposure Limit of 2.5 mg trifluoroacetic acid per litre blood, a breakdown product of halothane.

5 Kinetics

In studies with patients, 50 to 70% of the dosed halothane was still present in the lungs (alveoli) directly after stopping anaesthesia. Comparable results were obtained with rats.

Halothane quickly accumulates into body fat. According to *in vitro* studies, this accumulation may reach levels which are 110 to 220 times higher than the

concentration of halothane present in air. Furthermore, halothane has a low affinity for blood and passes the placenta barrier; neonatal umbilical vein concentrations can range from 44 to 71% of that found in the venous blood of an anaesthetised mother.

Halothane is enzymatically broken down in to particularly trifluoroacetic acid (TFA), a bromide and a chloride ion. The extent of the breakdown in humans varies between 5 and 55%. This variation is most probably inversely related to the exposure concentration and is influenced by environmental and genetic factors.

Halothane taken up is expired unchanged, whereas its breakdown products are excreted in the urine. Both human and animal data show that halothane is present in the body for a prolonged time; several days after exposure, halothane was still present in the blood.

6 Effects

Human data

The dose of halothane needed to induce incision insensitivity in half of the patients is expressed as the Minimal alveolar Concentration (MalvC) and varies between 57 and 65 g/m^3. Lower concentrations affected the behaviour and the psychomotor performance of volunteers. Although data demonstrate that patients receiving 3.3 g/m^3 halothane for 30 minutes showed decreased saccadic eye movements, indicating that they were lightly sedated, it is not known exactly at what dose patients do not experience sedation anymore. In general, performance is completely restored in about 5 to 30 minutes after stopping the anaesthesia.

A few cases of halothane-associated hepatitis have been reported after occupational exposure. This severe liver disease is most probably caused by an increased immune response against so-called TFA-proteins, because in patients suffering from halothane-associated hepatitis antibodies against TFA-proteins were found.

In several epidemiology studies, increased spontaneous abortions and malformations were described in newborn of women occupationally exposed to halothane. However, the committee noted that these studies were not carried out according to scientifically objective standards. The committee, therefore, believes that these studies are inadequate for risk assessment.

The committee did not find data suggesting that the anaesthetic effect of halothane is synergistically or antagonistically influenced by other anaesthetic gases, but concomitant exposure to halothane and nitrous oxide appear to be additive in causing anaesthesia.

Animal studies

The MalvC of halothane in animals varies between 73.9 and 114.9 g/m^3, which is higher than in humans. Lower doses of halothane caused behavioural changes in mice, whereas no behavioural changes were observed in mice exposed to 8.2-16.4 g/m^3.

As in humans, in several animal species TFA-proteins adducts were found in the liver after exposure to halothane (4 hours, 82.1 g/m^3). Furthermore liver lesions were found in mice — not in rats and guinea pigs -, which were continuously exposed to 123 mg/m^3 halothane for 35 days.

The committee did not find data on the carcinogenicity or genotoxicity of halothane.

Several investigators reported on the developmental toxic effects of halothane in the offspring of mice and rats exposed to a concentration of as low as 82 mg/m^3, such as histopathological changes in the liver, the kidneys and the brain, neurobehavioural changes and retardation.

Effects of halothane on the fertility of exposed male and female rats and mice are found as well, including death of embryo's (rats, 65.7 g/m^3; mice, 8.2 g/m^3), effects on the ovary and uterus (rats, 82 mg/m^3) and dose-related chromosomal aberrations in bone marrow and in spermatogonial cells in male rats (8,2 or 82 mg/m^3). However, the effects on males and on the ovary and uterus are observed after a concomitant exposure with nitrous oxide (90 or 900 mg/m^3). Although the nitrous oxide concentrations used were far below its NOAEL of 1,520 mg/m^3, and thus the observed effects on the male and female fertility may be ascribed to halothane alone, the committee believes that this study is not suitable as a starting point in deriving a HBR-OEL, because of the mixed exposure. However, the results of the mixed exposure study are a point of concern.

Several other studies have been published in which animals were exposed to mixtures of halothane and other anaesthetics, such as nitrous oxide and fluranes. These studies revealed no synergistic or antagonistic effects on various biochemical parameters, on the pathology of various organs, on the tumour incidence and on the reproduction.

7 Hazard assessment and recommended occupational exposure limit

For anaesthesia, halothane is often used in combination with nitrous oxide. From the toxicological point of view they could act synergistically or antagonistically, but the committee did not find any data that this is the case.

The committee considers the human data inadequate for a risk assessment. According to the committee, animal data show that the most sensitive effect of halothane is on the development of the offspring.

The lowest-observed-adverse-effect-level (LOAEL) of halothane in the offspring of rats is 82 mg/m^3. In deriving a health-based recommended occupational exposure limit (HBR-OEL), the LOAEL was adjusted by a composite uncertainty factor of 200. This factor was composed of a factor of 10 for taken into account intra- and interspecies variations and of a factor of 20 to compensate for the absence of a NOAEL and to take into account the committee's concern that in a mixed exposure study, the effects observed on the fertility at a concentration of halothane of as low as 8.2 mg/m^3 plus 90 mg/m^3 nitrous oxide may have been caused by halothane exclusively.

Applying this composite uncertainty factor of 200, the committee recommends a HBR-OEL of 0.41 mg/m^3 (0.05 ppm) for halothane, as a time-weighted average concentration of 8 hours.

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 air. Such an exposure limit cannot be derived when 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 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 procedures

The present document contains the assessment by DECOS, hereafter called the committee, of the health hazard of halothane. The members of the committee are listed

in Annex B. The first draft of this report was prepared by MA Maclaine Pont, Msc, from the Wageningen University, The Netherlands, by contract with the Ministry of Social Affairs and Employment.

In April 2001 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

Literature on the hazardous effects due to exposure to halothane was retrieved from the on-line database Superbase (October 1995 - July 1999). Further, literature was retrieved form CD ROMs of Toxline, Medline (1985 - June 2002) and Chemical Abstracts (1991 - November 1999).

Chapter 2

Identity, properties and monitoring

2.1 Identity

Halothane is a non-flammable highly volatile liquid with a sweetish pleasant odour (Mac94). Identification data are obtained from How92, NIA98, Bud96 and Rou86.

Preferred name	2-bromo-2-chloro-1,1,1-trifluoroethane
Synonyms	bromochlorotrifluoroethane; 1-bromo-1-chloro-2,2,2-trifluoroethane; Halothane; Chalothane; Fluothane; Haltan; Fluorotane; Halsan; Narcotane; Rhodialothan
CAS reg no	151-67-7
EINECS no	205-796-5
RTECS no	KH6550000

2.2 Physical and chemical properties

The chemical and physical properties are given on the next page. The data are obtained from Las94, Lew92, Mor92, NIA98, Kal91, Pez89, Rou86, Kor85, Gem84, Amo83, and Bud96.

Molecular formula	C_2BrClF_3H
Structural formula	F_3C-CHClBr
Molar mass	197.38 g/mol
Boiling point	50°C
Melting point	-118°C
Vapour pressure (20°C)	32 kPa
Relative density of saturated vapour in air (air = 1; 101 kPa; 20°C)	2.87 (32%)
Density	20°C/4°C: 1.871 kg/m^3 20°C/20°C: 1.868 kg/m^3
log $P_{oct/water}$	measured: 2.30; calculated: 2.45
Solubility in water	0.45 g/100 mL at 20°C; 0.345 g/100 mL
Miscible with	alcohol, chloroform, ether, petroleum ether and other fat solvents
Odour threshold	271 mg/m^3; recognition: 267 mg/m^3
Conversion factors (101 kPa; 20 °C)	1 ppm = 8.21 mg/m^3 1 mg/m^3 = 0.122 ppm

For commercial use, halothane is sold as a racemic mixture of the R- and the S-form. Halothane, may be stabilised with the antioxidant thymol (final air concentration of 0.01%). The vapour of halothane is three times heavier than air, and, therefore, accumulates close to the floor.

When halothane is heated above its boiling point, it decomposes in fumes of fluoride, bromide and chloride. These fumes may induce anoxia, which may finally lead to unconsciousness.

2.3 EU classification and -labelling

Not available.

2.4 Validated analytical methods

2.4.1 Environmental monitoring

In the procedure described by OSHA, 9 or 12 litres of air are sampled at a rate of 100 or 50 mL/min on coconut shell charcoal or on Anasorb® 747 tubes. The samples are desorbed with carbon disulphide (CS_2) and analysed by gas liquid chromatography (GLC) using a flame ionisation detector (FID) (SKC96). The reliable quantitation limit is 0.19 mg/m^3, which is the smallest reliable amount that can be spiked on a charcoal tube. Alternatively, an electrochemical detector (ECD) may be used. Using this detector, the concentration range of halothane is linear between 0.8 and 821 µg/m^3.

In practice, several (GLC) conditions and detection limits are described for analysing halothane in air (Heu85); with a simple analysis using a gas chromatograph and an ECD a linearity of between 1.8 and 1,642 mg/m^3 may be obtained (Lad87).

The two enantiomers of halothane (R- and S-form) can be separated on a special GLC column (Mei91).

2.4.2 *Biological monitoring*

In Germany, levels of halothane in blood are indirectly measured by monitoring the amount of trifluoroacetic acid (TFA), an important metabolite of halothane. For assessing TFA levels after occupational exposure, blood is collected directly or at several time points after a working period (DFG98).

Currently only one method has been described for the determination of halothane in blood, namely by gas chromatographic headspace analysis (Kou88).

Chapter 3

Sources

3.1 Natural occurrence

No data have been found suggesting that halothane occurs naturally.

3.2 Man-made sources

3.2.1 Production

Halothane is synthesised by the reaction of bromine with 1-chloro-2,2,2-trifluoroethane or chlorine with 1-bromo-2,2,2-trifluoroethane (Pat78). Furthermore, it can be obtained by rearrangement of $CBrF_2CHClF$ in the presence of $AlCl_3$ at 50°C (Wol79).

In the eighties, the quantity produced world-wide was estimated to be less than 12,000 tonnes (Rod89). No other production data have been found.

3.2.2 Uses

Since its introduction into clinical practice in 1956, halothane is exclusively used as an anaesthetic agent (Pat78).

The dose needed to prevent movement in response to a surgical incision in half of the patients, is expressed as the Minimal alveolar Concentration (MalvC) (Heu85). For halothane, the MalvC ranges between 47.62 and 77.17 g/m^3 (0.58 and 0.94%) in adults.

Included herein are older people, who are found to be more sensitive, and thus need lower doses for complete sedation (Ler86, Map96). However, others have not confirmed this age-related outcome (Dwy90).

Children are less sensitive to halothane than adults. This is because children have 1) a greater ratio of alveolar ventilation to functional residual capacity, 2) a greater fraction of cardiac output perfusing the vessel-rich group, 3) a greater ratio of alveolar ventilation to cardiac output per kg body mass, and 4) a significantly lower blood/gas partition coefficient than adults. Neonates have smaller fat depots and a lower solubility of volatile anaesthetics, and, therefore, higher concentrations of halothane are needed for sedation than for adults (Ler86).

The MalvC values for humans and for several animal species are summarised in Table 3.1 and 3.2.

Table 3.1 The Minimum alveolar Concentration (MalvC) of halothane in humans.

	MalvC halothane in		ref.
	g/m^3	%	
man	63.2	0.77	Dru85
	53.4	0.65	Kal91
	55.8-57.5	0.68-0.70[a]	Hou93
nonpregnant women	61.6	0.75	Cha96
pregnant women	47.6	0.58[b]	
14.4 month old infants	77.2	0.94	Mur90

[a] No differences in sensitivity of MalvC were found between Asiatic (China, Nepal) and Caucasian (European) populations.

[b] MalvC differed significantly from MalvC of non pregnant women ($p<0.0005$).

Table 3.2 The Minimum alveolar Concentration (MalvC) of halothane in several animal species.

species	MalvC halothane in		ref.
	g/m^3	%	
Java monkey	94.4	1.15	Dru85
Rhesus monkey	81.3	0.99	Whi94
dog	82.1	1.00	Tah91
dog	71.4	0.87	Dru85
cat	97.7	1.19	Dru85
New Zealand white rabbit	114.1	1.39	Dru85
	75.5	0.92	Sob93
	51.7	0.63	Mac77
hamster	94.4	1.15	Viv99
Sprague Dawley rat	101.8	1.24	Tah91
male F344 rat 5 months old	101.0	1.23[a]	Los89
14 months old	99.3	1.21[a]	
25 months old	86.2	1.05	
Sprague Dawley rat (pregnant and non pregnant female, male)	84.6	1.03	Maz85
mouse	82.1	1.00	Dru85
ddN mouse	79.6	0.97	Kom93
Swiss Webster mouse (pregnant and non pregnant female, male)	78.0	0.95	Maz85
male Hartley guinea pig	82.9	1.01	Sei89

[a] MalvC differed significantly from MalvC of 25 months-old rats (*p* not given).

Chapter 4

Exposure

4.1 General population

Because halothane is exclusively used for anaesthesia, the general population is only exposed to halothane during anaesthesia.

4.2 Working population

Halothane production workers and medical personnel, such as anaesthetists, anaesthetic nurses, technicians, surgeons, veterinarians, and workers with laboratory animals can be occupationally exposed to halothane.

Only one report on the exposure levels of halothane of workers manufacturing halothane was published and cited by the ACGIH (Svabova *et al.*, 1975, ACG92). Thirteen employees in a single plant were exposed to halothane at concentrations of averaging 5.4 g/m^3 (660 ppm).

On the other hand, many data are presented on the occupational exposure levels in operating theatres in the Netherlands and other countries, in paediatric and recovery rooms, and in veterinary hospitals (see Tables 4.1-4.5). The data show large variations of concentrations of halothane in the air. For instance, in hospitals concentrations between 2.5 and 378 mg/m^3 halothane were measured. Even higher concentrations were measured in paediatric rooms and veterinary hospitals (< 4.1 to 977 mg/m^3).

The presence of scavenging facilities decreases the concentrations of halothane in the air, but values lower than 0.6 mg/m^3 halothane have not been measured.

Table 4.1 Occupational exposure concentrations of halothane for hospital personnel in the Netherlands.

working place; persons exposed	sampling method	sampling time	concentration (range; no. of measurements)	ref.
1 operating room; anaesthetist	diffusive PAS[f]	30 - 120 min	Half-closed system: mean, 8.2 mg/m^3; peak, 24.6 mg/m^3 (n = 4); not found during 3/4 surgeries.	Ton80
1 operating room; anaesthetist	diffusive PAS	10 - 12 min	Face mask-narcosis: mean, 28.7 mg/m^3; peak, 148 mg/m^3.	
1 operating room; anaesthetist	diffusive PAS	45 - 75 min	open system: mean, 41-164 mg/m^3; peak, 123-493 mg/m^3 (n=4).	
3 operating rooms	ambient air at least 6 points	during anaesthesia	Mechanically ventilated room: 6.6-36 mg/m^3 (n=?)	Rej80
4 operating rooms;				Zwe83
1 anaesthetic assistant	active PAS	4 h	10 mg/m^3 (n=3)	
		8 h	2 mg/m^3 (n=4)	
3 - 4 other operating personnel	active PAS	4 h	2 mg/m^3 (n=2)	
		8 h	0.9 mg/m^3 (n=5)	
4 operating rooms plus ENT-dept;	active PAS	4 h	16 mg/m^3 (n=1)	
		8 h	5 mg/m^3 (n=1)	
1 anaesthetic assistant	active PAS	4 h	7 mg/m^3 (n=3)	
3 - 4 other operating personnel	not given	not given	33-330 mg/m^3 (n=?)	Smu85
1 polyclinic; anaesthetists working with a rollable anaesthesia apparatus				
1 sluder room	not given	not given	107-542 mg/m^3 (n=?).	Mar96
1 sluder room	not given	not given	Peak value 402 mg/m^3 (n=?)	
7 operating rooms[a]	active PAS	8 h	Median: 0.3 mg/m^3 (0.003-36 mg/m^3), n=69, 23 were above detection limit.	Pee99
7 sluder rooms	active PAS	during sluder sessions, generally 1.5 hours	Median: 6.1 mg/m^3 (0.5-135 mg/m^3), n=21, 15 were above detection limit.	

[a, f] See Table 4.5.

Table 4.2 Occupational concentrations of halothane for hospital personnel in other countries.

working place; persons exposed	air sampling	sampling time	concentration (number of measurements)	ref.
74 operating theatres[b,c] in Canada: all staff; n=364	active PAS	mean: 135 min	median, 0.9 mg/m^3 (n=503); 40 h TWA, 0.6 mg/m^3 (n=364); range, 0.04-769 mg/m^3 (n=505).	Raj89
40 operating theatres[c] in UK:	diffuse PAS	8 h		Gar 89b
anaesthetists[d]			2.5-378 mg/m^3 (n=35); GM (GSD), 33 mg/m^3 (4.0).	
surgeons[d]			2.5-58 mg/m^3 (n=12); GM (GSD), 10.7 mg/m^3 (1.3).	
anaesthetists[b]			1.6-82 mg/m^3 (n=21); GM (GSD), 9.0 mg/m^3 (2.4).	
surgeons[b]			1.6-15.6 mg/m^3 (n=9); GM (GSD), 4.9 mg/m^3 (2.2).	
2 operating theatres in Spain[c,d]	diffuse PAS	during anaesthesia	1otorhinolaryngology, 176-200 mg/m^3; 1 general surgery, 66-121 mg/m^3.	Unc89
Operating theatres[c] in Germany, all staff[d]:	diffuse PAS	during anaesthesia: 15.3 h in one week	7.4±8.2 mg/m^3 (n=37);	Weg90
7 air changes/hour:		14.6 h in one week	2.8±2.3 mg/m^3 (n=50);	
23 air changes/hour:		6.3 h in one week	0.6±0.7 mg/m^3 (n=24).	
5 operating theatres[c] in Italy; anaestehtists and technicians	diffuse PAS	4 h	0.35-75 mg/m^3; median (GSD), 10.4 (3.9) mg/m^3 (n=58).	Imb91
Operating theatres[c] in Czechoslovakia[e]; anaesthetists	PAS (not specified)	during anaesthesia	9-490 mg/m^3 (n=?, at least 24).	Kar92
2 operating theatres in France	diffuse PAS	8 h	85 mg/m^3 (18-317 mg/m^3; n=20)[d] 23 mg/m^3 (3.3-59 mg/m^3; n=25)[b]	Gan93
1 operating theatre in Japan; anaesthetists	not given	not given	face-mask narcosis[e]: 0-123 mg/m^3, mean, 40 mg/m^3 (n=60?); vertical laminar flow, 4.5±4.4 mg/m^3 (n=40); horizotal laminar flow, 49±31 mg/m^3 (n=80).	
102 operating theatres in Germany:			range, 4.1-82 mg/m^3;	Boh95
physicians	active PAS	30-240 min	90% values <28 mg/m^3 (n=108),	
other personnel	active PAS	30-240 min	90% values < 9 mg/m^3 (n=116).	

[b, c, d, and e] See Table 4.5.

Table 4.3 Occupational exposure concentrations of halothane for personnel in paediatric hospitals.

working place; persons exposed	air sampling	sampling time	concentration (range; no. of measurements)	ref.
2 Operating theatres (Finland); - respectively 6 and 4 nurses	active PAS	during anaesthesia 30-60 min sample periods	During intubation: 16.6 mg/m^3 (4.4-66.5 mg/m^3 (n=?) Using face masks: 274 mg/m^3 (n=?).	Kor78
2 Operating theatres (France); -3 anaesthetists	ambient air	1 h after last anaesthesia	One with scavenging: - 1 anaesthesia: 555 mg/m^3 (n=1), - 5 anaesthesias: increase to 975 mg/m^3 (n=1). One with scavenging and air filtering: - 1 anaesthesia: 40 mg/m^3 (n=1) - 3 anaesthesias: increase to 116 mg/m^3 (n=3).	Sti86
1 Hospital in the US	not given	not given	54% samples: >4.1 mg/m^3, (n=24)	Gun86
1 Operating theatre (Germany); - 5 anaesthetists[c] - 8 volunteers in an exposition chamber[d]	 active PAS active PAS	 during anaesthesia during exposition	 60-700 mg/m^3 (n=32) 10-800 mg/m^3 (n=22)	Sch95
1 Operating theatre (Austria); - anaesthetist (n=?)	 ambient air	 total 20 operations	Range during induction: 18-149 mg/m^3; median: 29 mg/m^3, range during maintenance: 1.6-19 mg/m^3; median: 4.9 mg/m^3.	Hoe97

[c and d] See Table 4.5.

Table 4.4 Occupational exposure concentrations of halothane for personnel in veterinary hospitals.

working place; persons exposed	air sampling	sampling time	concentration (range; no. of measurements)	ref.
16 Veterinary operating theatres (USA)	- continuous recording of breathing zone,	during anaesthesia	[d] mean: 16.4 mg/m^3 [b] mean: 11.9 mg/m^3	Sho83
	- continuous recording of general room air	during anaesthesia	[d] mean: 10.9 mg/m^3 [b] mean: 12.2 mg/m^3	
10 Veterinary surgeries (USA); 13 veterinarians[d,e]	diffusive PAS	during anaesthesia 9-85 min	31.6 mg/m^3 (9.4-75.5 mg/m^3) (n=10)	Pot88
		13-41 min	0.7 mg/m^3 (<0.6-1.2 mg/m^3) (n=6)	
Veterinary surgeries (UK); -anaesthetists and technicians (n=14)[c,d]	diffusive PAS:	during anaesthesia(<4 h)	- <4.1-977 mg/m^3 (n=19; TWA 44-431 min); GM (GSD): 91 mg/m^3 (5.3); or: <4.1-280 mg/m^3 (n=18; TWA 8 h); GM (GSD): 38 mg/m^3 (4.0)	Gar91
-anaesthetists and technicians[b,c]	diffusive PAS:	during anaesthesia(<4 h)	- <4.1-49 mg/m^3 (n=35; TWA during anaesthesia; GM (GSD): 9.9 mg/m^3 (2.2); or: <4.1-25 mg/m^3 (n=34; TWA 8 h); GM (GSD): 5.7 mg/m^3 (1.7).	
3 Veterinary surgeries (Canada); veterinarians and technicians	continuous recording of breathing zone,	during anaesthesia	Veterinarians (n=58): 5.7-98 mg/m^3; GM (GSD): 23.8 mg/m^3 (11.5); Technicians (n=41): 3.3-26 mg/m^3; GM (GSD): 12 mg/m^3 (14);	Kor99
	continuous recording of general room air	during anaesthesia(<4 h)	0.82-77 mg/m^3 (n=56): GM (GSD): 11.5 mg/m^3 (14).	

[b,c,d and e] See Table 4.5.

Table 4.5 Concentrations of halothane in recovery rooms.

recovery room	air sampling	sampling time	concentration (no. of measurements)	ref.
3 Recovery rooms (the Netherlands)	ambient air, at least 6 points	7 h	1.6-8.0 mg/m^3 (n=?)	Rej80
1 Recovery room (the Netherlands)	active PAS	8 h	0.6 mg/m^3 (n=2)	Zwe83
- other operating personnel	stationary	8 h	0.7 mg/m^3 (n=2)	
1 Recovery room (UK)	not given	each 5 min during 24 h	In absence of patients: 0.4 mg/m^3; at admission of patients: 8.2 mg/m^3; halothane level was decreased to background after 120 min, although patients left the room after 80 min.	Rit88
18 Recovery rooms (UK)[c,e]	diffusive PAS	8 h	range: <1.6-27 mg/m^3; GM (GSD): 1.7 mg/m^3 (2.2) (n=28); 96% values: <16 mg/m^3	Gar89b
2 Recovery rooms (Spain)	stationary	not given	1 After otorhinolaryngology: 14 mg/m^3; 1 after general surgery: trace amounts.	Unc89
16 Recovery rooms (Germany)	active PAS	0.5 - 2 h	range: 4.1-9.9 mg/m^3 (n=26); 90% values: <4.4 mg/m^3.	Boh95
16 Veterinary recovery rooms (USA)	-continuous recording of breathing zone	not given	Mean: 8.8 mg/m^3.	Sho83
	-continuous recording inside recovery cage	not given	Mean: 10.3 mg/m^3.	
3 Veterinary recovery rooms (Canada)	continuous recording of breathing zone	not given	3.4-1309 mg/m^3; GM (GSD): 55-347 mg/m^3 (24; 8.2) (n=180)	Kor99

[a] Whether personnel was also exposed to nitrous oxide is not known,
[b] With scavenging facilities.
[c] Persons were also exposed to nitrous oxide,
[d] Without scavenging facilities,
[e] Using a face mask,
[f] PAS, personal air sampling.

Simultaneous exposure

In a study, in which one German and twenty-five Swiss hospitals co-operated, air concentrations of nitrous oxide and halogenated anaesthetics were measured during a total of 114 operations. Halothane was not monitored separately. Of those operations, 55 were carried out on children younger than 10 years, 9 on children aged between 11 and 16, and 50 on adults.

Occupational exposure to anaesthetics during operations of very young children was on average fivefold higher than during operations of adults (41-90.2 mg/m^3 (5-11 ppm) children *versus* 8.2-16.4 mg/m^3 (1-2 ppm) adults). Furthermore, high occupational exposure levels were particularly found when high gas flows were used and when standard face masks were loosely held. The authors showed that an effective reduction of occupational exposure up to 85% can be achieved by efficient scavenging systems and lower fresh gas flows (Mei95).

In general a ratio of 50:1 of nitrous oxide:halothane is used for anaesthesia. In one report, the same ratio was measured in the breathing zone of the personnel (Gar89b), but several other studies demonstrated variations. For instance, in Canadian operating theatres the ratio varied from 48:1 for anaesthetists to 730:1 for surgeons (Raj89). In the Netherlands, ratios between 33:1 and 140:1 for anaesthesia assistants and between 574:1 and 596:1 for other operating personnel were measured (Zwe83). The results of the last study should be carefully interpreted, because sampling times differed for nitrous oxide (10 min) and halothane (4 hours).

Chapter 5

Kinetics

5.1 Absorption

In patients (n=10, average 27 yr; n=10; average 65 yr), anaesthetised for 20 minutes with halothane, the ratio of the alveolar (F_A) to the inspired (F_i) amount of halothane was estimated to be 0.52-0.53, and this was irrespective of their age (Dwy90). Others, however, reported higher alveolar uptake after anaesthesia, namely ratios of 0.67-0.75 (Shi90, Car86a, Reh67).

The uptake of halothane by the lungs varies with body size. For instance, in a study in which humans, rats and one whale (6,330 kg) were exposed to 1.15 g/m^3 halothane for 1 hour, the most rapid uptake was in rats (68.4±4.9%), followed by humans (50.4±4.2%) and the whale (40.6%) (Wah74).

In excised rat skin (n=17; 32°C) exposed to 0.34 or 1.67 g/m^3 halothane, the skin/air partition coefficient averaged 10.6±0.7, and the permeability constant measured 0.45 mm/h. These are relatively low values (Mat94). Approximately 0.2% of the halothane is taken up by the skin after whole body exposure (Mat94, McD90).

5.2 Distribution

The distribution of inhaled halothane among several organs and other parts of the body is studied *in vitro*. In summary, halothane is easily distributed in body fat. In fact, concentrations in body fat were found to be 110-220 times higher than in air.

Halothane does not distribute well in blood, whereas in other organs the partition coefficient varied between 3 and 10 (Cob86a/b, Fis86, Gar88, Gar89a, Ler86, Pat78, Ste90, Tah91, Wah74, Wan93, Wil96, Yas89, Zbi85).

A few human studies have been published about the placental transfer of halothane. In one study, women (n=8) scheduled for caesarean section received 41.05 g/m^3 halothane for about 13 minutes. Immediately after delivery, arterial blood from the mother and venous blood from the umbilical cord were taken. The ratio of the venous (0.22±0.03 mg/mL) to the arterial (0.52±0.07 mg/mL) blood calculated was 0.44 ± 0.05 (Sat95). In a similar study, 11 women underwent caesarean section, after which the partial pressure of the umbilical vein (P_{uv}), umbilical artery (P_{UA}) and of the maternal artery (P_A) was measured (Dwy95). The concentration of halothane was expressed as partial pressures. The ratio (P_{uv}/P_A) was 0.71±0.16, which was higher than in the study of Satoh *et al.* (Sat95). The committee finds it difficult to explain the different outcomes. Probably several explanations are applicable.

In another human study, the ratio P_{UV}/P_{UA} was calculated and found to be 0.38±0.12. Although the foetal washout was not measured, the so-called Apgar scores were satisfactory, suggesting that volatile agents are rapidly eliminated by the newborn, in this case within 5 minutes after delivery (Dwy95).

Placental transfer was also studied in mice. Groups of C57BL mice were exposed to halothane at concentrations of 0.37-26.24 g/m^3 for 1 hour on gestation day 18 (GD18). Directly following the exposure the maternal concentration of halothane in plasma decreased rapidly. In the amniotic fluid the concentration of halothane never reached more than 20% of maternal plasma levels. Metabolites of halothane (trifluoroacetic acid, bromide), which were formed mainly by maternal metabolism, accumulated in the foetus and amniotic fluid, reaching plateau levels in amniotic fluid between 4 and 24 h after exposure. No measurements were made after 24 hours (Gha86).

Pregnant C57BL/6 mice were exposed to radiolabeled halothane (Dan84) at a dose of approximately 80 μCi ^{14}C-halothane for 10 minutes on GD11, GD14 or GD17. Immediately after ending the exposure, a very high uptake of halothane in the brain, body fat, the nasal mucosa, blood, the liver, the kidneys and the lungs was measured in the pregnant mice. Metabolites of halothane were found in small but significant amounts in the same organs, except in body fat and brain. The concentration of these metabolites peaked after 4 hours in several organs, such as in the liver, the kidneys, the eyes, and the lungs, whereas the concentration of halothane decreased in these organs.

Halothane passed the placental barrier at all studied stages of gestation. At GD11, halothane was evenly distributed among the various embryonic structures, whereas at GD14 and 17 — the foetus being more differentiated — higher concentrations in the blood and the liver were found compared with other foetal organs. One hour after

ending the exposure, the major part of the volatile ^{14}C-halothane had left the foetus (Dan84).

5.3 Biotransformation

Halothane is enzymatically oxidised or reduced by enzymes of the cytochrome P450 monooxygenase system (P450), among others by P450-2B and -2E1.

Patients were given a single oral dose of disulfiram, an inhibitor of P450-2E1, 12 hours before anaesthesia with halothane. All patients showed a significantly reduced breakdown of halothane (Kha96). The extent at which halothane is metabolised in humans varied between 5 (Pez89), 6 (Shi90), 20 (Ken94), 30 (Cla95, study from 1978) and 55% (Cah81). The extent is inversely related to the exposure concentrations of halothane in humans (Cah81) (not in rats (Loi97)), is influenced by environmental and genetic factors (Cas71) and is sex-related (Loi97).

Under *aerobic* conditions (oxidation), halothane releases a bromide ion, after which the intermediate metabolite is hydrolysed to trifluoroacetic acid (TFA) and a chloride ion.

Under *anaerobic* conditions (reduction) the bromide ion is also released, whereas the intermediate metabolite is reduced by P450 enzymes to a reactive radical. This radical reacts with biomacromolecules, or abstracts a hydrogen atom from another molecule to yield 2-chloro-1,1,1-trifluoroethane (CTFE), or is further metabolised by P450 enzymes to 2-chloro-1,1- difluoroethylene (CDFE), releasing a fluoride ion (see Figure 5.1) (Goe95, Kam 90, Pez89). Whether in humans reduction of halothane does occur is currently unknown, but reductive metabolites of halothane have been detected in exhaled air from patients even after halothane anaesthesia in 100% oxygen (Kam90). In an animal study, in blood of rats given unrealistic low oxygen supply (14%) during halothane exposure, CTFE and CDFE were found (Tam87).

The formation of metabolites of halothane is studied in several human, animal and *in vitro* studies.

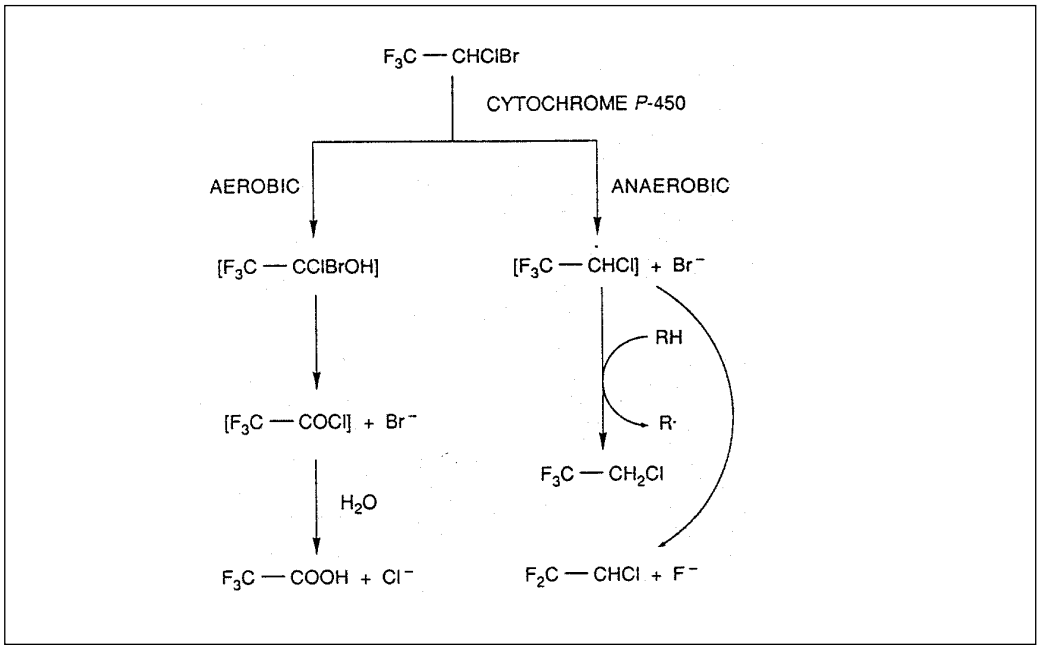

Figure 5.1 Metabolic pathways of halothane (Kam90). The last compound on the right-hand side of the figure should contain a double bond: $F_2C = CHCl$.

Human studies

In 1985, investigators determined serum bromide levels after exposure of halothane in 26 persons working in an operating theatre. In the first two weeks (day 1-10) only enflurane, a halogenated anaesthetic, was used for the anaesthesia of patients, but in the following three weeks (days 11-32) only halothane (air concentration not known). Blood samples (n=7-14) from the personnel were taken on days 1, 10, 18, 24 and 32. The highest individual bromide concentration measured was 1.24 mmol/L. On average, serum bromide levels were significantly higher on the exposure days (day 18, 24 and 32) than before exposure (day 1 and 10); mean difference 0.22±0.18 mmol/L (n=26). Overall, the mean plasma bromide levels were significantly higher in the personnel than in patients (n=11) not exposed to halothane for at least three months (0.48±0.05 mmol/L) (Car85).

In 1990, staff members of operating theatres in a German hospital donated voluntarily blood and urine to assess levels of TFA over a working period of one week (Weg90). Also personal air samples were taken. The results suggest that even at low exposure levels halothane is metabolised into TFA (Table 5.1), although on urinary samples no statistical analyses were performed. The committee has difficulty with interpreting the results, because workers exposed for a longer period to high concentrations of halothane showed nonsignificantly increased TFA levels in blood,

whereas workers exposed for a shorter period to low concentrations showed significantly increased TFA levels.

Table 5.1 Concentrations of halothane in air and of TFA in blood and urine taken from medical staff in a German hospital, at the beginning (B) and at the end (E) of the week (Weg90).

group exposed	halothane (mg/m^3)[a] personal air sampling	TFA in blood (μM)	TFA in urine (μM)
anaesthetists (n=7)	6.1 ± 9.8, (n=26)[b]	B: 0.60 ± 0.45	B: 0.02 ± 0.01
		E: 1.60 ± 1.48	E: 0.12 ± 0.06
surgeons (n=10)	4.4 ± 4.5, (n=31)[c]	B: 0.39 ± 0.10	B: 0.02 ± 0.007
		E: 0.83 ± 0.39 (p<0.001)	E: 0.04 ± 0.02
operation assistants (n=8)	3.0 ± 2.3, (n=31)[d]	B: 0.40 ± 0.17	B: 0.02 ± 0.01
		E: 0.97 ± 0.70	E: 0.07 ± 0.07
ambulatory nurses (n=6)	1.8 ± 1.6, (n=23)[e]	B: 0.38 ± 0.14	B: 0.01 ± 0.007
		E: 0.56 ± 0.61 (p<0.05)	E: 0.05 ± 0.03

[a] Measured during the hours that persons worked with halothane.

[b] 14.7±6.4 h in one week (n=7).

[c] 9.9±4.7 h in one week (n=10).

[d] 14.3±6.5 h in one week (n=8).

[e] 11.6±5.9 h in one week (n=6).

In patients (n=10) undergoing urological surgery, the end-tidal concentration and serum levels of halothane, TFA and inorganic fluoride were monitored at different time points during and after anaesthesia (Imb87), which lasted on average 76±45 minutes. In the first hour, patients received 61.6 g/m^3 halothane (=0.75%) combined with a N_2O/O_2 mixture (50/50), whereas during the rest of the operation time anaesthesia was maintained with nitrous oxide (N_2O) alone. As soon as halothane administration was stopped, the concentration of halothane in exhaled air quickly decreased. The concentration of halothane in blood reached a plateau at 20 minutes after starting the anaesthesia, but quickly decreased after stopping the administration. TFA increased till 23 hours after the anaesthesia, then slowly decreased, but was still not at control levels after three days. Inorganic fluoride remained nearly constant during and after the anaesthesia. The results are shown in Table 5.2.

Table 5.2 Plasma halothane, inorganic fluoride and TFA levels in patients anaesthetised with halothane (61.6 g/m^3 (=0.75%)) for 1 hour, during and after anaesthesia (Imb87).

time	halothane end-tidal (g/m^3)	halothane in blood (µg/mL)	inorganic fluoride (µM)	TFA (µg/mL)
0 min			2.4 ± 1.8	
10 min		63.5 ± 32.0		3.0 ± 1.9
20 min		81.2 ± 37.5		3.8 ± 1.9
40 min		70.6 ± 19.3		
1 h	7.14 ± 4.52	68.2 ± 35.7	1.7 ± 0.7	5.3 ± 3.2
1.5 h	1.40 ± 0.74			
2 h	0.90 ± 0.82	11.3 ± 6.7	2.2 ± 1.3	6.1 ± 1.6
3 h	0.57 ± 0.41	7.3 ± 5.0	2.0 ± 0.9	5.5 ± 1.9
6 h		6.3 ± 5.6	1.7 ± 0.7	7.4 ± 0.6
24 h				7.8 ± 2.0
48 h				4.1 ± 1.6
72 h				3.6 ± 2.0

Japanese patients (n=8) were anaesthetised with 69.8 g/m^3 halothane (=0.85%) for one hour, to undergo minor elective surgery (Shi90). They calculated that 66.9±5.7% of the halothane was taken up by the body and that 18.1±4.2% of the inhaled halothane was eliminated by the lungs. Within five days, 14.6±9.2 mmol fluoride was excreted in the urine. This amount correlated with 15.7±10.0% of the halothane taken up during anaesthesia.

Serum inorganic fluoride concentrations were also measured in patients (n=10), who were anaesthetised with halothane for prolonged head and neck surgery. Patients received a mean concentration of halothane of 109.2±6.3 g/m^3 (1.9±0.13 MalvC). The operation time averaged 10.2±0.59 hours. Plasma inorganic fluoride concentrations increased from a mean of 3.8 µmol/L (baseline) to a peak of 12.6 µmol/L at 24 hours after induction of anesthesia. Levels were still not back to control levels 48 hours later (Mur92).

Bentley *et al*. (Ben82) performed a study on the effects of obesity and metabolism of halothane in humans. Morbidly obese (n=17) and nonobese (n=8) patients, who were scheduled for surgery, received 73.9-246.3 g/m^3 halothane for a maximum of two hours. Both during and after anaesthesia, serum levels of TFA, inorganic fluoride and bromide were measured at different time points. TFA levels peaked at 24-72 hours after initiating the anaesthesia and this was the same in obese (613±66 µM) and nonobese patients (501±94 µM). One to two weeks later, however, obese patients showed still significantly elevated serum TFA levels, whereas nonobese patients were at control level. This suggests that halothane is released slowly from fat tissues. In obese patients, serum levels of inorganic fluoride peaked at two hours after initiating anaesthesia

(3.2±0.6 μM), whereas in nonobese patients concentrations of inorganic fluoride remained more or less constant (1.9±0.2 μM). The committee considers the results of the bromide measurements unreliable, because Pancuronium bromide was utilised for muscle relaxation during surgery. This may have interfered with the bromide measurements. Furthermore, data are lacking to calculate the half-life or the amount of halothane that is metabolised.

Kotlyar and Carson (Kot99) reviewed the literature on the effects of obesity on the cytochrome P450 system in humans. The authors concluded that the activity of cytochrome P450-2E1 was increased and that of cytochrome P450-3A4 was decreased in obese persons.

Animal studies

The metabolism of halothane was mainly studied on Fischer 344 rats, because this strain possesses higher contents of P450 isoenzymes than other strains.

Male Fischer 344 rats (n=42 total) were exposed to 82.1 g/m^3 halothane (=1%) during two hours. Before the exposure, the animals were pre-treated with either saline or with isoniazid to enhance oxidative hepatic metabolism. Serum levels of TFA and bromide were significantly elevated in rats pre-treated with isoniazid and not in those pre-treated with saline, whereas serum levels of fluoride differed not between the two groups. The amount of metabolised halothane was not calculated, but it was estimated that half of the halothane and its metabolites were cleared in 48 hours (Ric87).

Investigators described a study on young male Fischer 344 rats (n=3-4/group), which were exposed to 164.2 g/m^3 halothane during the first five minutes and to 82.1 g/m^3 for an additional 55 minutes in combination with 99% oxygen. Before the exposure, half of the rats were treated with phenobarbital to enhance oxidative hepatic metabolism. For making ^{19}F-NMR spectra, all animals received a nonfluorinated long acting anaesthetic 15 to 60 minutes after stopping the exposure. TFA was detected more rapidly and at higher levels in phenobarbital-treated animals than in non-treated animals. A steady state level was reached 2 hours after ending the exposure to halothane, and remained fairly constant over the time course of the study. The half-life's of halothane were 3.5 and 2.5 h for phenobarbital- and non-treated animals, respectively. The loss of halothane was a single exponential first order decay process with a rate constant of 0.20±0.01 h^{-1} in the non-treated rats, and 0.28±0.04 h^{-1} in pre-treated rats (Sel87).

In 1997, a study was published in which Sprague Dawley rat strains were used instead of Fischer 344 strains. Rats (n=3-5/sex) were exposed to 4.1, 8.2, 16.4, 24.6 or 32.8 g/m^3 halothane for 6 hours. Directly after exposure, the animals were placed in metabolic cages to collect urine for over 4 days. In both genders, the uptake of

halothane reached saturation at around 3.3 mg/m^3. The cumulative urinary TFA excretion was 2 to 3 times higher in males than in females (24.6-32.8 mg/m^3;p<0.005). The rate of metabolism was not concentration-dependent. With these results, the authors developed a physiologically-based pharmacokinetic model to describe concentration-dependent kinetics of halothane in rats (Loi97).

In one study, the influence of obesity on the metabolism of halothane was studied. Obese male Fischer 344 rats (n= 8 pairs), put on a high-fat diet and weighing approximately 20% more than normal rats (438±20 *versus* 365±18 g), and nonobese animals inhaled 64.1 g/m^3 halothane for four hours. Thirty-six hours following exposure, serum TFA, bromide and fluoride peaked at 7.3±1.1 mM, 9.1±1.0 mM and 4.9±0.6 µM respectively in obese animals, and at 4.7±0.7 mM, 6.9±0.6 mM and 4.1±1.0 µM respectively in nonobese animals. For fluoride these values did not differ significantly between the obese and nonobese animals. The total amount of TFA and bromide excreted in the urine, which was collected during 180 hours after the exposure, was 519±69 and 127±30 µmol respectively in obese animals, and 336±22 and 79±14 µmol respectively in nonobese animals; urinary excretion of fluoride was not elevated. These results show that the metabolism of halothane is significantly higher in obese animals than in nonobese animals. One of the explanations given by the authors is that storage of halothane in a much larger fat depot may result in sustained halothane release in the postanaesthetic period and thus prolonged availability of halothane for biotransformation. The presented data do not show increased anaerobic metabolic activity (reduction) in obese rats compared with nonobese animals. The half-life of halothane is estimated to be approximately 300 hours (12 days), whereas TFA was virtually completely excreted in the urine in 180 hours (Bie89).

Other investigators reported on the distribution of halothane metabolites in male Sprague Dawley rats (n=not given), which received a single intravenous injection of 10 µCi ^{14}C-halothane (9.4 mg/kg b.w.). Two, 20, 60 and 240 minutes later, the animals were killed and prepared for analysis. In short, sagittal freeze-dried sections of the whole body were made, before volatile radioactive halothane was removed. In half of the sections, also unbound radioactive metabolites of halothane were chemically removed before film exposure. Two minutes after starting the exposure, the labelling of bound radioactive metabolites was most marked in the nasal, oral, and oesophageal mucosa, liver, and the intestinal contents, whereas it was less marked in the kidneys, tongue mucosa, cheek and soft palate, and the pharyngeal mucosa. Similar distribution patterns were observed in sections of animals killed later. With time, however, the proportion bound radioactive metabolites increased in the relevant tissues. The localisation of halothane metabolites in the upper alimentary and respiratory tract is correlated to the presence of P450 enzymes at these sites (Gha88).

In the same study, the capacity to incorporate radioactive metabolites of halothane in various tissues was also investigated *in vitro*. The highest incorporation was found in the nasal olfactory mucosa, followed by in decreasing order, the liver, lateral nasal glands, cheeks, oesophagus, tongue, nasal respiratory mucosa and the trachea. The lowest incorporation was recorded for the heart, the lungs, the kidneys, and the walls of the intestines and stomach (Gha88).

Other investigators demonstrated that rat liver microsomes and human HepG2 cells, a human liver cell line expressing P450-2E1, metabolised halothane into among others TFA. For HepG2 cells the metabolic activity averaged 0.82±0.18 nmol/min/nmol P450-2E1, which was 5 to 20 times higher than rat hepatic microsomes could metabolise (Yin95).

5.4 Elimination

Human data

In two different hospitals, anaesthetists were occupationally exposed to 8-82 mg/m^3 halothane (hospital 1, n=7) or to 25-214 mg/m^3 halothane (hospital 2, n=8). Seven to 64 hours after exposure, halothane was still detected in end- expired breath. The detection limit was not mentioned (Cor73a).

The same investigators showed that patients (n=12-19) exposed to about 114.9 g/m^3 halothane for one to 5½ hours, exhaled the compound for 11 to 20 days after exposure. In one anaesthetist, the amount of exhaled halothane was measured after five single and one repeated occupational exposure(s). Breath decay curves showed that halothane was exhaled for 26 hours (70 min single exposure) to 64 hours (390 min single exposure), whereas before exposure no measurable levels in exhaled air were detected. Concerning the repeated exposure, the anaesthetist was first exposed for 300 minutes and 24 hours later for 30 minutes. Three days after his last exposure, halothane was still detected in the exhaled air. Half-life's were not calculated (Cor73b).

In 1978, nurses were occupationally exposed to a mixture of halothane and nitrous oxide. In nurses (n=4) exposed to 274 mg/m^3 halothane, blood levels averaged 2.98 µg/mL and end-tidal concentration averaged 88.7 mg/m^3 sixty minutes after exposure. The data showed that the higher the exposure level of halothane was, the higher the levels in blood and expired air were (Kor78). The committee concludes from this study that halothane is slowly excreted from the body.

Elimination of halothane by the lungs was studied in healthy volunteers (n=9) undergoing donor nephrectomy. The donors received premedications and 65-70% nitrous oxide for 30 minutes, before the exposure with 18.5±0.4 g/m^3 halothane. Halothane was given in combination with isoflurane, enflurane or methoxyflurane for 2

hours. Directly after the anaesthesia with halothane, the ratio of the alveolar (F_A) to the inspired (F_i) concentrations was estimated on approximately 0.7. The decline in the alveolar concentration after stopping the administration of halothane occurred rapidly; five days after anaesthesia, it was estimated at 0.1% of the alveolar concentration measured directly after anaesthesia. However, the half-life of halothane in the alveoli is not given (Car86a). The authors estimated that the quantity of halothane metabolised was 46.1 ± 0.9%, considering the following deductions and assumptions: 1) the rate of metabolism was estimated from the quantity recovered unchanged, 2) the recovery of halothane was related to the recovery of isoflurane, because they were simultaneously administered, and 3) it was assumed that isoflurane was not metabolised (Car86b). The committee believes that the method is too crude to measure the rate of metabolism correctly.

The same investigators performed a study on seven healthy volunteers, who received the same mixture of halothane, isoflurane, enflurane, and methoxyflurane, under similar conditions, but now for only 30 minutes instead of two hours. The end-tidal and mixed-expired anaesthetic concentrations were followed up to 9 days after exposure. Compared with the previous study, the alveolar concentrations decreased more rapidly. However, the duration of administration did not affect the time constants determined, the number of compartments identified and the percentages of anaesthetic metabolised (Car87b).

In 1991, elimination of halothane via the skin was studied in healthy male volunteers (n=8), who received a mixture of anaesthetics for 30 minutes. The percutaneous loss was estimated to be 0.23±0.03% of the total they received (16.4 mg/m^3 (0.2%) halothane, combined with 2% deflurane, 0.4% isoflurane and 65% nitrous oxide). A comparable result (0.25%) has been reported in 1969. In that study, patients (n=6) received anaesthesia for one hour; the end-tidal concentration of halothane was 73.9 mg/m^3 (Sto69). In the first study, percutaneous loss of halothane was higher than of for the other anaesthetics. The authors, however, expected the opposite, because of all the anaesthetics used in their study, halothane has the highest affinity for fat. The authors, therefore, suggest that the differences in fat solubility are too small to affect loss or that the fat reservoir in the body is so large that the capacity to store fat soluble compounds seems infinite and release follows other processes (Fas91).

The same group of investigators performed a study on the exhalation of halothane, desflurane and isoflurane. Healthy male volunteers (n=8) were exposed as described above. End-tidal concentrations (F_A), mixed exposure concentrations and minute ventilations were measured up to 7 days after exposure. Directly after stopping the exposure, the ratio F_A/F_i (inspired concentration) was 0.58±0.04. Five minutes later, the alveolar concentration declined to 0.25±0.02% of the alveolar concentration measured

directly after stopping anaesthesia. After 7 days, the total recovery of halothane was calculated at 64±9%. From these data, the authors argue that halothane is partially exhaled, but that approximately 40% is eliminated by the liver. The committee remarks that this is not necessarily true, because halothane could be accumulated in fat tissue (Yas91).

Carpenter *et al*. (Cas71) investigated the excretion of halothane in the urine of pharmacists (n=4) and anaesthetists (n=5). The volunteers received an intravenous injection of 3.4 mg radiolabeled ^{14}C-halothane, dissolved in 5 mL saline solution (total 25 µCi). Total urine was collected at 2, 4, 6, 8, 12 and 24 hours after injection, and thereafter daily up to 7 days. During the following 14 days, single samples of urine were collected. In general, the pharmacists excreted less metabolites of halothane than the anaesthetists. Four days after the injection, the pharmacists had excreted 14-17% of the dose, whereas the anaesthetists excreted 10-23%. Twenty one days after the injection, trace amounts of halothane metabolites could still be demonstrated in the urine.

In the same study, the urinary excretion of halothane was also assayed in 5 identical and 5 fraternal twins. They probably received the same dose as mentioned above. Identical twins metabolised considerably more halothane than fraternal twins. The normalised intrapair differences in the halothane metabolites excreted were smaller in identical (2.4 to 17.3%) than in fraternal twins (2.9 to 70.0%). The authors concluded that the metabolism of halothane is primarily genetically controlled (Cas71).

Animal data

Male Sprague Dawley rats inhaled 123.2 g/m^3 halothane for one hour. After ending the exposure, rats were killed at different timepoints (3 animals per timepoint). Elimination of halothane from the blood and the brain was biexponential. The T_1 elimination time constants in these organs were 1.29±0.15 and 2.70± 0.66 minutes, respectively. The T_2 were 36± 5.6, 34±7.8 and 246±51 minutes for the blood, the brain, and adipose tissue, respectively. These results are consistent with the notion that the initial rate of elimination of anaesthetics from tissue is considered to be a function of its blood/gas partition coefficient, whereas the long-term elimination rate of anaesthetics from body stores is considered to be a function of its tissue/blood partition coefficient (Ste90).

In another study, male Wistar rats (n=5) were exposed to 1.2 g/m^3 (150 ppm) halothane for 7 weeks (8 hours/day, 5 days/week). During exposure, blood samples were taken on Monday mornings and Friday evenings. As a result, the level of halothane in blood gradually accumulated during exposure and gradually decreased after ending the exposure. No half-life was calculated (Mar84). Data are presented in Table 5.3 and 5.4, and in Figure 5.2.

Table 5.3 Concentrations of halothane in blood of rats, which were exposed to 1.2 g/m^3 halothane for 7 weeks (8 h/day, 5 d/week) (Mar84).

week	halothane (µg/mL (SD); n=5)	
	monday	friday
1	0.00	0.20 (0.02)
2	0.04 (nd)	0.32 (0.02)
3	0.05 (nd)	0.43 (0.03)
4	0.07 (nd)	0.47 (0.03)
5	0.14 (0.02)	0.55 (0.03)
6	0.16 (0.02)	0.70 (0.04)
7	0.25 (0.02)	0.73 (0.04)

nd = not determined.

Table 5.4 Concentrations of halothane in blood of rats after ending the exposure to halothane (Mar84).

time (days)	halothane (µg/mL (SD); n=5)		time after last exposure (h)	
	8.00 am	4.00 pm	8.00 pm	4.00 am
1st	0.200 (0.010)	0.180 (0.015)	64	72
2nd	0.140 (0.013)	0.100 (0.090)	88	96
3rd	0.080 (0.060)	0.070 (0.050)	112	120
4th	0.045 (nd)	0.030 (nd)	136	144
5th	nd			

nd = not determined.

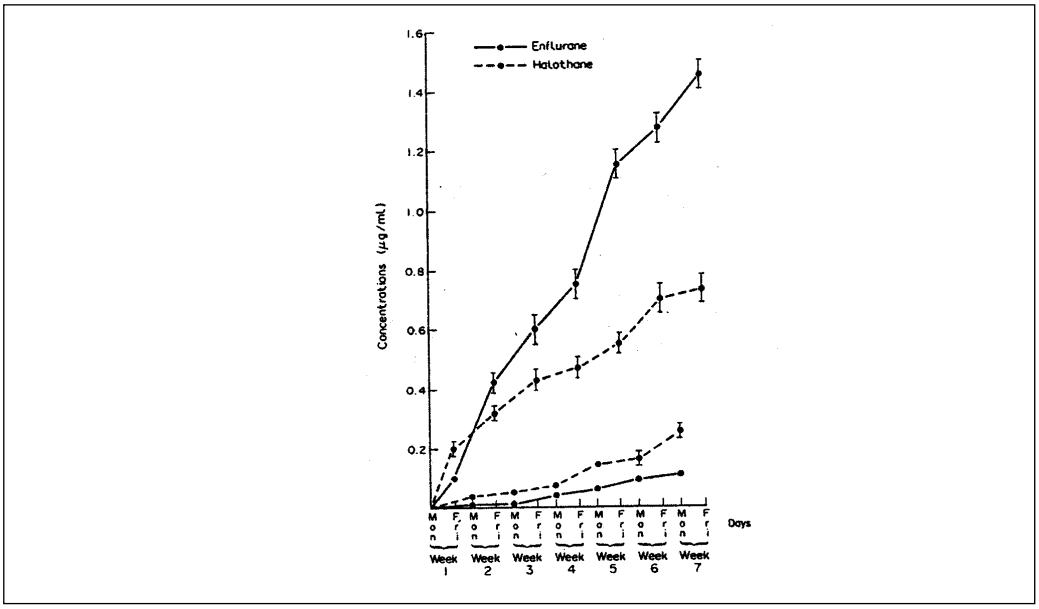

Figure 5.2 Blood levels (µg/mL) of halothane and enflurane in rats, exposed to 1.2 g/m^3 (150 ppm) halothane or 200 ppm enflurane for 7 weeks (Mar84). The lower two lines represent data obtained on Mondays, the upper two lines represent data obtained on Fridays.

5.5 Simultaneous exposure

A few studies, in which patients were anaesthetised with a mixture of anaesthetics, are described in the previous sections.

Carpenter *et al*. (Car86a/b) anaesthetised patients with a mixture of 60-65% nitrous oxide and halothane, enflurane, isoflurane and methoxyflurane (see also section elimination), and speculated on a possible pharmacokinetic modulation among the anaesthetics by comparing their results with results found by others. The authors concluded that the degree of metabolism found in their study is comparable or exceeding those found by others, indicating no interference among halothane and the fluranes in humans. The committee remarks that this conclusion is doubtful, because different kind of data were compared with each other, such as comparisons of individual exposure data with group exposure data. Moreover, the observations made by Carpenter *et al*. were in contrast with observations made in two studies on rats. In the one, halothane inhibited the metabolism of enflurane (Fis83), whereas in the other animal study, the oxidative metabolism of halothane itself was inhibited by isoflurane (Fis84). Furthermore, Carpenter *et al*. pointed out that in their study, the metabolism of halothane was not inhibited by fluranes, because it was measured during anaesthesia. The committee, however, notes that the break down of halothane is expected to occur after exposure, when concentrations of halothane may be below those producing significant inhibition.

The absence of interference between halothane and other anaesthetics have also been reported by others. For instance, Imbenotte found that the metabolism of halothane was not significantly altered, when simultaneously used with nitrous oxide, barbiturates, narcotics, and pancuronium (Imb87, see section biotransformation). Furthermore, the metabolism of halothane in man, who were simultaneously exposed to anaesthetic concentrations of nitrous oxide and other drugs, was not altered (Sak78; see section elimination). In addition, nitrous oxide (dose, 2.2-50%) did not influence the metabolism of halothane (dose, 5.09 g/m^3; 0.062%) in the liver of rats (Fis84).

Fujii (Fuj95) investigated the metabolic interaction between halothane and isoflurane *in vitro*. Liver microsomes from male Hartley guinea pigs were pre-incubated with various concentrations of isoflurane (0-7.4 mM) for 5 min (37°C), after which 1 mM halothane was added. Ten minutes later, samples were prepared for analysis. Isoflurane inhibited the oxidative dehalogenation of halothane and accelerated the reductive dehalogenation in a dose-dependent way.

In another *in vitro* study, nitrous oxide decreased the solubility of halothane in blood from human donors (n=4). This blood was exposed to 82.1 g/m^3 halothane combined with various concentrations of nitrous oxide. The blood-gas partition coefficient of halothane decreased from 2.37±0.04 (0% N_2O), 2.29±0.05 (70% N_2O; p<0.05), to 2.25±0.08 (100% N_2O) by increasing the concentration of N_2O (Xie93).

5.6 Possibilities for biological monitoring

Based on occupationally exposed subjects (n=58; anaesthetists, surgeons, and nurses), investigators found a significant correlation between the halothane concentration in urine (C_u, µg/L) and the environmental concentration (C_i, mg/m^3). Air was sampled for 4 hours, whereas urine was collected both at the beginning and at the end of the exposure period. Data retrieved from urine samples taken at the end of exposure gave the following equation for the regression line: $C_u = 0.242\ C_i + 3.51$ (r=0.92, p<0.00001). The regression line does not start from the origin of the Cartesian co-ordinates, but intercepts the y axis at 3.51 µg/L. This means that some workers showed halothane in their urine before exposure and thus halothane was not totally cleared during the overnight period (Imb91). In 1995, the same authors refined the equation for the regression line: $C_u = 2.103\ C_i + 2.684$ (n=165, r=0.81, C_i in ppm, p<0.001). The authors concluded that the urinary anaesthetic concentration of halothane can be used as an appropriate biological exposure index; the biological value proposed for halothane is 97 and 6.2 µg/L, which corresponds to 411 and 16.4 mg/m^3 (50 and 2 ppm) of environmental exposure, respectively (Imb95).

In contrast with the previous study, Schaffernicht *et al.* (Sch95) did not find halothane in urine of anaesthetists (n=5) before they started to work. Their equation for the regression line was: $C_u = 0.1330C_i$ (n=58; C_u= concentration of halothane in urine (µg/mL); C_i= concentration of halothane in air (mg/m^3)). The occupational exposure range to which this regression line was based was broader (10-800 mg/m^3) than the range used by Imbriani *et al* (up to 75 mg/m^3; Imb91).

Another study indicates that the body clearance of halothane is still not complete on the Monday after a weekend break; in anaesthetists (n=9), levels of 77 µg halothane per g creatinine (median 19 µg/g creatinine) were found in urine samples. During working hours, air concentrations of halothane averaged 295 mg/m^3 (n=15; range 0.09-13.72 g/m^3, measured at 40 cm from the anaesthetist) (Ste87).

The rate of metabolism of halothane shows large individual differences. These differences are influenced by environmental and genetic factors (Cas71). Because genetic predisposition of individuals cannot be assessed, it is advised to perform the biological monitoring on a group of individuals. Furthermore, in assessing the internal

burden of halothane, excretion of both halothane and TFA should be determined the same time.

5.7 Summary

Halothane is absorbed by the lungs. Estimates of alveolar uptake in man varies between 50% (low exposure) and 75% (anaesthesia). Data from studies on rats show that approximately 0.2% of the halothane is taken up by the skin after whole body exposure.

Halothane is a highly lipophilic compound that easily accumulates in the adipose tissues of the body, whereas it has a low affinity for blood and other tissues; *in vitro* studies showed that in human adipose tissue, the concentration of halothane was 110 to 220 times higher than in air.

Halothane crosses the placenta. In humans, the serum concentration of halothane in the umbilical vein is 0.44-0.71 times that of the maternal vein. In amniotic fluid, the fraction of halothane did not exceed 0.2 times that of the maternal levels in pregnant mice. Unchanged halothane leaves the foetus within one hour. On the other hand, metabolites of halothane accumulate in the mouse foetus and reach a plateau between 4 and 24 hours.

In man, probably 5 to 55% of halothane is broken down by enzymes of the cytochrome P450 system. The rate of this break down is inversely related to the exposure concentration (not in rats), is influenced by environmental and genetic factors, and may be elevated in obese persons. The main breakdown products (metabolites) of halothane are trifluoroacetic acid (TFA) and bromide, and to a lesser extent fluoride. All these metabolites are found in occupationally exposed man. Animal studies showed that stimulation of the cytochrome P450 system increased the break down of halothane.

The elimination of halothane and its metabolites from the body is slow and may not be completely cleared from the body overnight or even during the weekend. For instance, investigators still detected halothane in the alveolar air of occupationally exposed anaesthetists 7 to 70 hours (3 days) after a single exposure to 8-214 mg/m^3 (1-26 ppm). Other investigators detected trace amounts of radiolabeled halothane or its metabolites in the urine, 3 weeks after an intravenous injection of 3.4 mg ^{14}C-halothane. The rate of elimination varies among people, as was shown in twins: identical twins excreted 2.4-17.3% metabolites of halothane, whereas fraternal twins excreted 2.9-70.0%. Serum levels of fluoride have been demonstrated to remain nearly constant after exposure to anaesthetic concentrations of halothane, except by prolonged anaesthesia. During intermittent exposure (7 weeks) to 1.23 g/m^3 (150 ppm) halothane, blood levels of halothane in rats increased and gradually decreased after stopping the

exposure, but were still measurable 6 days later. The T_2 elimination constant for adipose tissue was calculated at 246±51 min. In rats, the half-life of halothane is estimated at 48 hours and for bromide more than 12 days.

From a study on human volunteers, who were exposed to a mixture of anaesthetics, among others halothane (16.4 g/m^3), for 30 minutes, investigators calculated that about 64% of the inhaled halothane is exhaled again, meaning that approximately 35% is excreted in the urine or has accumulated in adipose tissue. Elimination of halothane via the skin is negligible (0.23-0.25%).

For anaesthesia, often a mixture of halothane and other anaesthetics are used. So far known, the only interference between the action of halothane and those other anaesthetics is the observation that nitrous oxide decreases the solubility of halothane in human blood.

For biological monitoring, more data are needed on the excretion of halothane and TFA. These data should come from groups of people rather than from individuals, to exclude interindividual variations.

Chapter 6

Effects

6.1 Observations in man

6.1.1 Irritation and sensitisation

The severity of airway irritation is scaled between 1 (least) and 4 (most). Male volunteers (n=11), who were exposed to 1 MalvC (63.3 g/m^3) halothane for 15 seconds, showed a severity score of 2.09±0.51, whereas a score of 1.91±0.94 was calculated in volunteers exposed to 2 MalvC. The severity score correlated with a decreased tidal volume and increased cough reflex. Compared with enflurane and isoflurane, halothane was less irritating (Doi93).

6.1.2 Toxicity due to acute and short-term exposure

Currently, a concentration of 57.5 g/m^3 (7,000 ppm) halothane — inhaled for 3 hours — is the lowest lethal concentration ever reported in humans. Autopsy showed adverse effects of halothane in liver and gastrointestinal tract (Lew92). The same investigators described a lethal case after an intravenous injection of 129 mg/kg b.w. halothane, which affected the central nervous system, the cardiovascular system and the lungs (Lew92).

Hepatitis

In Europe, except Denmark, halothane is the most common drug associated with hepatic failure after paracetamol (Neu87).

Patients exposed to halothane may develop a mild or a severe form of liver damage. The mild form occurs most commonly (1:5), resolves easily and is of minor clinical importance. It is probably induced by a direct chemical insult, caused by one or more reactive metabolites of halothane.

The severe form, or halothane-associated hepatitis, is a result of massive and potentially fatal centrilobular necrosis. Its clinical and pathological pattern is identical to viral hepatitis. It predominates in obese middle-aged patients and is more common in patients after a second exposure to halothane (Kam90).

In Denmark, 1,188 cases of suspected drug-associated hepatitis were reported between 1978 and 1987. Of these cases, 280 were caused by exposure to halothane, whereas 18 were caused by exposure to paracetamol. Most patients (97.5%) developed hepatitis 1 to 28 days after exposure, with a median latency period of 6 days. Halothane was responsible for 14 (27%) of the 52 lethal cases recorded (Fri92). Overall, the incidence figures reported in the literature vary between 1:8,000-1:15,000 (Kul94), 1:10,000 (Ric87), to 1:10,000 (adults) and 1:200,000 (children) (Mar95), to 1:30,000 (Kam90). In one study, the mortality rate ranged between 15 to 50% (Kul94). For comparison, the overall mortality after anaesthesia as a whole is 1:10,000 to 1:20,000 (Ber92).

Most probably, halothane-associated hepatitis is an immune response disease against so called TFA-proteins, which are formed in the liver.

The presence of antibodies against these TFA-proteins in patients with halothane-associated hepatitis, has been investigated in several studies. In one study, in most of the fifteen patients studied, antibodies were found in their serum against various non-TFA modified human hepatic polypeptides with a molecular mass between 60 and 80 kDa. The antibodies were also found in 3 of the 6 sera of patients exposed to halothane without developing hepatitis, whereas no antibodies were found in sera of non-exposed blood donors (n=16) (Kit95). In another study, about 70% of sera from patients with halothane-associated hepatitis contained antibodies against several halothane-associated antigens, such as TFA-proteins (Ken95). Furthermore, sera of 10 halothane-associated hepatitis patients reacted positive with several of the purified rat TFA-proteins with a molecular weight ranging from 57 kDa (2), 58 kDa (5), 59 kDa (2), 63 kDa (1), 80 kDa (5), and 100 kDa (9) (Poh91).

Current evidence indicates that all individuals produce TFA-proteins when exposed to anaesthetic level of halothane. However, an antibody response to these adducts appears to be restricted to a small subset of susceptible individuals who develop

halothane-associated hepatitis (Gut92, Smi93). Persons exposed to subclinical doses of halothane probably do not form antibodies against TFA-proteins, because in occupationally exposed workers (anaesthetists, critical care technicians, and recovery nurses (n = 5)), very few or no antibodies against TFA-58 kDa proteins were found, compared with patients (n=40) having halothane-associated hepatitis (p<0.001) (Mar93).

Persons with a familial, constitutional susceptibility factor may be more vulnerable to develop halothane-associated hepatitis (Hof81, Far85).

Behavioural tests

Data on the behaviour after occupational exposure to halothane are presented in Table 6.1.

Table 6.1 Behavioural tests in volunteers exposed to halothane.

n=	dosing regimen halothane (g/m^3)	effects	LOAEL g/m^3	ref.
10 males	0, 4.1, 8.2 and 16.4; end-tidal for 30 min	*During exposure*: dose-related decrease in digit span memory test, choice response test and psychomotor performance; dose-related increase in choice reaction time test, amnesia for word pairs; *After exposure*: all tests returned to normal within 30 min after discontinuation.	4.1	Coo78
6 volunteers	0, 3.3 and 5.8; end-tidal for 30 min	*During exposure*: dose-related decrease in peak saccadic eye movement. *Five min after exposure*: returned to base-line values.	3.3	Yos91
6 males	±3.3 ; for the length of the experiment	*During exposure*: increase in response time to auditory stimuli; no effect on pain threshold.	3.3	Tom 93

6.1.3 Case reports

Several cases of hepatitis and other liver disorders have been described after occupational exposure to trace amounts of halothane. All cases are described in Annex D, including a few cases on sniffing halothane, which in one case resulted in death.

6.1.4 Epidemiological studies

Most of the epidemiological studies described below involves concomitant exposure of halothane with nitrous oxide or other anaesthetic compounds.

Effects on the reproduction

Concerning fertility, Andersen *et al*. (And92) did not find differences in sperm quality in subjects (n=17) before and after exposure to halothane (61.6-123.2 g/m^3 (0.75-1.5%) in combination with nitrous oxide. Samples were tested according to WHO criteria. Peelen *et al.* (Pee99) reported that the time to pregnancy was not affected in operation chamber assistants. In this study, the concentration of several anaesthetic gases were measured; the maximal halothane concentration measured was 135 mg/m^3 (16 ppm).

Several epidemiological studies were performed, in which female anaesthetists, operating nurses and wives of male anaesthetists were inquired about the course and outcome of their pregnancies, with specific attention for miscarriages and congenital anomalies (Ask70, Coh71, Ame74, Cor74, Kni72, Kni75, Pha77, Sch77, Ros78, Eri79). In all studies, except one, some effects on these parameters were suggested. However, the studies were criticised by several authors, among them Baeder and Albrecht (Bae90), for the following reasons: studies were retrospective and often loaded questionnaires were used, age differences occurred between the exposed and control group and no consideration was given to social factors, medication, illness and possible stress. Furthermore, the composition of the anaesthetic mixtures was not always reported, nor the duration of the exposure and its timing in pregnancy. Overall, the committee considers the quality of these studies insufficient for definite conclusions about the causality of the observed association between human exposure to halothane and adverse pregnancy outcome. In a well described study, an increased risk for spontaneous abortion, preterm birth and congenital abnormalities were observed (Pee99), but the committee could not interpret the results because the operation personnel was exposed to a mixture of anaesthetic gases.

Guirguis *et al.* (Gui90) reported on the adverse effects of a mixture of anaesthetics on pregnancy in occupationally exposed women. However, concentrations to which the women were exposed are not measured.

Another study described the adverse effects of halothane on among others pregnancy (Ste89 (only abstract), Wil98). The investigators reported in the abstract that of the 2,174 pregnancies, conceived when having a position as veterinarian, 50.7% involved exposure to halothane. However, exposure to other anaesthetics, radiation or pesticides cannot be excluded. Agent-specific spontaneous abortion risks were estimated for the exposed/nonexposed pregnancies, and risk ratios adjusted for gravity, history of spontaneous abortion, age, and alcohol and tobacco use were derived by logistic regression (Ste89). In the full article 2.489 pregnancies were evaluated (Wil98). The investigators reported that in the cohort of 816 female veterinarians, working exclusively in small animal practice, 538 had been exposed to among others halothane. In this cohort no increased relative risk was found for preterm delivery

(PTD), small-for-gestational age (SGA) or clinical practice type. But, absolute risks of PTD and SGA births among cohort members were overall lower than in the general female population.

Psychomotor and behavioural performances

Volunteers (2 females, 9 males, mean age: 23 yr) received halothane/nitrous oxide/oxygen anaesthesia for 5.8±0.7 min. The concentration of halothane was gradually increased to 123.2 g/m^3 (1.5%) and, when the subjects no longer reacted to lower-abdominal pinching, continued at 61.6 g/m^3 (0.75%). The concentration of nitrous oxide is not mentioned, but had to be 60-70%. After the end of the exposure, double-blind tests were carried out using a driving simulator (2, 4.5 and 7 hr later) and a psychomotor test battery (1 and 5 hr later). Psychomotor performances (p<0.05-0.001 after 5 hr) and driving skills (p<0.05 after 4.5 hr) were significantly lowered in exposed volunteers compared with the values from non-exposed volunteers (n=12). The investigators did not find evidence that halothane interacted with nitrous oxide (Kor77).

The same investigators reported on a study, in which nurses (n=19) working in the operating theatre, received psychomotor tests at the end of a working day and in the rooms they were exposed to nitrous oxide and halothane. Fifteen and 60 minutes after stopping the work, levels of nitrous oxide and halothane were measured in workplace air, in blood and in exhaled air (exposure data, see Table 4.3). The results of the test were compared with nurses (n=11) working in the wards of the same hospital. Despite their higher age and exposure to the operating room environment, the driving skills of the operating room nurses were similar to those of the ward nurses. The results suggest that tolerance to anaesthetic gases develops among operating room personnel (Kor78).

Around the same time, a study was performed with male volunteers (5 groups; n=20/group) exposed to nitrous oxide alone (45, 90 or 900 mg/m^3) or combined with halothane (8.2 or 82 mg/m^3) by a gas mask for 4 hours (Bru76). Within each group, 10 volunteers were exposed first to air, whereas the others were first exposed to the anaesthetic. Data showed contradictory results on tests for visual acuity, recognition of changed patterns, vigilance and for digit span memory. From these results, the committee cannot conclude on any modulation between the two compounds, because exposure to halothane alone was not investigated.

Anaesthetists (n=22) exposed to 11.5 mg/m^3 (1.4 ppm, range 0-6.4 ppm) halothane combined with 58 ppm (range 23-216) nitrous oxide, performed behavioural tests (mood and cognitive function tests) in the middle and after the working day. On a non-specified reference day, they worked in an area where anaesthetic gases were expected to be absent; only low amounts of 0.25 mg/m^3 (0.03 ppm) halothane and 15

ppm nitrous oxide were inhaled during that day. Overall, no impaired performances of the tasks were observed (Sto88). Because of the possible prolonged presence of residuals of halothane in the body of the subjects, the committee concludes that there is very little difference in circumstances between the working and the reference day.

Other effects

In a cross-sectional survey, 16,995 patients, who underwent inhalation anaesthesia, were questioned. The list of complications surveyed comprised effects on heart rate and arterial pressure, myocardial ischemia and airway obstruction and other respiratory effects, increase in secretions, vomiting, anaesthesia depth, shakes/shivers and idiosyncrasy. Hepatitis and malignant hyperthermia were first selected but subsequently excluded from the study, because of their extreme rarity. In 46.6% of the cases halothane was used as an anaesthetic. The use of nitrous oxide is not mentioned. In 86.7% of the cases no complications occurred, in 10.6% of the cases 1 complication occurred, and in 2.7% of the cases more than one complication occurred. The main complication was cardiac arrhythmia, followed by bradycardia and shakes/shivers (Lew91).

Effects of halothane (amount unknown) and nitrous oxide on the immune system were investigated in occupationally exposed (anaesthetists, anaesthetic assistants and nurses; n=32) and non-exposed workers (n=20). The occupational period averaged 6.5±4.4 working years. No differences in the serum concentrations of IgG, IgM, and IgA peripheral blood lymphocytes and lymphocyte subpopulations between the groups were observed (Kar92a).

In another study, effects of simultaneous exposure to halothane and nitrous oxide on haematological parameters were investigated in anaesthetists (n=17; control group n=35), at the peak of the working season and after a three week lasting holiday. During the working period, air concentrations of halothane ranged between 10 and 350 mg/m^3 and for nitrous oxide between 85-1500 ppm. Before the holiday, red and white blood cell count, haemoglobin, haematocrite values, differential leukocyte count, and natural killer cell activity were significantly decreased compared with the values measured after the holiday. After the holiday, the parameters were the same as those from the control group. Changes in Ig values could not be interpreted statistically, because of the small group size (Per91, Per94). Furthermore, the authors tried to establish a relationship between the haematological changes and the age of the staff, but they did not succeed. The way they did that was also criticised, because they used incorrect statistical tests and no error bars were shown in the charts, making conclusions meaningless (Mat95). The committee concludes that the evidence for age-dependency on the effects are too small to be convincing.

Murray *et al*. (Mur90) investigated the combined effects on anaesthesia of halothane and nitrous oxide. Infants and small children (age 14.7±7 months) were anaesthetised by endotracheal intubation with halothane combined with O_2 (n=11), 25% N_2O (n=13), 50% N_2O (n=13) or 75% N_2O (n=14). For assessing the MalvC of halothane, the move or no move in response to a surgical incision was measured. The assumed MalvC for halothane alone was set on 1.0 vol%. The MalvC of halothane was significantly reduced with the combined exposure of N_2O: 0.8±0.1 vol%, 0.4±0.1 vol% and 0.3±0.06 vol% for 25, 50 and 75% N_2O respectively (r^2=0.87). The authors concluded that the contribution of N_2O to the MalvC of halothane in children is additive. Combined with O_2, the MalvC for halothane was calculated at 0.9±0.08 vol%. Older data published in 1964 suggest that also in adults the contribution of nitrous oxide to the MalvC of halothane may be additive (Tor74).

6.1.5 *Genotoxicity*

Data on mutagenic and genotoxic effects of halothane in humans and in human cells *in vitro* are presented in Table 6.2. From these studies, the committee concludes that halothane has potential to induce sister chromatid exchanges and chromosomal aberrations in lymphocytes.

6.1.6 *Other studies*

Samples of ciliated epithelium from the nose of non-smoking healthy volunteers (n=24) were exposed to 73.9, 147.8, 184.7 and 468.4 g/m^3 halothane for 3 hours. Over a 4 hour lasting observation period, the cilia beat frequency was significantly reduced compared with control sample exposed to air alone. But, after stopping the exposure, the values returned to control values. Comparable results were found in nasal epithelial brushings samples taken from healthy adult volunteers (n=18; 184.7 g/m^3 (2.25%) halothane for 4 hours) or patients (n=11; 246.3 g/m^3 (3%) halothane for 1 hour) (Gyi94, Rap96a/b), and in nasal samples taken from 13 healthy newborn infants (147.8 g/m^3 for 2 hours) (OCa94).

McDiarmid and Wallis (McD95) reported on the reduction of aggregation of platelets in human blood of healthy volunteers (n=10), whose blood samples were exposed to 164.2 g/m^3 halothane for 10 minutes (37°C). In fact, a reduction of 61% (8-73%) regarding the control value was found.

Table 6.2 Mutagenicity and genotoxicity data of halothane in humans *in vivo* and *in vitro*.

species	dose halothane	result	test in/remarks	ref.
Ames assay				
S. typhimurium TA98 and TA100, using urine from 24 anaesthetic workers, collected at the end of the workweek	9-490 mg/m^3 and N_2O	-	with and without metabolic activation	Kar92
induction of DNA strand breaks				
Healthy volunteers	1 vol/%, 1 h	-	peripheral lymphocytes	Rei93
Xeroderma pigmentosum patients[a]	1 vol/%, 1 h	+	lymphocytes	
Healthy male volunteers	0, 0.1 or 1 mM for 10 or 30 min	+	peripheral lymphocytes	Jal99
66 Operating theatre personnel (32 smokers, 34 nonsmokers), compared with 41 controls (11 smokers, 30 nonsmokers)	not known, plus N_2O and other anaesthetics	+	peripheral lymphocytes; no difference between smokers and nonsmokers	Sar98
induction of sister chromtid exchanges (SCEs)				
Women (n=21) receiving anaesthesia for 75-180 min.	MalvC + N_2O	-	peripheral lymphocytes immediately after and five days after the operation.	Hus81
Human smokers (n=20) receiving anaesthesia for 63 min.	MalvC + 67% N_2O	-	peripheral lymphocytes one day after the operation.	Hus85
Anaesthetists (n=16), supportive staff (n=4) and control (n=20): 1-33 years worked	dose unknown: halothane, N_2O or combined.	+	peripheral lymphocytes:	San89
15 Operating theatre personnel.	not known, + N_2O and diethylether	-	peripheral lymphocytes.	Lam89
15 Operating theatre personnel.	not known, + N_2O and other anaesth.	-	peripheral lymphocytes.	
29 Anaesthesia unit technicians and 20 anaesthetic nurses, occupationally exposed;	the same + X-ray	+	peripheral lymphocytes	
18 anaesthetists	not known, + N_2O + isoflurane	-	peripheral lymphocytes	Sar92
14 Anaesthetists and 10 anaesthetic nurses, occupationally exposed	the same; 9-490 mg/m^3 + N_2O	+	peripheral lymphocytes	Kar92

[a] Cells deficient in DNA repair

Table 6.2 Continued.

species	dose halothane	result	test in	ref.
induction of chromosomal aberrations				
Anaesthetists occupationally exposed.	not known + N_2O + diethylether	+	peripheral lymphocytes	San89
15 Operating room personnel.	not known + N_2O + other anaesthetics	-	peripheral lymphocytes	Lam89
15 Operating room personnel.	the same plus X-ray	+	peripheral lymphocytes	
14 Anaesthetists and 10 anaesthetic nurses occupationally exposed.	9 - 490 mg/m^3 + N_2O	+	peripheral lymphocytes	Kar92
20 Anaesthetists and 16 theatre staff occupationally exposed.	21 mg/m^3 + 180 mg/m^3 N_2O	+	peripheral lymphocytes	Pad95
induction of spindle polymerisation				
human venous blood	80 µg/mL	+		Cor87

6.2 Animal experiments

6.2.1 *Irritation and sensitisation*

A dose of 100 mg halothane irritated severely the eyes of rabbits (Lew92). No details of the study were given.

6.2.2 *Toxicity due to acute exposure*

The lethal doses and concentrations of halothane in several animal species are listed in Table 6.3. According to the EC criteria, halothane does not need to be classified (EC93, NIA98).

Table 6.3 Toxicity data after acute exposure to halothane.

species	parameter	dose/concentration	ref.
rat	LD_{50} oral	5.7 g/kg b.w.	Lew92
	LC_{50} inhalation	238.1 g/m^3 (=29,000 ppm)	
mouse	LC_{50} inhalation (10 min)	180.6 g/m^3 (=22,000 ppm)	Lew92
CD-1 male albino mouse	LC_{50} inhalation (10 min)	346.9 g/m^3 (=42,255 ppm)	Mos85
	LC_{50} inhalation (30 min)	281.8 g/m^3 (=34,320 ppm)	
	LC_{50} inhalation (60 min)	202.0 g/m^3 (=24,601 ppm)	
guinea pig	LD_{50} oral	6.0 g/kg b.w.	Lew92

Table 6.4 Appearance of TFA-protein adducts in several animal species after exposure to halothane.

species	exposure regimen	result	ref.
male Sprague Dawley rat (n=1/experiment)	1.97 g/kg bw ip injection, single	Appearance of TFA-protein adducts in Kupffer cells (liver macrophages) 18 h after injection	Chr91
male Sprague Dawley rats (n=?), pretreated with phenobarbital	1.97 g/kg bw ip injection, single	Appearance of TFA-protein adducts in heart, liver and kidney 6 h after injection (relative quantity: <0.5, 100, 5, respectively)	Huw92 a/b
male C75Bl/10 mice (n=2/experiment)	1.97 g/kg bw ip injection, single	Appearance of TFA-protein adducts in liver, olfactory epithelium and lung 4 h after injection; still present 48 later	Hei93
rat, pretreated with phenobarbital (n=?)	82.1 g/m^3 in 100% O_2	Appearance of TFA-protein adducts in liver 24 h after exposure	Dyk92
male New Zealand white rabbits (n=6)	82.1 g/m^3 in 80% O_2 for 4 h	Appearance of TFA-protein adducts in liver, 24 h after exposure	Hub89
male Hartley guinea pigs (n=?)	82.1 g/m^3 in 21% O_2 for 4 h	Appearance of TFA-protein adducts in liver, 6 h after exposure, still apparent 90 h later	Hub89
male Hartley guinea pigs (n=8) male Sprague Dawley rats (n=8)	82.1 g/m^3 in 40% O_2 for 4 h the same	In all cases: appearance of TFA-protein adducts in liver 15 h after exposure, increasing up to 48 h after exposure; in guinea pigs almost 5-fold greater than that in rats after inhalation, and approx 10-fold greater 48 h after ip injection	Cla95
male Sprague Dawley rats (n=8)	1.97 g/kg bw ip injection, single		

6.2.3 *Toxicity due to short-term exposure*

Liver: TFA proteins.

As described in a previous section (see section 6.1.2) halothane-associated hepatitis may be triggered by an immune response against TFA-proteins. The appearance, half-life's and characterisation of TFA-proteins in animals is reported by several investigators (see Table 6.4 and text below).

TFA-protein antigens, which were isolated from rat liver, were identified as the trifluoracetylated forms of disulfide isomerase (57 kDa), phosphatidylinositol- specific phospholipase C-alpha (58 kDa), microsomal carboxyl esterase (59 kDa), the Ca^{2+}-binding protein calreticulin (63 kDa), and of the stress proteins ERp72 (80 kDa) and ERp99 (100 kDa) (Huw92a, Poh91).

A few authors reported on the half-life of TFA-proteins. For instance, a t½ for the 70 kDa TFA-protein was found to be less than 12 hours (Huw92a); t½ values between 18 and 90 hours were found for a variety of TFA-proteins, isolated from rat kidneys (Huw92b). In Kupfer cells of rats (liver macrophages), 74, 110 and 220 kDa TFA-proteins disappeared within 24 or 48 hours, whereas other TFA-proteins were persistent for at least 48 hours (Chr91).

Investigators reported on the different levels of antibodies against TFA-proteins in three strains of guinea pigs; in the Hartley strain, 4- to 11-fold higher levels of antibodies against TFA-proteins were found than Strain 2 and the Amana strain (Sia87).

In rats exposed to the halothane-analogue 2,2-dichloro-1,1,1-trifluoroethane (refrigerant HCFC-123), TFA-protein adducts were detected in hepatic microsomal and cytosolic fractions. This finding raises the possibility that individuals sensitised to HCFC-123 may be at risk of developing hepatitis after anaesthesia with halothane (Har91).

Liver: general effects

Sprague Dawley rats (n=8/sex/group, control n=36/sex/group), ICR mice (n=11-24/sex/group, control n=8-24/sex/group) and guinea pigs (n=5-8/sex/ group, control n=2-4/sex/group) were examined for histopathological changes in several organs after being exposed to 0.12, 0.41, 1.23 and 2.46 g/m^3 halothane or air (control) for 7, 14 or 35 days (24 hours/day). Body weight was clearly decreased in the two highest-dose groups, but not in the lowest-dose groups on the seventh exposure day. Guinea pigs and mice in the highest-dose group were sacrificed after 21 and 8 days respectively, because by this time approximately 25% of each of these species died.

Furthermore, macro- and microscopic examination on a limited number of animals showed only lesions in the liver (focal granulomatous or suppurative inflammation, vascular abnormalities such as passive congestion, pigment deposition, extramedullary hematopoeisis and ductal hyperplasia). These lesions were found in all exposed groups, but not in air-exposed controls, and increased with increasing the dose (Ste75).

When adult male Fisher 344 rats (n=3-5) were exposed to 82.1 g/m^3 (=1%) halothane for 2 hours, no changes on the hepatic microsomal cytochrome P450 content and aminopyrine demethylase activity were observed, even not when halothane was given in the presence of low oxygen supply (14%). When rats (n=5) were pre-treated with an enzyme inducer (phenobarbitone) both parameters decreased and this was irrespective of the amount of oxygen supplied (10, 14 or 21%). The decrease was abolished in the highest oxygen supplied group 12 hours after stopping the exposure,

whereas the parameters were still decreased in the lowest oxygen supplied group after 48 hours (Kni87).

A study comparable to the latter was performed by Rice *et al*. (Ric87). Adult male Fischer 344 rats (n=42 total), with or without pretreatment with isoniazid, an enzyme inducer, were exposed to 82.1 g/m^3 halothane for 2 hours. One day later, the hepatic and renal SGOT and SGPT levels were significantly elevated compared with saline-pretreated animals (controls), whereas three days later no differences were observed between the two groups. Other serum parameters indicative of hepatic or renal function were not changed.

Plummer *et al*. (Plu88) described a study, in which male Fisher 344 rats (n=8/group) were pre-treated with phenobarbitone (inducer of both oxidative and reductive pathways), isoniazid (induces only oxidative pathways *in vitro*) or saline (non enzyme-inducer), before they were exposed to 411 mg/m^3 (50 ppm) halothane for 4 weeks (24 hours/day, 7 days/week). Exposure to halothane with only saline-pretreatment resulted in increased serum bromide levels and in liver injury, *i.e.* increased SGPT activity, focal hepatocellular necrosis and fatty change. In isoniazid pre-treated animals, however, the serum bromide levels were 33% lower and no liver injury was observed, whereas in phenobarbitone-pretreated rats, serum bromide levels were slightly increased and liver injury was partly prevented. The authors suggested that the metabolism of halothane plays an important role in the toxic effects of halothane on the liver.

The same investigators performed a study on male Fisher 344 rats (n=4/group), which were exposed to 164 mg/m^3 (20 ppm) halothane for 6, 19 or 30 weeks (24 hours/day, 7 days/week), before they were killed. Several parameters in liver and kidney were investigated. One rat died during the 19-week exposure period because of respiratory tract infection. Serum bromide levels were clearly increased, with the highest increase found in week 6, compared with non-exposed animals in whom bromide levels were always below the detection limit (0.5 mmol/L). In kidneys, no indications of toxic effects of halothane were observed for the different time periods. In liver, the following observations were made. Liver weights were increased after 6 weeks, but became normal as exposure continued. All animals showed minimal changes in hepatic microvesicular fat content, that slightly became more prominent at 30 weeks. Furthermore, SGPT activity was increased after 6 weeks. Cytochrome P450 content increased uniformly over the total exposure period. The last observation is remarkable, because in other studies, in which anaesthetic levels of halothane were used, neither a decrease nor a change in the cytochrome P450 content of the liver was found. The authors suggest that acute exposure to high concentrations of halothane results in other effects than those seen from chronic exposure to low concentrations of halothane. The committee noted that the results are based on only 4 rats per exposure

period and that only one concentration was tested. The influence of halothane exposure at subanaesthetic concentrations on the hepatic cytochrome P450 content, therefore, has to be further investigated.

Behavioural effects

Data of different behavioural tests on mice, which were exposed to low concentrations of halothane are summarised in Table 6.5 on the next page.

Investigators reported on a study, in which male CD rats (n=not given) were anaesthetised once or repeatedly with halothane (dose not given), before they received one to five times an electroconvulsive shock (ECS) or a convulsive footshock over a ten day period. Twenty four hours after the shock treatments, passive avoidance tests were carried out. No significant effects of halothane and convulsive footshocks were observed on learning and memory. However, ECS, given repeatedly resulted in impairment of learning and memory (Gos95).

6.2.4 Toxicity due to long-term exposure and carcinogenicity

Kunz *et al*. (Kun69) investigated the carcinogenic properties of halothane combined with diethylnitrosamine (DENA), an inducer of liver cancer and haemangioendotheliomas. NMRI mice (n=40/group) were exposed to 57.5 g/m^3 (7,000 ppm) halothane for 30 weeks (1 hour/day, 6 days/week). At the end, body weight gain and growth rate was a little bit lower than in non-exposed animals. One group also received continuously 240 mg/kg b.w. DENA by the drinking water during the whole exposure period. The survival time, tumour frequency and tumour localisation by DENA were not altered by halothane. However, DENA alone induced haemangioendotheliomas and liver cancer in a ratio of 26:1, whereas administration of halothane changed the ratio to 2:1. The committee cannot make any firm conclusions from this study, because of imperfections in study design.

Pregnant Swiss ICR mice (n=80) were exposed to 0, 2.6, 10.3 or 41.1 g/m^3 halothane, combined with 25 or 100% oxygen, for 2 hours at GD 11, 13, 15 and 17. Exposure to halothane of the dams was resumed 5 days after delivery for 8 weeks (2 hours/day, 3days/week). Exposure to the offspring continued after weaning, till they were about two and a half months old. The offspring was killed at 9 and 15 months of age (n=1973). At both time points, no effects of halothane exposure were found on delivery, litter size, sex ratio and tumour incidence of the offspring. However, at 15 months of age, the incidence of lymphomas was significantly decreased in males of the highest-dose group (41.1 g/m^3 with 25% oxygen). The authors concluded that halothane, when used at anaesthetic concentrations, is not carcinogenic (Ege78).

Table 6.5 Results of behavioural tests in mice after exposure to halothane.

mouse strain	exposure regimen	results[b]	ref.
male CD-1 (n=9/group)	8.2-41.1 g/m^3 for 15 min	EC_{50}: 30.0 g/m^3, 50% decrease in a handle-press test for getting drinking water[c].	Mos85a
male CD-1 albino (n=12/group)	range not given, at least 3 concentrations; for 10, 30 or 60 min	Motor performance was tested by rotating a horizontal screen; EC_{50} 10 min: 39.7 g/m^3; EC_{50} 30 min: 34.4 g/m^3; EC_{50} 60 min: 35.9 g/m^3.	Mos85
male CD-1 (n=15/group)	9.0-54.2 g/m^3 for 30 min	EC_{50}: 34.0 g/m^3, 50% decrease in a handle-press test to receive milk.	Mos86
male ddN (n=20-35/group)	0, 19.9, 39.8 or 79.6 g/m^3 for 2 hours	Avoidance training session before exposure was repeated 0.5 h after exposure; performance after exposure to 19.9 g/m^3 was enhanced, but decreased in a dose-related way.	Kom91
male ddN (n=18-20/group)	0, 24.7 or 82.0 g/m^3 for 2 hours	Avoidance training session before exposure was repeated 22 h after exposure; performance after exposure did not differ from that before exposure.	Kom93
male ddN (n=12; own control)	8.2-82.1 g/m^3 for 30 min	8.2 and 16.4 g/m^3, increased frequency of pressing a lever to obtain some food; 32.8 - 82.1 g/m^3, dose-related decreased frequency.	Kom97

[b] EC_{50}, concentration predicted to decrease mean response rates by 50%.

[c] Because the concentration-effect curve was sigmoidal, only the linear portion of the curve was analysed. Halothane *increased* rates of responding at low concentrations (8.2 and 16.4 g/m^3) in 5 of the 9 mice, but only rate-*decreasing* effects were statistically significant.

[a] It is possible that animals were not yet recovered from the halothane exposure.

Investigators reported on a study, in which pregnant Swiss ICR mice (n=15/group) were exposed to 4.1 g/m^3 (500 ppm) halothane for 2 hours a day between GD 10 and 19. Five days after delivery, the exposure on the offspring continued for two hours a day and three times a week, until mice were 78 weeks old. Ten weeks after ending the exposure, the offspring (n=161, halothane exposed; n=120, non-exposed) was sacrificed and subjected to gross and histopathologic examination on abnormal tissues and tumours. The incidences of malignant tumours and benign tumours (pulmonary adenomas, ovarian cysts, others) in the halothane-exposed offspring were 7,7 and 22%, respectively. These incidences were almost similar with those incidences found for non-exposed mice (8,4 and 19%, respectively). The authors concluded that lifetime exposure to halothane was not associated with an increased incidence of neoplasia in Swiss/ICR mice (Bad79).

Table 6.6 Mutagenicity and genotoxicity data of halothane in bacteria and in animals *in vivo* and *in vitro.*

species	dose range	result	and remarks	ref.
Ames assay				
S. typh. TA98/100/1535/1537	0-100-10,000 µg/plate	-	with and without metabolic activation	Mor86
S. typh. TA98/100/1535	Not reported	-	with and without metabolic activation; measures to prevent evaporation are not reported	Khu87
induction of DNA strand breaks				
L5178Y mouse lymphoma cells	1% for 1 h	-		Rei93
induction of micronuclei				
male Sprague Dawley rats	790 mg/kg bw, oral, 1x	+	test in kidney cells 48 h after dosing	Rob98
induction of SCEs				
CHO cells	160-1,600 µg/mL	-	without metabolic activation	Gal87
	500-5,000 µg/mL	-	with metabolic activation	
induction of chromosomal aberrations				
CHO cells	160-1,600 µg/mL	-	without metabolic activation	Gal87
	500-5,000 µg/mL	-	with metabolic activation	
induction of sex-linked recessive lethality				
D. melanogaster	1,300 ppm, food/inj.	+/-		Woo85
D. melanogaster	82.1 or 164.2 g/m^3, for 1 h	-	After exposure, males were mated with untreated virgin females.	Kun85
D. melanogaster	82.1 or 164.2 g/m^3 + up to 75% N_2O, for 1 hour	+	After exposure, males were mated with untreated virgin females.	Bad87a/b
induction of reciprocal translocation				
D. melanogaster	1,300 ppm, feeding	-		Woo85
induction of aneuploidy				
Microtus oeconomus	1.6 g/m^3 + N_2O, for 10 days and 7.5 h/day	±	Test on spermatids; no significant increase compared with 2% N_2O; only nondisjunction and diploidy measured.	Tat79
pregnant mouse	0 or 82.1 g/m^3	-	Test on 16-day old embryos.	Mai86
V79 cells	0.5% (least effective dose tested)	+	Induction of C-mitoses and multipolars.	Gal86
D. melanogaster	3.45 mg/mL in 0.1% sucrose for 24 h	±	Only positive for total, not for each individual experiment (test for non-disjunction).	Cle81
V. faba root tips	82- 328 g/m^3 (1-4%) for 2 h	±	Inconclusive.	Gra77

6.2.5 *Genotoxicity*

From the data on the mutagenicity and genotoxicity (see Table 6.6), the committee concludes that halothane neither induces mutations in bacteria, nor induces sister chromatid exchanges or chromosomal aberrations in mammalian cells *in vitro*, nor induces aneuploidy in mice. However, it induced micronuclei in rats 48 hours after oral dosing.

6.2.6 *Reproductive toxicity*

Fertility and developmental toxicity studies with low concentrations of halothane in experimental animals are summarised in Tables 6.7 and 6.8.

Baeder *et al.* (Bae90) evaluated studies on the reproductive toxicity of halothane in animals. In this review also studies with high concentrations of halothane are described. Exposure to »0.2 and 41 g/m^3 halothane for several days (8 hrs/day) during gestation, resulted in among others behavioural disorders, retardation and reduction in brain weight in the offspring of rats; death embryos were found at 65.7 g/m^3. Mice were less sensitive, in that these effects were observed from 8.2 g/m^3 upwards.

Table 6.7 Fertility and developmental toxicity rats exposed to halothane.

species	exposure regimen	results	NOAEL	ref.
female Sprague Dawley rats (n=20; controls=20)	0 or 82 mg/m^3, 8 h/day, 5 d/week for 31 days before conception and during pregnancy.	No maternal toxicity at necropsy. No effect on female fertility[a]. Increased proportion of male litter; no gross structural anomalies or skeletal defects[b].	±82 mg/m^3	Hal81
male Sprague Dawley rats (n=20; controls=20)	0 or 82 mg/m^3, 8 h/day, 5 d/week for 36 or 64 days before mating to unexposed females.	No paternal toxicity at necropsy. Male fertility was not presented. After 36 days, increased litter size; no gross structural anomalies or skeletal defects[b)]; after 64 days, decreased foetal/placental weight ratio.	±82 mg/m^3	

[a] Consisting of the following parameters: number of rats pregnant, number of life foetuses per litter, percentage of resorptions, total number of life foetuses, number of life foetuses per litter, foetal weight, placental weight, foetal/placental weight ratio, crown-rump length.

[b] Consisting of the following parameters: anomalies of phalanges, ribs (supplementary, reduced), sternebrae, cranium and vertebrae, potential artefacts.

Table 6.8 Developmental toxicity studies in animals exposed to halothane.

species	exposure regimen	results	NOAEL	ref.
female Wistar rats (n=26; control=15)	0 or 74 mg/m^3, 4h/day on gestation days 1-21	Increase in early and late resoptions (p<0.05); in offspring no effect on body weight, no gross structural anomalies or skeletal defects.	74 mg/m^3	Pop79
male Wistar rats, (n=10-12; control=5)	0 or 74 mg/m^3, 4h/day for 6 or 8 months before mating with virgin females (48 with 12 males, 30 with 10 males, 20 with 5 males)	After 6 months, increased number of early resorptions (p<0.05); after 8 months, decreased fertility (decreased %age of pregnancy, p<0.10); in offspring, no effect on body weight and no gross strucural anomalies or skeletal defects; two sc heamatomas.	74 mg/m^3	Pop79
female Sprague Dawley rats(n=8; control=not known)	0 or 82 mg/m^3, 8 h/day, 5 d/week during gestation; within 24 hours after birth 4 randomly selected pups were killed	*Effects on neonatal livers*: fatty changes, leukocytic infiltration, accumulation of lysosome, and cellular necrosis; *effects on neonatal kidneys*: ultrastructural changes, mainly in proximal convoluted tubules; *ultrastructural changes in the cerebral cortex of the brain*, mainly in neurons and Golgi complex; *after 100 days after birth*: degenerative changes in cerebral cortex were still observable.	82 mg/m^3	Cha75 a/b Cha76 Cha77
female Sprague Dawley rats (litters=10-12)	0 or 82 mg/m^3, 8 h/day, 5 d/week; UU, undosed group; UD: exposed at postnatal days 60-150 (312 in Dud77); DU, exposed at postnatal days 0 to 60; DD, exposed at postnatal day 0-150 (312 in Dud77)	Group DU and DD showed a decrease in performance in a shock avoidance test (n=12 pups/group)[a], and in seeking food in a maze at postnatal days 140-145. Decreased response (startle and jump) to electric footshocks (a higher m-amperage was necessary) (n=20)[a].	82 mg/m^3	Qui74 Qui75 Bow77 Dud77
female Sprague Dawley rats (n=not known)	0 or 103 mg/m^3, 8 h/day, 5 d/week; females and their litter from gestation day 2 to postnatal day 30 or 60	Litter at postnatal day 30 and 60: decreased response in a shock avoidance test (n = 8)[a]; no effect on response to electric footshocks or acoustic shocks; decreased performance in an exploration test.	103 mg/m^3	Lev86

[a] It is possible that halothane has not only anaesthetic but also analgesic properties, and, therefore, decreased response of the animals does not necessarily has to be described as a developmental effect.

Table 6.8 Continued, developmental toxicity studies in animals exposed to halothane.

species	exposure regimen	results	NOAEL	ref.
female Albino Norwegian rats (litters exposed =6-12)	A: 821 mg/m^3, 8 h/day, 5 d/week, GD2[a]to PND 60[a]; B: 205 mg/m^3, 24 h/day, 7 d/week, GD2 to PND 60; C: 821 mg/m^3, 24 h/day, 7 d/week, GD2 to PND 60; D: control; E: 821 mg/m^3, 8 h/day, 5 d/week, GD2 to PND 30; F: 821 mg/m^3, 8 h/day, 5 d/week, PND 31-90; G: 821 mg/m^3, 8 h/day, 5 d/week, GD2 to PND 90	Litter size was culled to 8; rats were tested on postnatal day 150; decreased radial arm maze performance in all groups except in group B. Performance deficit in the male litter of group F.	821 mg/m^3	Lev90
Male Wistar rats (n=10-12; control=5)	0, 410 or 4105 mg/m^3, 6 h/day on gestation days 10-20	reduced maternal body wight gain in high dose group; no changes in maternal liver and kidney weight.	4105 mg/m^3	Sai97
female DUB/ICR mice (n= not known; dams or litters)	0 or 41 g/m^3 (0.5%) for 6 hours on gestation day 14 or for 4 hours on postnatal day 2; total activity tests were performed on postnatal days (PND) 20-23, 44-48 and at 6 months.	The total activity was decreased at pre-weaning, young and mature adulthood when exposed on PND 2 (litter size 8-14), and at young adulthood when exposed *in utero*.	41 g/m^3	Rod86
female DUB/ICR mice (n=5-6/group; dams or litters)	the same exposure regimen; tests were performed on PND 6-10, 14-18 and 20-23	No effect on body weight; when exposed *in utero*; decreased number of pups with open ears on PND 5 and decreased number of pups with open eyes on PND 15; decrease in righting reflex on PND 6; decreased locomotion on PND 8.	41 g/m^3	Koe86
female Sprague Dawley rats (n=18-26; control=39-50)	0 or 65.7 g/m^3, 6 h/day on gestation days 8-10 (A), 11-13 (B), or 14-16 (C)	Group A, decreased maternal growth, decreased foetal weight; increase in developemental visceral variants, most frequently rudimentary ribs; group B, no effects; group C, decreased foetal weight; all groups, no effect on reproductive indices, no teratologic effects.	65.7 g/m^3	Maz86
female Sprague Dawley rats (n=34; control=34) the same (n=44; control=43)	0, 75, or 150 mg TFA/kg bw oral doses on gestation days 10-20	TFA, reduced maternal weight gain in high dose group, increasedrelative and absolute liver weight in both dose groups, slight and transient changes in neonatal rat liver	75 mg/kg bw	Sai97

[a] GD, gestation day; PND, postnatal day

The committee does not take the study of Popova *et al.* (Pop79) into consideration in deriving a HBR-OEL, in which the lowest dose of halothane (74 mg/m^3) is used, because of the questionable quality; the body weight of females was very low (75-80 g) and probably the age of the females was too young. Further, the pregnancy rate was low: 7/15 in the control group (47%) and 18/26 in the treatment group (69%). The committee, therefore, concludes from several other studies that 82 mg/m^3 (10 ppm) halothane is the lowest concentration at which developmental toxicity, such as fatty changes in the liver, and ultrastructural changes in kidneys and brain, are observed.

6.2.7 *Simultaneous exposure*

General effects

Investigators performed a study to investigate the influence of nitrous oxide on the MalvC of halothane. Male Sprague Dawley rats (n=9) received 90.3 g/m^3 halothane simultaneously with 0, 15 or 75% N_2O for 90 minutes. Halothane requirement decreased in a non-linear way as the concentration of N_2O increased (Col90). Comparable results were found in horses; N_2O larger than 25% provided no additional requirement (Tes90). The committee noted that these data are contradictory to the human data; the human data show that the contribution of nitrous oxide to the MalvC of halothane is additive (Tor74, Mur90).

In a short-term study, rats (n=10/sex) were exposed to ambient air, 411 mg/m^3 (50 ppm) halothane, 720 mg/m^3 N_2O, or to a combination of 411 mg/m^3 halothane and 720 mg/m^3 N_2O for 180 days (6 h/day). After this exposure period, animals were killed and checked for changes in biochemical parameters, such as blood cells (segmented leukocytes, lymphocytes, eosinophils, monocytes), electrolytes (sodium, potassium, calcium), SGOT and SGPT, prolactin, estradiol, creatinine and glycaemia and weight gain. Furthermore, histopathologic examinations were carried out on the brain, the heart, the liver, the kidneys, the testicles, the aorta, the stomach, and the bone marrow. Interpretation of the biochemical data, shown in graphs only, suggested that some parameters were decreased in the exposed groups when compared with non-exposed animals. The results of simultaneous exposure were suggestive of additive effects. The authors did not make any calculations on the biochemical parameters. Furthermore, no pathological changes in the examined organs were observed (Van95).

In 1979, the group of Coate *et al*. (Coa79a) published the results of a long-term study on the carcinogenicity of low concentrations of halothane-nitrous oxide. They exposed Fischer 344 rats (n=50/sex) to:

- 8.2 mg/m^3 (1 ppm) halothane + 90 mg/m^3 N_2O, or to
- 82.0 mg/m^3 (10 ppm) halothane + 900 mg/m^3 N_2O

for 104 weeks (7 h/day, 5 d/week). No evidence for exposure related effects on body weight, appearance, behaviour, survival, or haematological findings was found. Histopathologic examination of the reticuloendothelial system and other major organs showed neither enhancement of the spontaneous tumour rate nor unusual neoplasm in any of the groups. The authors noticed that their results did not support those found by others (studies from 1968 and 1973; study from 1968 could not be corroborated by the same investigators in 1974). The other investigators hypothesised that low levels of these anaesthetics are responsible for the higher incidence of reticuloendothelial malignancies found in personnel working in operating theatres.

Reproduction toxicity

The same group of Coate *et al*. (Coa79b) performed a long-term study to investigate the effect of chronic exposure of low concentrations of halothane- nitrous oxide on fertility, gestation, foetal development, and in males, damage on chromosomes *in vivo*. Sprague Dawley rats (n=20/sex) were exposed to:

- 8.2 mg/m^3 (1 ppm) halothane + 90 mg/m^3 N_2O, or
- 82.0 mg/m^3 (10 ppm) halothane + 900 mg/m^3 N_2O

for 12 weeks (7 h/day, 5 d/week), before the animals were allowed to mate. After mating, pregnant females were divided in two groups; one group received the same exposure regimen as before mating between gestation days 1-15 and were designated for natural delivery, and a second group received the same exposure regimen as before mating, but between gestation days 6-16 and were designated for cesarean-section delivery. After the mating period of 3 weeks, exposure to the males was continued for an additional 40 weeks. After ending the exposure period, bone marrow and spermatogonial cells were screened for cytogenetic damages.

In Table 6.9, data are shown on the fertility of the female animals. Exposure to halothane/nitrous oxide decreased the ovulation and implantation efficiency in all female groups, but this was only statistically significant in the high-dose group. In both the low- and high-dose female groups, which were allowed to deliver naturally, the number of *corpora lutea* was significantly decreased compared with non-exposed females.

Effects on the development of the offspring were marginal. All male and female litter born by caesarean section showed lower birth weight and birth length, whereas only male litter born naturally showed lower birth weights. No major teratogenic or abortifacient effects were observed in the litter of exposed females, irrespective of the dose they received.

Table 6.9 Mean ovarian, uterine and litter data (± SD) of SD rats designated for natural delivery. Animales were exposed to 8.2 mg/m^3 halothane plus 90 mg/m^3 nitrous oxide (low dose), or to 82.1 mg/m^3 halothane plus 900 mg/m^3 nitrous oxide (high dose) for 7 h/day, 5 d/week during 12 premating weeks on and GD1-15 (Coa79b).

	non-exposed	low dose	high dose
ovarian c*orpora lutea*	19.5 ± 3.8	15.4 ± 5.9[a]	13.2 ± 5.2[a]
uterine implantation sites	10.9 ± 5.5	7.5 ± 6.2	3.8 ± 6.2[a]
implantation efficiency %)	56	49	29[a]
post-implantation loss index (%)	14	15	28[a]
number of pregnancies (total)	17	13	7[a]
number of full-term litters born (total)	16	12	4[a]
gestation index (%)	94	92	57[a]
number of pups born (total)	178	115	46[a]
number of pups born alive (total)	177	110	44[a]

[a] $p<0.05$

In male rats, cytogenetic analyses of bone marrow and spermatogonial cells revealed a significant dose-dependent increase of aberrant cells ($p<0.05$) in both exposure groups. Furthermore, chromatid breaks, dicentrics or other abnormalities in the chromosomes were found.

From these results, the committee concludes that exposure to 8.2 mg/m^3 halothane + 90 mg/m^3 N_2O induced significant reproductive effects in both female (decreased *corpora lutea*) and male (chromosomal abnormalities) rats. Furthermore, dose-dependent effects on various reproductive parameters were evident and were even statistically significant in groups exposed to higher doses of halothane-nitrous oxide (82 mg/m^3 - 900 mg/m^3). The committee expects that the observed effects were not caused by nitrous oxide, because for nitrous oxide alone a NOAEL of 1520 mg/m^3 (800 ppm) was set for adverse reproductive effects in rats (SZW92).

Investigators reported on a study, in which pregnant female Sprague Dawley rats (n=7-10) were exposed to graded concentrations of halothane (13.1- 26.3 g/m^3 (0.16-0.32%)), nitrous oxide (1-50%), or nitrous oxide (10%) + halothane (13.1 g/m^3) throughout the gestation period of 21 days for 8 hours a day, or only during the gestation days 1-8 (= implantation period).

Females exposed to 26.3 g/m^3 halothane throughout the gestation period had a low food intake and, therefore, did not gain weight; only one of them gave birth. Females exposed to the same dose of halothane but only during the gestation days 1-8 showed normal weight gain and 9 out of the 10 of these females gave birth. The placental weights were only significantly decreased in groups exposed to nitrous oxide. Foetal weights, however, were decreased in all exposed groups, except in those exposed to 1% N_2O. Developmental effects, such as foetal loss and skeletal anomalies were not observed, nor changes in sex ratios. The investigators did not find indications of

synergistic or antagonistic activity with simultaneous exposure of halothane and nitrous oxide (Pop78).

6.3 Other relevant studies

Halothane stereo selectivity

Halothane and its isomer R-halothane were tested for their ability to decrease the intracellular K^+-content in Hartley male guinea pig liver slices (n=5/cage). Both compounds decreased the intracellular K^+-content at a concentration of 2.1 mM (n=2-4 samples/value; exposure times 1 to 24 hours), but R-halothane was less potent (Gha90).

The R- and S-isomers of halothane were tested for their potency to immobilise wild type and several mutant strains of the nematode *Caenorhabditis elegans*. The investigators found that R-isomers were more potent than the S-isomers, and suggested that steric differences of halothane are expressed in anaesthetic effects (Sed94).

Female $B6C3F_1$ mice (n=2/group) were administered R-halothane, S-halothane or racemic halothane at a dose of 10 mmol/kg b.w. by a single ip injection in sesame oil. The findings show that the R-isomer of halothane produces significantly higher amounts of TFA adducts than does the S-isomer or the racemic halothane. Because the anaesthetic potency of the isomers appears to be similar, the authors suggest that S-halothane may be a safer inhalation anaesthetic than R-halothane or racemic halothane (Mar95). The committee noted that no explanation was given why the difference in amount of TFA adducts between the S-halothane and racemic halothane was negligible.

Halothane cross-sensitisation

A few cases have been reported of unexplained hepatic dysfunction and hepatitis after isoflurane anaesthesia. For instance, one case of cross- sensitisation has been reported after exposure to anaesthetic concentrations of various anaesthetics. A 35-yr old obese woman, with a previous history of halothane hepatitis, developed hepatitis after receiving general anaesthesia with halothane 6 years later, and with isoflurane one year thereafter. The SGPT levels were increased at each occasion (Has98). Cases of cross sensitisation after occupational exposure have not been found.

Pairs of male Sprague Dawley rats (n=not given) were injected intraperitoneally with halothane (10 mmol/kg b.w.), enflurane (21 mmol/kg b.w.) or isoflurane (21 mmol/kg b.w.) dissolved in sesame oil. Five hours later, a second dose of enflurane and isoflurane was administered, followed seven hours later by a third dose of enflurane or isoflurane. Only a single dose of halothane was given. Hepatic immunereactive

TFA-protein adducts were formed in all three treatment groups. The relative amounts of the adducts formed were halothane >> enflurane >> isoflurane and correlates directly with the reactive extents of metabolism of these anaesthetics (Chr88).

In another study two animals models were used, namely male Sprague Dawley rats and Hartley guinea pigs (n=2/group). The animals were injected intraperitoneally with halothane (10 mmol/kg b.w., single dose), enflurane (50 mmol/kg b.w., three doses) or isoflurane (50 mmol/kg b.w., three doses) dissolved in sesame oil. Other groups were exposed by inhalation of the anaesthetics (halothane, 82.1 g/m^3 (1%)), enflurane (2.5%) or isoflurane (2,5%)) for 4 hours. Animals were killed at different time points after the exposure. As was found in the previous study, the relative amounts of hepatic immunereactive TFA-protein adducts formed were halothane >> enflurane >> isoflurane. Proportionally, hepatic adduct formation following halothane, enflurane and isoflurane administration correlated with their biotransformation rates. However, a greater amount of adducts were demonstrated in guinea pigs compared with rats, which may be the result of an increased biotransformation rate of the anaesthetics in the guinea pigs (Cla95).

Mucociliary activity

Rabbits (n=6), which were exposed to 90.3 g/m^3 (=Malv$C_{rabbits}$) halothane, showed increased mucociliary activity in the maxillary sinus, with a first peak within 2 minutes and a second peak at 3-8 minutes after starting anaesthesia (Cer98).

Immune response

Investigators performed a study, in which adult male CBi mice (n=14/group) were exposed to 123.2 g/m^3 (1.5%) halothane for 40 minutes, once a week during 3 weeks. Halothane treatment increased the amount of specific antibody secreting B-cells, without affecting the delayed type hypersensitivity reaction to the same antigen (Ele97).

6.4 Summary

Human data

Inhalation of anaesthetic levels of halothane may cause moderate airway irritation. The lowest lethal inhaled concentration reported is 57.4 g/m^3 after exposure for three hours. In human studies with volunteers the lowest observed effect on the neurobehaviour ranged between 3.3 and 4.1 g/m^3.

In patients, several cases of halothane-associated hepatitis have been reported. However, only a few cases have been reported after occupational exposure. The hepatitis is most probably caused by the production of immuno- reactive TFA-protein adducts in the liver after inhalation of halothane, which in a small subset of susceptible individuals may trigger an immune response.

Halothane may induce sister chromatid exchanges and chromosomal aberrations in lymphocytes obtained from occupationally exposed personnel, although not all tests are positive.

Epidemiological studies cause concern about the effect of anaesthetic mixtures containing halothane on foetal development, miscarriages and sperm quality. However, it was not clear whether or not the effects described were caused by halothane. Confounding factors such as exposure to gas mixtures, differences in age between control and the exposed groups may have played a role.

Overall, no interactions, including potentiation and immunological effects, have been found between halothane and other anaesthetics.

Animal studies

Eye irritation tests in the rabbit showed that a dose of 100 mg halothane was severely irritating. The acute lethal toxicity of a single exposure is low; in mice the lethal concentration at which 50% of the animals died ranged between 180 and 350 g/m^3, depending on strain and exposure duration, and 238 g/m^3 in rats.

Several short-term exposure studies showed that halothane at anaesthetic levels produced TFA-protein adducts in the liver of rats, mice, rabbits and guinea pigs. Estimates on the half-life of a variety of these adducts varied between 19 and 90 hours. TFA-proteins were also found after exposure to other anaesthetics, such as enflurane, isoflurane and halothane analogues, suggesting that cross-reaction with halothane may occur.

Continuous exposure to 123 mg/m^3 halothane for 35 days did not affect the body weights of rats, mice and guinea pigs, and only induced liver injury in mice. In another study, continuous exposure to 164 mg/m^3 halothane increased the relative liver weight of rats after 6 weeks of exposure, but not after 19 or 30 weeks. All three exposure periods induced minimal fatty changes in the liver. Investigators reported that continuous exposure to 411 mg/m^3 halothane for 28 days induced liver injury in rats, and that the injury was completely abolished when a reducer of halothane metabolism was given before the halothane exposure. Neurobehavioural effects have been observed in mice exposed to halothane at relative high concentrations (30-40 g/m^3) for 10 minutes to 2 hours.

No long-term carcinogenicity studies have been performed at satisfactory concentrations and exposure durations. In one long-term study no carcinogenic effects were found in rats exposed to a low concentration of halothane (82.1 mg/m^3) combined with nitrous oxide (900 mg/m^3) for 2 years. In *in vitro* test systems, halothane did neither induce mutations in bacteria, aneuploidy, sister chromatid exchanges nor chromosomal aberrations in mammalian cells.

In several studies in rats and mice, effects on the development, such as fatty changes in neonatal livers, ultrastructural changes in the kidneys and brain, and neurobehavioural changes were observed after pre- and postnatal exposure to halothane at concentrations of as low as 82 mg/m^3 by inhalation. In one study, death of the embryos occurred at 65.7 g/m^3 halothane in rats and at 8.2 g/m^3 in mice. In another study, fertility in exposed male rats was decreased, although this was not confirmed by others. Investigators exposed male and female rats to 8.2 or 82.1 mg/m^3 halothane combined with 90 or 900 mg/m^3 N_2O for 12 weeks. In males, chromosomal aberrations in bone marrow and spermatogonial cells were found, and in females decreased ovarian *corpora lutea* (p<0.05 in both dose groups). Although the nitrous oxide concentrations used were far below its NOAEL of 1,520 mg/m^3, and thus the observed effects on the male and female fertility may be ascribed to halothane alone, the committee believes that this study is not suitable as a starting point in deriving a occupational exposure limit, because of the mixed exposure. However, the results of the mixed exposure study are a point of concern.

In another study, female rats were exposed to 13.1 g/m^3 (0.16%) halothane combined with 10% nitrous oxide during the gestation period. Foetal weight was decreased, but this was not larger than in litter of females exposed to halothane or nitrous oxide alone. No other effects on the reproduction were observed.

Investigators did not find any synergistic or antagonistic action between halothane and other anaesthetic gases, when given simultaneously. However, results of a few studies suggest that simultaneous exposure with halothane may be additive, although in one study the requirement of halothane was non-linear with increasing the dose of nitrous oxide.

Preliminary studies show that R-halothane was more potent in immobilising nematodes than S-halothane. In another study, R-halothane induced more TFA-protein adducts in mice than S-halothane.

Chapter 7

Existing guidelines, standards and evaluations

7.1 General population

Guidelines for the general population have not been found.

7.2 Working population

7.2.1 Occupational exposure limits

Current occupational exposure limits in the Netherlands and some other countries are presented in Table 7.1.

The Netherlands

Recently, the Committee for Compounds Toxic to Reproduction of the Dutch Health Council evaluated the effects on the reproduction after occupational exposure to halothane (Hea00). In this report several epidemiological studies are evaluated, but this committee considered all these human studies insufficient for classification. However, in view of the animal data, the Health Council recommended classifying halothane in category 3 (substances which cause concern for humans owing to possible developmental toxic effects) and to label halothane with R63 (possible risk for harm to the unborn child). This classification was performed according to the guidelines of the

Table 7.1 Occupational exposure limits in several countries.

country - organisation	concentration mg/m^3	ppm	time-weighted average	type of exposure limit	note	reference
The Netherlands	40	5	8 h	administrative force	R[b]	SZW02
Germany						
- DFG	41	5	8 h	MAK	peak , R	DFG01
Sweden	40	5	8 h	LLV		SNB00
	80	10	15 min	STV		
Finland	8.2	1	8 h	HTP-arvot		SOS00
	25	3	15 min	HTP-arvot		
United Kingdom						
- HSE	82	10	8h	OES		HSE02
USA						
- ACGIH	404	50	8 h	TLV	A4	ACG02
- NIOSH	16.2	2	ceiling 1 h	REL		ACG02
- OSHA	-	-				

[b] R = compound toxic to reproduction, see text

European Union (Directive 93/21/EEC). Halothane could not be classified with respect to fertility due to a lack of appropiate data.

France

In 1988, the Technical and Medical Services of the 'Institut National de Recherche et de Sécurité' of France prepared a toxicological data sheet on halothane (INR88). The MAC (8 h TWA) is set at 16.4 mg/m^3 (2 ppm) halothane. Regulations are given concerning ventilation of operating theatres, medical examination of exposed persons, protection of the general population and transport of halothane. Furthermore, safety recommendations are given with respect to instruction of personnel, storage of the compound, maintenance of the anaesthesia apparatus, and first aid measures after massive exposure.

Germany

Recently, the 'Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area' of the Deutsche Forschungsgemeinschaft has discussed a change of the MAK value of halothane and suggested setting a MAK value of 41 mg/m^3 (5 ppm) (8h TWA). Because halothane is metabolised to among others TFA, which has accumulation properties, the formation of TFA should be restricted (DFG02).

Furthermore, halothane is classified in pregnancy group B. This means that currently available information indicates that a risk of damage to the developing embryo or foetus should be considered to be probable. Damage to the developing organism cannot be excluded when pregnant women are exposed, even when MAK and BEL values are observed.

IARC

The IARC evaluated the carcinogenicity, mutagenicity and genotoxicity of volatile anaesthetics as a group (IAR87). At that time, it was not possible considering exposure to different volatile anaesthetics separately. IARC concluded that the evidence in humans and animals (mice, rats) for any carcinogenic properties of these anaesthetics was inadequate.

Norway

In 1985, The Nordic Expert Group evaluated the toxicity of halothane (NEG85). From the available literature, the NEG concluded that the critical effect of halothane was on the central nervous system. Also possible adverse effects of halothane on pregnancy outcome should be taken into account. In rats, the lowest air concentration tested, which induced slight microscopic changes in CNS and kidneys of the offspring, was 90 mg/m^3 halothane, when given for 8 weeks (8 h/day, 5 d/week). No further conclusions were drawn.

USA (NIOSH)

In 1977, NIOSH evaluated the toxicity data of waste anaesthetic gases (NIO77). According to the NIOSH, primary health concerns are the adverse effects of halothane, whether simultaneously used with other anaesthetics or not, found in occupationally exposed women and congenital abnormalities found in new born of exposed personnel. These effects are considered guidelines for recommending exposure limits of

halogenated anaesthetics alone or when used as a mixture. Based on the available health information, a safe level of exposure to the halogenated agents cannot be defined. NIOSH, therefore, recommends that occupational exposure levels should be no greater than the lowest detectable concentration by using sampling and analysis techniques recommended by NIOSH. A maximum air concentration of 2 ppm (=16.4 mg halothane/m^3) as a ceiling (1 hour) for all halogenated gases together is recommended.

USA (ACGIH)

The ACGIH recommended a TLV (8 h TWA) of 404 mg/m^3 (50 ppm) for halothane (ACG92). The recommendation is made amongst others from observations that halothane possesses only low carcinogenic potential in animals, and from the low severity of the hepatotoxic and adverse effects on the reproduction. The ACGIH recommends no STEL, until additional toxicological data and industrial hygiene experience become available. A 10 ppm TLV was adopted for surgical suites using 50:1 nitrous oxide to halothane.

Furthermore, the ACGIH has classified halothane in category A4 for carcinogenicity, which means that it is 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 classifiy the agent into one of the other categories.

7.2.2 Biological limit values

Because halothane is metabolised in amongst others TFA, which has accumulation properties, Germany believes that the formation of TFA should be restricted. Therefore, Germany has set a Biological Exposure Limit (BEL) for halothane of 2.5 mg TFA per litre blood, which results from an exposure to 41 mg/m^3 (5 ppm) halothane in air during a normal working week of 40 hours. For assessing the biological exposure, blood samples are taken at the end of the exposure period or shift, or after several shifts in case of long-term exposure (DFG98).

Chapter 8

Hazard assessment

8.1 Assessment of health hazard

Halothane is a widely used anaesthetic agent and its properties are well investigated. However, human data on the adverse effects of halothane after occupational exposure are limited in number (see Table 8.1). There is no evidence in humans for the carcinogenicity of halothane. Evidence for effects on the reproduction in humans is inadequate.

In animals, short-term exposure to halothane at concentration levels of 123-410 mg/m^3 caused liver injury in mice and rats, and, furthermore, loss of the relative liver weight and minimal fatty changes in the liver (see Table 8.1). Exposure concentrations in the order of 30,000-40,000 mg/m^3 affected the neurobehaviour of mice. Animal data on the carcinogenicity is very limited and do not demonstrate that halothane is carcinogenic at low exposure levels.

In practice, halothane is often used in combination with other anaesthetic gases, such as nitrous oxide and fluranes. A few animal data suggest that the influence of these anaesthetic gases on the effects of halothane is no more than additive.

Table 8.1 Relation between exposure levels of halothane and observed adverse effects.

dose mg/m^3	exposure regimen	effects	ref.
55,000-63,000		MalvC for anaesthesia in humans.	§ 3.2.2
4,105	30 min, end-tidal	Neurobehavioural effects in volunteers.	Cor78
3,284	30 min, end-tidal	Decrease in peak saccadic eye movement in volunteers.	Yos91
1,232	8 h/day, 5d/week, 7 weeks	Halothane blood levels increased over the weeks of exposure in male Wistar rats.	Mar84
164	contineous for 7 weeks	Increased relative liver weight, minimal fatty changes and increased P450 in male F344 rats.	Plu86
123	several exposure regimen	Liver lesions in mice.	Ste75
82	with 900 mg/m^3 N_2O; 7 h/day, 5 d/week for 12	Several studies showed reproductive effects in rats and mice (see text).	Several
82	weeks, and on GD1-15 with 90 mg/m^3 N_2O; 7h/day, 5d/week for 12	Lower birth weight of male offspring; Decreased ovulation and implantation efficiency in Sprague Dawley rats.	Coa79b
8.2	weeks, continued for 40 weeks or on GD 1-15	Aberrant chromosomes in bone marrow and spermatogonial cells in male Sprague Dawley rats (40 weeks); decreased number of *corpora lutea* in female Sprague Dawley rats after GD15.	Coa79b

Effects on the reproduction, more specifically on the development (fatty changes in neonatal livers, ultrastructural changes in kidneys and brain, and neurobehavioural changes), were observed in many studies (see Table 6.8; Bow77, Cha75a/b, Cha76, Cha77, Dud77, Hal81, Qui74, Qui75), in which rats and mice were exposed to halothane at concentration levels of as low as 82 mg/m^3. Based on these data, the committee considers the effects on the development of the offspring as the most sensitive effects of halothane that should be prevented by setting an health-based occupational exposure limit.

In one study, effects on the fertility of both male (dose-related increase of chromosomal abnormalities in bone marrow and spermatogonial cells) and female rats (decreased number of ovarian *corpora lutea*) have been reported after exposure of halothane at a concentration of as low as 8.2 mg/m^3 (Coa79b, see Table 8.1). Because the animals were simultaneously exposed with nitrous oxide, the committee considers this study not suitable as a starting point in deriving a HBR-OEL. However, the committee is concerned that the effects on the reproduction observed at this concentration level may have been caused by halothane alone, because the concentrations of nitrous oxide were below its NOAEL. In the study, no data have been presented on the effects of halothane or N_2O exposure exclusively.

Based on the developmental effects in the offspring of rats, the committee considers the lowest-observed-advers-effect-level (LOAEL) of 82 mg/m^3 (10 ppm) halothane as a starting point in deriving an HBR-OEL.

For the assessment of this HBR-OEL, the committee takes the following considerations into account: dose-response curve, differences between experimental conditions and the exposure pattern of the worker, intra- and interspecies variation, and the confidence of the data base. Considering the dose-response curves of the animal studies, the committee concludes that a factor of 2 is too low for the extrapolation from the LOAEL of 82 mg/m^3 to a NOAEL, because application of this factor would result in a NOAEL of 8.2 mg/m^3, whereas at this level the committee is concerned that halothane may induce effects on the fertility. Therefore, the committee recommends using a factor of 20 in stead of 2. The committee, furthermore, considers a factor of 10 sufficient for intra- and inter- species variations. These considerations result in a composite uncertainty factor of 200.

By applying this uncertainty factor, the committee recommends a HBR-OEL of 0.41 mg/m^3 (0.05 ppm) halothane as an eight-hour time-weighted average concentration.

8.2 Groups at extra risk

No groups at extra risk need to be indicated.

8.3 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit (HBR-OEL) for halothane of 0.41 mg/m^3 (0.05 ppm) as an eight-hour time-weighted average concentration.

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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 Boogaard
  toxicologist; Shell International B.V., The Hague
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; IRAS, Utrecht University, Utrecht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- TM Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of 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
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of the present advisory report was prepared by MA Maclaine Pont, Msc, from the Wageningen University, The Netherlands, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: R Aksel-Gauri. Lay-out: J van Kan and M Javanmardi.

Annex C

Comments on the public review draft

A draft of the present report was released in April 2001 for public review. The following organisations and persons have commented on the draft document:

- A Aalto, Ministry of Social Affairs and Health, Occupational Safety and Health Division, Tampere, Finland;
- RD Zumwalde, National Institute for Occupational Safety and Health, Cincinnati, USA.

Annex **D**

Case reports

Several cases of hepatitis and other liver disorders are described after occupational exposure:

- A 33-yr old anaesthetist had worked for 3.5 months with halothane, when he fell ill with fatigue and nausea and developed jaundice. His serum hepatic enzyme levels were high. His jaundice abated in about 3 weeks. Two months later the man was anaesthetised with halothane for a few minutes in a provocation test. Five hours later he fell suddenly ill, with chills, nausea, headache, and myalgia. The next day the patient's general condition soon improved. Again, serum transaminase levels increased, with peaks at 2 to 4 days after exposure, which did not normalise untill three weeks after the provocation (Bel66).
- A 44-yr old anaesthetist with a history of hay fever and asthma had recurrent hepatitis which led to the development of cirrhosis. Each of the relapses coincided with the patient's return to work and re-exposure to halothane. Challenge with a nonanaesthetic dose of halothane (8.2-16.4 mg/m^3 in O_2 for 5 minutes) provoked an identical relapse characterised by chills, fever and acute hepatitis (Kla69).
- A 26-yr old female laboratory technician developed hepatitis within 2 months of starting to use halothane to anaesthetise rats. Her initial illness was one of malaise, anorexia and vomiting. On re-exposure she developed an accelerated reaction characterised by fever, rigors, rash, body aches and malaise, followed by jaundice. Liver biopsy showed a hepatitis picture with prominent eosinophilic infiltrate. The patient made a rapid uneventful recovery (Joh71).

- A previously healthy 28-yr old female nurse had her appendix removed under halothane anaesthesia. Five years later she started to work in a department where halothane was administered to several patients daily. Three months later, she complained that she needed more sleep than normal, and her serum transaminase levels were increased. After stopping her work, the transaminase levels returned to normal. She resumed work and again the same symptoms returned. After discontinuation and resuming work this was repeated once more. She was advised to stop her work in the department (Lun74).
- A 41-yr old surgeon developed liver damage after occupational exposure to trace amounts of halothane. He had been working for 15 years in operating theatres. Blood analysis showed increased level of SGOT (±200 iu/L *versus* ±20 iu/L normal range) and halothane-related antibodies in his serum. The serum level of transaminase returned to normal upon cessation of exposure (Neu81).
- A 26-yr old doctor had an increased plasma level of SGOT after 3 months exposure to halothane (51-90 iu/L *versus* ±20 iu/L normal range). Also he had halothane-related antibodies in his serum. Upon avoidance of further exposure to halothane he has remained well since then (Neu81).
- A 43-yr old nurse had more than ten times elevated value of SGPT on routine analysis when serving as a blood donor. She did not feel ill and appeared healthy at physical examination. Clinical analyses revealed that she had mild pruritus and dark urine. SGPT normalised within 20 days. Analysis of the workplace air revealed concentrations of nitrous oxide and halothane, several times above the occupational exposure limit (halothane, 41 mg/m^3). The authors, therefore, concluded that the liver injury probably was caused by occupational exposure to halothane (Kei84).
- A 54-yr old anaesthetist developed halothane-related hepatitis after several years of occupational exposure to unknown quantities of halothane. There was a causal relationship between halothane exposure and an increased level of SGPT (Lin88).
- A 40-yr old anaesthetist developed hepatitis about 5 years after starting this work. He had elevated levels of SGPT and SGOT. Upon discontinuation of exposure to halothane, the effects disappeared (Eft88).
- A 34-yr old nurse developed an icteric and feverish hepatitis after 3 years of occupational exposure to halothane. The levels of SGPT and SGOT were elevated (Eft88).
- A 51-yr old nurse, a teetotaler, showed elevated levels of SGPT and SGOT for 5 years after a viral hepatitis. Two successive removals from work, after these 5 years, decreased the enzyme levels to normal values. An isolated enzyme induction experiment with halothane led to the conclusion that halothane was the most probable cause (Eft88).

- A 42-yr old male biochemist developed hepatitis after using for 3 years repeatedly halothane for sedation and euthanasia of rats. He suffered recurrent episodes of epigastric discomfort, culminating in an episode of malaise, anorexia, jaundice and elevated liver associated enzymes that promptly resolved after removal from exposure to halothane (Sut92).

A few cases of adverse effects have been reported after sniffing haltohane:

- A 25 yr old female scrub technician complained of sore throat, headaches, malaise, nausea, vomiting, and fever of 40 °C. Upon admission to the hospital she developed jaundice, became comatose and hypotensive within 12 h. She died after an episode of supraventricular and ventricular tachycardia. Postmortem examination revealed diffuse bilateral pulmonary haemorrhage and pneumonia. The liver showed acute yellow atrophy. There was extensive hepatic necrosis. Information from friends revealed that the patient had been intermittently sniffing halothane for more than 1 year and in the month before her death had again been sniffing, usually on multiple occasions daily, consuming about 1250 mL of halothane in this manner (Kap79).
- A 25-yr old female operating room technician became involved in halothane sniffing during the previous month, and had consumed between 250 and 400 mL of halothane. She experienced nausea, vomiting, anorexia, abdominal pain, chills and fever to 40°C. Eight days later her serum transaminase level were still elevated, but she felt well and refused further follow-up (Kap79).
- A 23-yr old woman had sniffed halothane only five to six times over the previous 3 weeks. Her SGPT level elevated during the following days and peaked at day 6. She continued to do well clinically and the enzyme levels returned to normal over the next 4 months. Liver size has not changed (Kap79).

In one case report, two lethal cases of a homicide by smothering with halothane-moistened towels is described. Analysis of halothane in the blood and other tissues revealed halothane concentrations of about 3.5-6 (blood, mg/L), 1.7-21.3 (liver), 11.7-14.5 (kidney) and 103.6-120.2 (brain) mg/kg. Of the 100 mL bottle only 25 mL was left (Mad99).

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 Administration (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

Additional abbreviations in the present report

MalvC	maximum alveolar concentration
N_2O	nitrous oxide
PND	postnatal day
SGOT	serum glutamic oxaloacetic transaminase (aspartate aminotransferase)
SGPT	serum glutamic pyruvic transaminase (alanine aminotransferase)
TFA	trifluoroacetic acid
t½	half-life; is the amount of time before the concentration of the compound is halved

Annex **F**

DECOS-documents

DECOS has produced documents on the following substances. To be ordered from the Health Council of the Netherlands:

Aanpassing van grenswaarden bij flexibele werktijden	2001/06OSH
Acetone cyanohydrin	1995/05WGD
p-Aramid fibres	1997/07WGD
Azathioprine	1999/04OSH
Aziridine (ethyl imine)	2000/13OSH
Azobisisobutyronitril	2002/01OSH
1,2,3-Benzotriazole	2000/14OSH
Bisphenol A and its diglycidylether	1996/02WGD
Bromoethane	1998/10WGD
1,2-and t-Butanol	1994/10WGD
n-, iso-, sec-, tert-Butylacetaten	2001/03OSH
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
Chlorotrimethylilane	2001/05OSH
Chromium and its inorganic compounds	1998/01WGD
Chromium VI and its compounds	2001/01OSH
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
Ejthylene oxide	2001/11OSH
Ethylene thiourea	1999/03OSH
Formamide and dimethylformamide	1995/08WGD
Hydrazinoethanol, phenylhydrazine, isoniazid, maleic hydrazide	1997/03WGD
Isopropyl acetate	1997/04WGD
Lactate esters	2001/04OSH
Lindane	2001/07OSH
Man made mineral fibers	1995/02WGD
Manganese and its compounds	2001/02OSH
2-Methylaziridine (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
Styrene	2001/08OSH
Quartz	1998/02WGD
Toluene	2001/09OSH
1,1,1-Trichloroethane	1995/03WGD
1,2,3-Trichloropropane	1994/25
1,2,3-Trichloropropane	1998/14WGD
Urethane (ethyl carbamate)	200012OSH

Vinylbromide	1999/15OSH
Xylene	2001/10OSH
Wood dust	1998/13WGD