The Nordic Expert Group for Criteria Documentation
of Health Risks from Chemicals

141. Isoflurane, sevoflurane and desflurane

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Preface

The main task of the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) is to produce criteria documents to be used by the regulatory authorities as the scientific basis for setting occupational exposure limits for chemical substances.

For each document, NEG appoints one or several authors. An evaluation is made of all relevant published, peer-reviewed original literature found. The document aims at establishing dose-response/dose-effect relationships and defining a critical effect. No numerical values for occupational exposure limits are proposed.

Whereas NEG adopts the document by consensus procedures, thereby granting the quality and conclusions, the author is responsible for the factual content of the document.

The evaluation of the literature and the drafting of this document on *Isoflurane, sevoflurane and desflurane* were made by Dr Anne Thoustrup Saber and Dr Karin Sørig Hougaard, National Research Centre for the Working Environment, Denmark. The draft document was discussed within the group and the final version was accepted by NEG on January 9, 2009 as its document. The following experts participated in the elaboration of the document:

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All criteria documents produced by the Nordic Expert Group may be downloaded from *www.nordicexpertgroup.org*.

Gunnar Johanson, Chairman of NEG

Contents

Abbreviations and acronyms

CI	confidence interval
EC	electron capture detection
EEG	electroencephalography
EPSC	excitatory postsynaptic current
F_A	alveolar blood concentration of flurane
FDVE	fluoromethyl 2,2-difluoro-1-(trifluoromethyl)vinyl ether (also called compound A)
fEPSP	field excitatory postsynaptic potential
FEV_1	forced expiratory volume in one second
F_I	concentration of inspired flurane
GC	gas chromatography
HFIP	hexafluoroisopropanol
IL	interleukin
LC_{50}	lethal concentration for 50% of the exposed animals at single inhalation exposure
LOAEL	lowest observed adverse effect level
MAC	minimum alveolar concentration of anaesthetic that produces immobility in 50% of subjects exposed to a supramaximal painful/noxious stimulus
MS	mass spectrometry
NOAEL	no observed adverse effect level
OR	odds ratio
PET	positron emission tomography
PS	population spike
SCE	sister chromatid exchange
SD	standard deviation
TFA	trifluoroacetic acid
TNFα	tumour necrosis factor alpha
TWA	time-weighted average

1. Introduction

Isoflurane, sevoflurane and desflurane are halogenated ethers and are used as inhalation anaesthetic agents. These agents are either used separately or in combination with nitrous oxide, intravenous anaesthetics, and muscle relaxants. Enflurane is also a member of this family but is no longer sold in the Nordic countries. For that reason enflurane is not included in this criteria document.

This document was made on initial request from the Danish Working Environment Authority due to a report indicating exposure of personnel at Danish hospitals to these agents and the lack of occupational exposure limits (16).
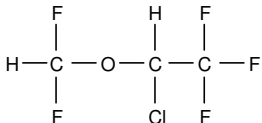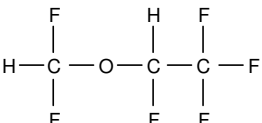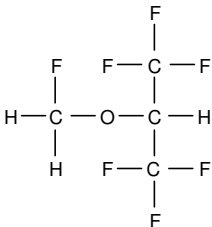
Until the 1990s, halothane and enflurane together with nitrous oxide were the primary anaesthetic gases. During the last decade, halothane and enflurane have been phased out and gradually replaced by isoflurane, desflurane and sevoflurane which, compared to halothane and enflurane, are less prone to metabolism causing immune hepatitis and other side-effects. As an example from the Nordic countries, isoflurane has been used in Denmark since 1984, while desflurane and sevoflurane are relatively new anaesthetics, introduced in 1994 and 1996, respectively (150-152).

In this document, the term fluranes is used as a common term for isoflurane, sevoflurane and desflurane. Some selection of literature has been performed. As opposed to most chemicals that occur in the work environment, the fluranes are meant to exert effects in humans at high dose levels. Thus, a large body of literature exists on pharmacological and toxicological effects in humans, mostly after a single exposure at anaesthetic dose levels, i.e. at exposure levels far exceeding the levels met in the working environment. Where relevant, effects of such high acute exposures in humans are described as summarised in reviews. Animal studies are generally described in detail for effects where human data are lacking or scarce, or if the studies were conducted at subanaesthetic concentrations or included repeated exposures.

2. Substance identification

The three fluranes are all ethers. Isoflurane and desflurane are halogenated methylethyl ethers with a difluoromethyl group and a fluorinated ethyl group. In isoflurane, one of the fluorine atoms in the ethyl group is substituted with chlorine. Sevoflurane is a polyfluorinated methyl isopropyl ether (55). Substance identification data for the fluranes are given in Table 1.

Table 1. Substance identification for the fluranes (46).

Generic name	Isoflurane	Desflurane	Sevoflurane
CAS No.	26675-46-7	57041-67-5	28523-86-6
EINECS No.	247-897-7	Not available	Not available
IUPAC names	2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane	2-(difluoromethoxy)-1,1,1,2-tetrafluoro-ethane	1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)pro-pane
Synonyms	1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, forane, isofluorane	1,2,2,2-tetrafluoroethyl difluoromethyl ether, difluoromethyl 1,2,2,2-tetrafluoroethyl ether, suprane	fluoromethyl 1,1,1,3,3,3-hexafluoroisopropyl ether, sevorane
Molecular formula	$C_3H_2F_5ClO$	$C_3H_2F_6O$	$C_4H_3F_7O$
Molecular weight	184.5	168.0	200.1
Structural formula			

3. Physical and chemical properties

Isoflurane, sevoflurane and desflurane are stable, clear, colourless volatile liquids at room temperature (desflurane boils at 23 °C). They neither burn nor explode.

Sevoflurane seems the least pungent of the three fluranes and the odour has been described as pleasant, much like chloroform. The odour of desflurane has been described as ether-like and unpleasant. Isoflurane has a mildly pungent, musty ethereal odour (55, 160). During exposure to 1 200 ppm of isoflurane, three of six volunteers rated the isoflurane smell as absent, whereas the other three rated it to be present (79). Some physical and chemical properties of the three fluranes are given in Table 2.

To minimise waste and decrease costs, flurane anaesthetics are delivered through a system which recirculates the anaesthetic after removal of carbon dioxide by an absorbent. The absorbent is a strong base such as calcium hydroxide.

Fluranes containing $-CHF_2$, such as isoflurane and desflurane, react with strong bases in carbon dioxide absorbents resulting in the formation of carbon monoxide (as reviewed by Anders (10). Carbon monoxide is a highly toxic gas. The simplified mechanism for the degradation of desflurane into carbon monoxide and hydrogen fluoride is shown in Figure 1. The chemical degradation occurs only in the recirculation system and not in biological tissues.

Table 2. Physical and chemical properties of the fluranes (69, 91, 160, 193).

Anaesthetic	Isoflurane	Sevoflurane	Desflurane
Tissue:gas partition coefficients [a] at 37 °C			
Blood	1.4	0.65	0.45
Brain	2.2	1.1	0.55
Heart	2.2	1.1	0.55
Liver	2.6	1.3	0.67
Kidney	1.4	0.78	0.4
Muscle	3.6	1.7	0.78
Fat [b]	70	37	13
Olive oil	98	47	19
Other properties			
Boiling point (°C) at 101.3 kPa	48.5	58.5	22.8
Saturated vapour pressure (kPa) at 20 °C	32.0	21.3	89.2
Gas density (kg/m^3) of 1 MAC of flurane in 25% oxygen and 75% nitrogen at 0 °C, 101.3 kPa	1.35	1.45	1.70
Conversion factors at 25 °C, 101.3 kPa	1 ppm = 7.55 mg/m^3 1 mg/m^3 = 0.13 ppm	1 ppm = 8.2 mg/m^3 1 mg/m^3 = 0.12 ppm	1 ppm = 6.9 mg/m^3 1 mg/m^3 = 0.15 ppm

[a] Human data.
[b] Estimated from the assumption that 70% of fat has the solubility of oil and 30% that of blood.
MAC: minimum alveolar concentration that produces immobility in 50% of the subjects exposed to supramaximal painful/noxious stimulus.

$$F_3C-CHF-O-CHF_2 + OH^- \rightleftharpoons F_3C-CHF-O-\overset{\bullet}{C}F_2 + H_2O$$

$$F_3C-CHF-O-\overset{\bullet}{C}F_2 \longrightarrow F_3C-CH=O + :CF_2 + F^-$$

$$:CF_2 + H_2O \longrightarrow CO + 2HF$$
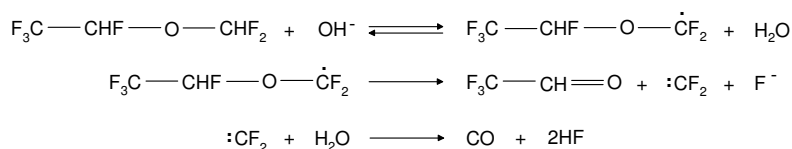
Figure 1. Simplified mechanism of carbon monoxide (CO) and hydrogen fluoride (HF) formation from desflurane (52).

Sevoflurane is partly degraded by strong bases in the carbon dioxide absorbent in clinical anaesthesia machines to fluoromethyl 2,2-difluoro-1-(trifluoromethyl)-vinyl ether (FDVE, also called compound A) (134). FDVE may thus contaminate the anaesthetic gas phase in the anaesthetic circulatory system (Figure 2). The chemical degradation occurs only in the recirculation system and not in biological tissues.

The formation of carbon monoxide increases as the water content in the absorbent decreases (10). It has been shown that the use of new carbon dioxide absorbents (without strong bases) decreases the formation of carbon monoxide and the formation of FDVE (136).

4. Occurrence, production and use

High doses of isoflurane, sevoflurane and desflurane induce anaesthesia and all three compounds are used as anaesthetic gases. The fluranes do not appear naturally, but are synthesised in closed systems. Fluranes are often used in combination with oxygen/nitrous oxide (O_2/N_2O) and other anaesthetics to induce and maintain general anaesthesia.

Fluranes are volatile liquids and are delivered using an anaesthesia machine. An anaesthesia machine allows for composition of a mixture of oxygen, anaesthetics and ambient air, delivers the mixture to the patient, and monitors the patient. The vapourisation of liquid anaesthetics takes place in the anaesthesia machine. The regular doses for the induction of anaesthesia are 5 000-25 000 ppm isoflurane in oxygen/nitrous oxide and 60 000-80 000 ppm sevoflurane in oxygen/nitrous oxide. Because of its airway irritating effect, desflurane is not normally used for induction of anaesthesia. The corresponding doses for the maintenance of
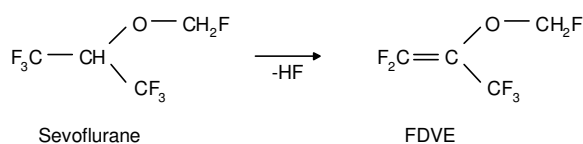
Figure 2. Chemical degradation of sevoflurane to fluoromethyl 2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE) (223).

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anaesthesia are 10 000-25 000 ppm isoflurane, 5 000-30 000 ppm sevoflurane and 20 000-60 000 ppm desflurane (147-149).

Occupational exposure to anaesthetic gases occurs in operating rooms at hospitals and in practises of specialists, recovery rooms, dental operatories, and veterinary facilities. Some decades ago, excess anaesthetic gases were usually exhausted into the surrounding air. Although excess gases are nowadays usually collected by a local exhaust system, some pollution of ambient air by anaesthetic gases is difficult to avoid completely. Thus, leaks around a patient's face mask, in the breathing system, or the ventilator may cause release of anaesthetics. Other sources of pollution include liberation of anaesthetics through leaks in connection tubes and accidental spillage of liquid anaesthetics when filling vapourisers. This makes dermal exposure to liquid anaesthetics possible. In addition, the patient will continue to exhale anaesthetics for some time after termination of the anaesthesia. This may contaminate the air in the recovery room (40). As described in Chapter 3, toxic degradation products are formed due to reaction of fluranes with carbon dioxide absorbents in the anaesthetic recirculation system. Exposure of personnel to these compounds may thus only occur due to leakage from the anaesthetic recirculation system. It is unknown whether personnel are exposed to FDVE. Since the fluranes are synthesised in closed systems, exposure during production occurs only through leaks in the synthesis equipment.

The degree of pollution in operating theatres depends mainly on the efficiency of the gas scavenging system, but also on other factors such as the number of air exchanges per hour, anaesthetic techniques, and the inspiratory and expiratory concentrations of the inhalation anaesthetics. An anaesthetic gas scavenging system collects and removes waste gases at the site of overflow from the breathing circuit and disposes of these gases to the outside atmosphere (40).

Local exhaust equipment is mandatory by law in e.g. Denmark and Sweden (15, 18, 19). No specific rules regarding occupational exposure to anaesthetics for pregnant women exist in Denmark but it is recommended that the exposure of pregnant women to anaesthetics is kept below 1/10 of the occupational exposure limit (17).

The annual use of isoflurane, sevoflurane and desflurane in the Nordic countries for the period 2000-2005 is listed in Table 3. During this period, the annual use of isoflurane in humans has been halved in Denmark, Norway, and Sweden, and in Finland, the use of isoflurane has diminished by 75%. Sevoflurane is the most widely used of the anaesthetics in humans and has primarily been used for the induction of anaesthesia in children because of its lack of irritative effect. The use of sevoflurane during 2000-2005 has been stable in Denmark and Norway, while a slight increase is observed in Sweden and Finland. In Denmark and Sweden, the amount of desflurane was largely unchanged from 2002 to 2005. In contrast, the use of desflurane in Norway doubled during this time period. The use of des-flurane in Finland has been fluctuating with its maximum use in 2002-2003 and the lowest use in the end of the time period. Isoflurane is the only flurane used for veterinary purposes, and data have only been obtained for Denmark and Sweden.

Table 3. Annual uses of the fluranes in the Nordic countries (litres).

Anaesthetic/ country	Year						Reference
	2000	2001	2002	2003	2004	2005	
Human use							
Isoflurane							
Denmark	720	550	395	360	400	275	(146)
Sweden	1 950	1 680	1 515	1 624	1 248	1 101	(11)
Norway	1 142	899	805	730	665	571	(176)
Finland	1 221	883	600	570	363	266	(177)
Sevoflurane							
Denmark	2 700	2 825	2 825	2 900	2 925	2 875	(146)
Sweden	6 934	7 416	7 826	7 732	7 816	8 110	(11)
Norway	2 331	2 668	2 602	2 729	2 771	2 696	(176)
Finland	3 830	4 277	4 251	4 140	4 593	4 822	(177)
Desflurane							
Denmark	125	225	500	550	500	552	(146)
Sweden	657	637	620	663	671	620	(11)
Norway	281	276	421	576	613	641	(176)
Finland	298	496	878	728	183	166	(177)
Veterinary use							
Isoflurane							
Denmark	No data	164	246	167	307	315	(54)
Sweden	158	479	555	475	1 144	1 357	(11)
Norway	---------------------------------- No data ---						
Finland	---------------------------------- No data ---						
Sevoflurane	---------------------------------- Not used ---						
Desflurane	---------------------------------- Not used ---						

In numbers for the Norwegian use of fluranes it is assumed that the number of inhabitants is 4 681 134 (185).

In the described time period, the veterinary use of isoflurane has increased 2- and 8-fold in Denmark and Sweden, respectively.

It should be noted that the anaesthetic potency of sevoflurane is only about half that of isoflurane, whereas desflurane is the least potent of the three. This is reflected by the minimum alveolar concentration (MAC) that denotes the concentration of an anaesthetic that produces immobility in 50% of the subjects exposed to a supramaximal painful/noxious stimulus. MAC-values for the three fluranes are given in Table 4.

Table 4. MAC-values for the fluranes.

MAC (ppm)	Anaesthetic			Reference
	Isoflurane	Sevoflurane	Desflurane	
Humans [a]	11 500-12 200	21 000	60 000	(69)
Rats	14 000, 14 200	25 000	72 100	(31, 66)
Mice	13 100 - 17 700 [b]	25 000	65 500 - 91 200 [b]	(194, 222)

[a] The tabulated values are stated for humans 36-49 years of age, as the MAC-values are age dependent and decrease with age.
[b] The tabulated ranges are stated for 15 inbred mouse strains.
MAC: minimum alveolar concentration that produces immobility in 50% of the subjects exposed to supramaximal painful/noxious stimulus.

5. Measurements and analysis of workplace exposure

5.1 Environmental exposure monitoring

Monitoring of the anaesthetics in ambient air was performed by discontinuous gas chromatography (GC) until about 1980. Since then, infrared spectrophotometry has been the almost exclusively used method for monitoring of air concentrations of the fluranes. Infrared spectrophotometry holds many advantages compared to GC, e.g. continuous measurement and measurement of peak exposure levels (40).

In most cases, flurane concentrations have been measured in the breathing zone using a photoacoustic infrared spectrophotometer connected to sampling tubes fitted at the operating theatre personnel's masks. The majority of measurements using this technique have been performed by employing two different analysers: 1302/1309 Multigas Monitor (Brüel and Kjær, Denmark) and Miran 1B2 (Foxboro, East Bridgewater, Massachusetts). The detection limits of the method for the respective flurane are given in Table 5.

5.2 Biological exposure monitoring

Biological monitoring of unmodified urinary volatile anaesthetics (84) as well as breakdown products of anaesthetics excreted in urine (106) has been described.

Several studies have indicated that the urinary isoflurane concentration might be used as an appropriate biological exposure index (119). Accorsi *et al* used gas chromatography-mass spectrometry (GC-MS) to detect both isoflurane and sevoflurane in urine supernatants. The limits of detection for isoflurane and sevoflurane were 0.02 and 0.03 µg/l, respectively (1). Isoflurane in urine has also been determined using head-space GC with electron capture detection (GC-EC). The sensitivity for this method has been reported to be 1 µmol/m^3 (120).

Urinary sevoflurane (1, 2) as well as its urinary metabolite hexafluoroiso-propanol (HFIP) (97) have been proposed for the evaluation of occupational sevoflurane exposure. Recently, Accorsi *et al* analysed connected data for concentrations of airborne sevoflurane, urinary sevoflurane and HFIP (3). The authors of the study concluded it advantageous to measure urinary sevoflurane compared to HFIP. The urine samples were analysed by headspace GC-MS. The limit of detection for urinary sevoflurane was 0.03 µg/l (1-3).

Table 5. Detection limits for the fluranes in photoacoustic infrared spectrophotometry.

Anaesthetic	1302 Multigas Monitor	Reference	Miran 1B2	Reference
Isoflurane	0.008-0.1 ppm	(33, 104, 108, 217)	0.1 ppm	(217)
Sevoflurane	0.01 ppm	(33)	0.1 ppm	(217)
Desflurane	0.05-0.1 ppm	(33, 104, 217)	0.1 ppm	(217)

6. Occupational exposure data

Occupational exposure to the fluranes occurs primarily via inhalation. Isoflurane, sevoflurane, and desflurane concentrations have been measured at different work-sites in hospitals and veterinary clinics (Table 6-8). The measurements have been performed either as room measurements or measurements in the breathing zone of personnel. No exposure data from the production of flurane anaesthetics have been located.

A number of studies show that the level of exposure depends on the type of mask used during anaesthesia (93) and that the highest exposure of health care personnel occurs during mask induction of anaesthesia (103).

The occupational inhalation exposure to anaesthetics in operating rooms at selected Danish hospitals from 1986 to 2000 was evaluated in a report elaborated on the initiative of the Danish Working Environment Authority (16). Measurements of isoflurane were initiated in 1990, while sevoflurane and desflurane measurements began in 1996. The use of isoflurane increased steadily from 1990, and was used in approximately 50% of the surgical operations in the year 2000. At the same time, sevoflurane was used in approximately 30% of the procedures. Desflurane concentrations were very low throughout the period. During the years of measurement, the average exposure to isoflurane and sevoflurane ranged from approximately 0.2 to 1 ppm (average of 2-31 measurements per compound/year) (16).

Exposure to the fluranes was measured in operating and recovery rooms in nine Swedish hospitals. A total of 7 desflurane, 21 isoflurane and 100 sevoflurane measurements were performed in 1999, 2002 and 2003 on personnel participating in different kinds of operations and examinations. Air concentrations were 0.04-0.22 ppm for isoflurane, 0.02-14.4 ppm for sevoflurane and 0.04-0.21 ppm for desflurane (117).

Isoflurane
Most measurements relate to exposure to isoflurane in hospitals (Table 6). In general, the levels of exposure to isoflurane have decreased over time and in the most recent studies performed at hospitals, the exposure levels stayed below 2 ppm. Not surprisingly were air values lower when scavenging was applied.

In two studies evaluating exposure to isoflurane at veterinary clinics (Table 6), where some of the highest exposures to isoflurane have been registered (105, 141), exposure levels reached several ppm.

Sevoflurane
As for isoflurane, the levels of exposure seem to be decreasing over time. Thus, the exposure levels stayed below 1 ppm in the most recent investigations (Table 7).

In one interesting study, the breath was screened of 40 operating room staff members (mixed gender) before operating room duty, 0, 1, 2, and 3 hours after

duty, and before commencing duty on the following day, and of 370 control persons. Staff members exhibited significantly increased sevoflurane levels in exhaled air at all times. The average after duty value was 0.80 ppb (0 hours), decreasing to 0.24, 0.27, 0.28, and 0.11 ppb, respectively, at the other measured time points. Significant concentrations of sevoflurane may be continuously present in persons exposed to sevoflurane on a daily basis (226).

Desflurane
Most of the occupational exposure levels of desflurane listed in Table 8 are below 1 ppm. However, higher levels of desflurane have been measured in postanaesthesia care units, intensive care rooms and recovery rooms (36, 217).

Table 6. Occupational exposure levels of isoflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)	
Intensive care unit	Stationary (0.5 m above patient's head).	15 patients	IR	Area	0.0-0.5 (range of means)	2005	(210)
	Personal (8-h shift)	10 nurses	GC on passive lapel dosimeters	Nurses	0-0.16 (range of means) (8-h TWA)		
Operating and recovery rooms at 9 Swedish hospitals	Personal (8-h shift)	21 samples	GC	Hospital staff	0.04-0.22 (range of means)	2004	(117)
Operating rooms at 14 Danish hospitals	Not described	154 samples	GC	Nurses	0.1-0.3 (range of medians)	2001	(16)
Postanaesthesia care unit (PACU)	Stationary	16 patients	Proton transfer reaction-MS	PACU personnel	0.0095 (mean)	2001	(204)
Operating room	Personal	Not described	Photoacoustic IR	*Without active scavenging, 1996:* Anaesthetists: Workplace A Workplace B Workplace C *With active scavenging, 1997:* Anaesthetists: Workplace A Workplace B Workplace C	Median: 1.7 3.8 25.9 Median: 1.4 1.7 0.3	2000	(242)
Anaesthetic room Operating room Recovery room	Stationary and personal	12 locations [a] 3 locations [a] 6 locations [a]	IR	Area Anaesthetists Recovery room personnel	1 (0-2) 3 (2-3) 1 (0-2)	1999	(101)
Veterinary clinics	Personal and stationary	178 samples 132 samples 229 samples	Single-beam IR	Veterinarians Assistants Area	5.3 ± 2.7 4.7 ± 2.5 4.6 ± 2.2	1999	(141)

Table 6. Occupational exposure levels of isoflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)	
Postanaesthesia care unit (PACU)	Personal	19 patients	IR	Nurses	1.1 ± 0.7	1998	(217)
Recovery room	Stationary	207 patients	Real-time IR	Recovery room personnel	1.4 ± 0.31	1998	(239)
Veterinary clinic	Personal (breathing zone)	Not described	Photoacoustic IR	Veterinary personnel	1.9 ± 2.5 (8-h TWA) 5.3 ± 8.1 (8-h TWA, open mask) Peak values > 300	1998	(105)
Operating room	Personal	15 patients	Photoacoustic IR	Anaesthetists Surgeons Nurses Patients' mouth	0.19 (0.01-0.78) 0.15 (0.01-0.69) 0.27 (0.02-1.57) 0.41 (0.01-3.15)	1998	(102)
Operating room	Personal	10 patients 10 patients	Photoacoustic IR	*Without scavenging:* Anaesthetists Perfusionists *With scavenging:* Anaesthetists Perfusionists	0.31 (0.15-2.08) 0.33 (0.15-2.10) 0.14 (0.13-0.19) 0.16 (0.13-0.20)	1997	(104)
Operating theatre	Stationary	100 + 165 measurements	Photoacoustic IR	Area	0.35 (0.1-6.2)	1997	(159)

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Table 6. Occupational exposure levels of isoflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)
Operating room	Personal (breathing zone) and stationary (3 leakage related locations)	10 patients	Photoacoustic IR	*Laryngeal mask airway anaesthesia:*		1996 (108)
				Anaesthetist	0.50 (0.28-2.28)	
				Surgeon	0.36 (0.20-3.93)	
				Nurse	0.64 (0.22-26.98)	
				Operating area	2.54 (0.22-31.02)	
				Patients' mouth	32.81 (0.46-2150)	
				Anaesthesia machine	0.37 (0.26-3.36)	
		10 patients		*Tracheal tube anaesthesia:*		
				Anaesthetist	0.35 (0.02-0.73)	
				Surgeon	0.29 (0.01-0.50)	
				Nurse	0.31 (0.02-1.07)	
				Operating area	0.32 (0.06-0.76)	
				Patients' mouth	0.89 (0.32-11.42)	
				Anaesthesia machine	0.30 (0.05-0.92)	
Operating room	Personal and stationary: Diffusive sampler Orsa 5 (Dräger) for TWA	Measurements	GC-MS	*Open circuit without scavenging:*		1995 (119)
		16		Anaesthetists and nurses	5.0 ± 0.4	
		5		Ventilator zone	6.3 ± 0.3	
				Open circuit with scavenging:		
		29		Anaesthetists and nurses	1.7 ± 0.2	
		13		Ventilator zone	1.5 ± 0.1	
				Low flow anaesthesia without scavenging:		
		29		Anaesthetists and nurses	2.0 ± 0.3	
		12		Ventilator zone	1.4 ± 0.3	
				Low flow anaesthesia with scavenging:		
		19		Anaesthetists and nurses	0.6 ± 0.04	
		12		Ventilator zone	0.9 ± 0.1	

[a] Measurements took place at 8 different hospitals in anaesthetic rooms, operating theatres and recovery rooms at 12, 3 and 6 different locations, respectively.
GC: gas chromatography, IR: infrared spectrophotometry, MS: mass spectrometry, PACU: postanaesthesia care unit, SD: standard deviation, TWA: time-weighted average.

Table 7. Occupational exposure levels of sevoflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)
Operating room	Personal	78 measurements	GC-MS-SIM	Anaesthetists Surgeons Nurses Auxiliary personnel	0.69 ± 2.23	2005 (3)
Operating room	Stationary Personal	6 hospitals 14 samples	Photoacoustic IR	Area: Central operating theatres Intervention rooms Anaesthetists	0.09-0.21 (range of medians) 0-24.8 (range of medians) 0.19 (median)	2005 (215)
Operating room	Personal	22 samples 15 samples 30 samples 11 samples	GC	Anaesthetists (n=10) Surgeons (n=10) Nurses (n=12) Auxiliary (n=4)	0.65 (median) 0.07 (median) 0.17 (median) 0.04 (median)	2004 (81)
Operating and recovery rooms at 9 Swedish hospitals	Personal (8-h shift)	100 measurements	GC	Hospital staff	0.02-14.4 (range of means)	2004 (117)
Operating room	Personal	5 patients	Photoacoustic IR	Anaesthetists Surgeons Perfusionists	Before surgery (during surgery) 0.03 ± 0.02 (0.04 ± 0.01) 0.16 ± 0.05 (0.14 ± 0.05) Not determ. (0.18 ± 0.03)	2003 (173)
12 operating areas	Personal	61 personnel	GC-MS	Operating room staff members	0.28 (0-1.88, all) 0.41 (0.02-1.88, open circuit) 0.18 (0-1.4, semi closed circuit)	2003 (232)

Table 7. Occupational exposure levels of sevoflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)	
Operating room	Personal	33 patients	Photoacoustic IR	Patients' mouths	*Cuffed oropharyngeal airway:* 8.1 ± 12.2 *Conventional face mask:* 46.5 ± 19.6 *Laryngeal mask airway:* 18.5 ± 25.8	2002	(93)
				Anaesthetists' breathing zone	*Cuffed oropharyngeal airway:* 0.5 ± 0.2 *Conventional face mask:* 2.2 ± 0.9 *Laryngeal mask airway:* 1.0 ± 0.9		
Operating rooms at 14 Danish hospitals	Not described	102 measurements	GC	Nurses	0.1-2 (range of medians)	2001	(16)
Postanaesthesia care unit	Stationary	16 patients	Proton transfer reaction-MS	Area	0.0159 ± SD (not given)	2001	(204)
Operating room	Personal	20 patients, children <10 yrs 5 patients, teenagers ≥10 yrs	Real-time photoacoustic IR	Surgeons Anaesthetists Surgeons Anaesthetists	0.95 ± 1.25 0.87 ± 1.05 0.39 ± 1.20 0.35 ± 1.03	2001	(39)
Operating room -heart-thorax operation	Personal	10 patients	Photoacoustic IR	Anaesthetists Surgeon/nurses	0.01 ± 0.002 0.04 ± 0.01	1999	(36)
-child operation room		20 patients		Anaesthetists Surgeon/nurses	0.99 ± 32 1.10 ± 0.34		
Child clinic		20 patients		Anaesthetists Surgeon/nurses	3.07 ± 0.97 1.93 ± 0.59		
Recovery room		33 patients		Nurses	2.50 ± 0.31		

Table 7. Occupational exposure levels of sevoflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)	
Operating room	Personal	25 patients	Photoacoustic IR	Anaesthetist	0.75 (0.13-1.95)	1997	(109)
				Surgeon	0.59 (0.13-1.80)		
				Auxiliary nurse	0.61 (0.13-3.81)		
Operating room	Personal	20 patients	Photoacoustic IR		*Mask induction:*	1997	(103)
				Anaesthetists	5.4 (3.7-11.9)		
				Nurse	2.9 (2.3-3.6)		
					During maintenance:		
				Anaesthetists	0.6 (0.2-1.6)		
				Nurse	0.5 (0.1-1.2)		
					Total anaesthesia time:		
				Anaesthetists	0.9 (0.4-4.6)		
				Nurse	0.5 (0.3-2.2)		
Operating room	Personal	25 patients	Photoacoustic IR	Anaesthetist	0.75 (0.13-1.95)	1997	(109)
				Surgeon	0.59 (0.13-1.80)		
				Nurse	0.61 (0.13-3.81)		
Anaesthetic room	Stationary	23 patients	Single beam IR	Area	1.1 (0.6-1.7) (8 h-TWA)	1997	(94)
	Personal	6 samples		Anaesthetist	1.2 (0.8-2.1)		

GC: gas chromatography, IR: infrared spectrophotometry, MS: mass spectrometry, SIM: selected-ion monitoring, SD: standard deviation, TWA: time-weighted average.

Table 8. Occupational exposure levels of desflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)	
Operating and recovery rooms at 9 Swedish hospitals	Personal (8-h shift)	7 measurements	GC	Hospital staff	0.04-0.21 (range of means)	2004	(117)
Operating room	Personal	5 patients	Photoacoustic IR		Before surgery (during surgery)	2003	(173)
				Anaesthetists	0.02 ± 0.01 (0.02 ± 0.003)		
				Surgeons	0.21 ± 0.10 (0.62 ± 0.28)		
				Perfusionists	Not determ. (0.82 ± 0.26)		
Operating rooms at 14 Danish hospitals	Not described	20 measurements	GC	Nurses	0.1-1 (range of medians)	2001	(16)
Operating room	Personal	10 adult patients	Photoacoustic IR	Anaesthetists	0.02 ± 0.03	2000	(38)
				Surgeons	0.21 ± 0.24		
		10 child patients		Anaesthetists	0.02 ± 0.03		
				Surgeons	0.30 ± 0.14		
Operating room	Personal		Photoacoustic IR			1999	(36)
-heart-thorax operation		5 patients		Anaesthetists	0.004 ± 0.001		
				Surgeon/nurses	0.18 ± 0.06		
-eye operation		10 patients		Anaesthetists	0.07 ± 0.04		
				Surgeon/nurses	0.48 ± 0.24		
-ear, nose, throat operation		10 patients		Anaesthetists	0.001 ± 0.002		
				Surgeon/nurses	0.02 ± 0.01		
Intensive care room		5 patients		Nurses	6.04 ± 2.80		
Recovery room		34 patients		Nurses	2.20 ± 0.34		
Operating room	Personal	20 child patients	IR	Surgeons	2.8 ± 1.42	1999	(37)
				Anaesthetists	0.43 ± 0.23		
Operating room	Personal	15 patients	Photoacoustic IR	Anaesthetists	0.47 (0.05-4.89)	1998	(102)
				Surgeons	0.43 (0.02-2.51)		
				Nurses	0.48 (0.01-7.53)		
				Patients' mouth	0.76 (0.01-7.82)		

Table 8. Occupational exposure levels of desflurane at different worksites.

Worksite	Sampling	No. of samples/ patients	Measurement	Personnel	Exposure level, ppm median (range), mean ± SD	Publication year (Reference)	
Postanaesthesia care unit	Personal	31 patients	IR	Nurses	2.1 ± 1.2	1998	(217)
Operating room -ear, nose, throat operation	Personal	Patients: 10 children 10 adults	Photoacoustic IR	Surgeons	0.3 ± 0.14 0.4 ± 0.22	1998	(240)
-cleft palate operation		10 children 10 adults			0.6 ± 0.23 0.2 ± 0.24		
Operating room	Personal	10 patients	Photoacoustic IR	*Without scavenging:* Anaesthetists Perfusionists	0.90 (0.56-6.08) 0.93 (0.54-6.10)	1997	(104)
		10 patients		*With scavenging:* Anaesthetists Perfusionists	0.24 (0.09-0.81) 0.26 (0.10-0.79)		

GC: gas chromatography, IR: infrared spectrophotometry, SD: standard deviation.

7. Toxicokinetics

Uptake, distribution, biotransformation, and elimination of the fluranes are primarily studied in patients exposed to high doses of anaesthetics rather than health care personnel exposed to low concentrations during work.

The toxicokinetics of the fluranes has been reviewed by Delgado-Herrera and co-workers (55) and this paper forms the basis for the following sections, supplemented with original studies when relevant.

7.1 Uptake

Fluranes are volatile liquids that are administered to patients in a vapourised state. Therefore, exposure to health care personnel occurs most likely via inhalation of waste gas. Isoflurane, sevoflurane and desflurane are all characterised by having low blood:gas partition coefficients (Table 2). This is associated with a rapid induction of and rapid recovery from anaesthesia, as the fluranes equilibrate rapidly between air and blood in the airways. The uptake of fluranes was evaluated in healthy volunteers 30 minutes after initiation of anaesthesia by measuring the ratio between the alveolar blood concentration of flurane (F_A) and the concentration of inspired flurane (F_I), i.e. F_A/F_I. After 30 minutes, the F_A/F_I ratio was 0.9 (desflurane), 0.85 (sevoflurane) and 0.73 (isoflurane), showing that the uptake of desflurane and sevoflurane is faster than that of isoflurane.

Uptake of isoflurane was estimated in pieces of male rat skin. After exposure to 41 ppm (302 mg/m^3) or 202 ppm (1 496 mg/m^3) isoflurane for 6 hours at 32 ˚C, the partition coefficient for skin:air was 4.5 ± 0.3 (mean ± standard deviation (SD)) irrespective of concentration tested (165).

Dermal uptake of the fluranes may occur to a small extent. The dermal absorption of isoflurane vapour *in vivo* was evaluated in rats (171). Male Fisher 344 rats with a closely clipped fur were exposed whole body to 50 000 ppm isoflurane for 4 hours while breathing fresh air through a latex mask. The flux was calculated to be 0.0096 mg/cm^2/hour, and the permeability constant for isoflurane was estimated to 0.025 ± 0.004 cm/hour. Mean blood concentrations reached 1.8 μg/ml. According to these experiments and calculations, the dermal uptake in rats is only about 0.1% of the inhaled amount upon whole-body exposure to vapours. The dermal uptake is likely to be lower in humans than in rats. In addition, the fluranes have low boiling points (23-59 ˚C) and high volatility, therefore any spill on the skin will quickly evaporate into the air. Thus, although human data are lacking, the potential for significant systemic uptake of fluranes via the skin seems low.

7.2 Distribution

Following uptake, the fluranes are rapidly distributed in the body. The partitioning in human fat from air is much higher (20-50 times, Table 2) than in any other human tissue or blood from air. When the distribution of sevoflurane to different

body compartments (lungs, vessel-rich organs, muscles, fat adjacent to the vessel-rich organs and peripheral fat) was estimated in a physiologically based pharmaco-kinetic model for human distribution of anaesthetics, muscle tissue received the highest volume of fluranes followed by vessel-rich organs (reviewed in Delgado-Herrera *et al* (55)).

Both isoflurane and sevoflurane pass the placenta and transfer from maternal to foetal blood, as assessed during late pregnancy (described below).

Isoflurane

Partial pressures of isoflurane in maternal and umbilical blood were measured in 12 healthy pregnant women undergoing caesarean section. The pregnant women were exposed to 8 000 ppm isoflurane to supplement nitrous oxide/oxygen anaesthesia. Maternal and umbilical blood was sampled for isoflurane measurements at delivery. The maternal mean arterial pressure of isoflurane as a fraction of the inspired partial pressure was 0.44. Umbilical venous partial pressures of isoflurane as a fraction of maternal arterial partial pressures averaged 0.71, indicating that foetal arterial blood contained more than two thirds of the concentration measured in maternal blood. The mean time from induction of anaesthesia until delivery was 11.7 minutes (61). In another study, in which 10 pregnant women were exposed to 6 000 ppm isoflurane, an average maternal blood concentration of 2.4 mg/dl and a foetal umbilical blood concentration of 0.7 mg/dl were reported. Thus the ratio of the anaesthetic concentration between the umbilical vein and maternal arterial blood was 0.27 for an average inhalation time of 8 minutes. This ratio correlated positively with inhalation time, and was calculated to be higher (approximately 0.4) for a delivery time of 13 minutes (213). When the solubility of isoflurane was determined *in vitro* in paired samples of maternal and mixed placental (foetal) blood, the blood:gas partition coefficients were significantly higher in maternal blood than in blood from the foetal compartment (1.51 ± 0.10 SD versus 1.35 ± 0.09 SD), resulting in a foetal:maternal ratio of 0.89. Similar ratios were obtained for plasma and erythrocytes (82).

Fisher *et al* experimentally determined human milk:blood partition constants using a vial equilibrium method, by introducing anaesthetic into the head space of vials with 500 μl fresh blood (n=6) or 200 μl thawed milk (n=25). The partition coefficient for blood:air was 3.40±3.76 SD, and that for milk:air 6.03 ± 4.56 SD. From these values, the milk:blood partition coefficient was calculated to be 1.77. These values were entered into a physiologically based pharmacokinetic lactation model. This model predicted that an infant would ingest 0.37 mg isoflurane through breast milk during a 24-hour period if the woman worked 9 hours at an air concentration of 50 ppm (75).

Sevoflurane

Ten pregnant women were exposed to 8 000 ppm sevoflurane from the time of initiation of caesarean section until delivery. Maternal blood concentration reached 5.2 mg/dl after 13 minutes, compared to an average concentration in foetal umbilical vein blood of 2.0 mg/dl, resulting in a ratio between the

Table 9. Human biotransformation of inhaled anaesthetic doses of the isoflurane, sevoflurane and desflurane (30, 130, 134).

Anaesthetic	Biotransformation %	Major metabolites
Isoflurane	0.2	F⁻, trifluoroacetic acid
Sevoflurane	2-5	F⁻, hexafluoroisopropanol
Desflurane	0.02	F⁻, trifluoroacetic acid

sevoflurane concentration in blood from the umbilical vein and maternal arteries of 0.38 (213).

Desflurane
No publications regarding distribution of desflurane have been identified.

7.3 Biotransformation

All the fluorinated anaesthetics undergo biotransformation to some small extent to inorganic fluoride and fluoroorganic metabolites. Human metabolism of isoflurane, desflurane, and sevoflurane has been characterised both *in vitro* and *in vivo* (135). The metabolism of the fluranes takes place mainly in the liver, and, to a lesser extent in the kidneys and the lungs. The fluranes are metabolised in the liver by cytochrome P450, especially by the isoenzyme CYP2E1, and the oxidative route is dominant. The induction of liver microsomal enzymes, by e.g. phenytoin, isoniazid, phenobarbital, alcohol and halothane, may increase the metabolism of the fluranes (190). Smoking does not seem to influence fluoride formation from the metabolism of sevoflurane (153). Table 9 lists the degree of biotransformation and major metabolites. It is apparent that the rate of metabolism is 10-100-fold greater for sevoflurane than for isoflurane and desflurane.

The metabolism of isoflurane and desflurane is similar; they are both metabolised to inorganic halide ion (chloride or fluoride) and trifluoroacetic acid (TFA). The simplified metabolic pathway for isoflurane and desflurane is shown in Figure 3.

The main pathway of metabolism of sevoflurane is depicted in Figure 4. Sevoflurane is metabolised in the liver by CYP2E1 to a transient intermediate that decomposes to equimolar concentrations of HFIP and inorganic fluoride ions.
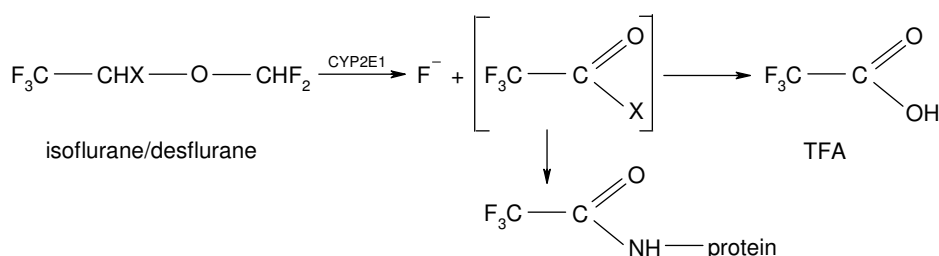

Figure 3. Metabolism of isoflurane (X=Cl) and desflurane (X=F) (modified from (130, 135). TFA: trifluoroacetic acid.

F$_3$C —— CH ⟨ O —— CHF$_2$ / CF$_3$ ⟶[CYP2E1] F$^-$ + F$_3$C —— CH ⟨ OH / CF$_3$ ⟶ HFIP-glucoronide

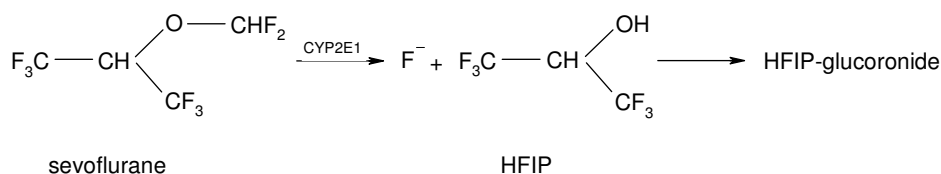sevoflurane   HFIP

Figure 4. Metabolism of sevoflurane (135). HFIP: hexafluoroisopropanol.

HFIP accounts for more than 80% of the organic fluorinated metabolites. HFIP conjugates rapidly with glucuronic acid, forming HFIP-glucoronide (134). The metabolism of sevoflurane is dose-dependent and related also to the duration of anaesthesia. In rats exposed to sevoflurane, serum levels and urinary excretion of fluoride and HFIP increased linearly up to 1.25%, but did not increase further above this exposure level. In human patients, peak plasma inorganic fluoride ion concentrations have been shown to correlate with the duration of sevoflurane administration. However, no difference in the post-anaesthetic decrease in plasma fluoride was seen in patients anaesthetised for less than 7 hours, compared to those anaesthetised for more than 7 hours (55).

7.4 Excretion

Exhalation is the major elimination route for the fluranes, due to low metabolism and low blood solubility, as shown for isoflurane (43). Percutaneous loss estimated in human volunteers amounted to less than 0.4% for all three fluranes (73, 158). A minor fraction of the fluranes is excreted unchanged in urine.

Approximately 90 minutes after cessation of anaesthesia, the ratio between the alveolar blood concentration of flurane (F_A) and the concentration of inspired flurane (F_I), i.e. F_A/F_I had approached 0.01 or less for the three fluranes (55). The elimination of isoflurane has been described to occur in three phases, i.e. a short phase with a half-time of 2 minutes, where the elimination is determined by the speed of elimination over the alveolar membrane. A middle phase with a half-time of 19 minutes can be ascribed to the elimination of isoflurane from the inner organs. The half-time of the long phase is 233 minutes and relates to elimination of isoflurane from muscle and adipose tissue (56).

The sevoflurane metabolite HFIP-glucoronide is excreted in the urine with a half-time of approximately 14 hours (113).

Isoflurane does not seem to accumulate in the body during the working week (159).

8. Biological monitoring

Isoflurane
Several studies indicate that the urinary isoflurane concentration might be used as an appropriate biological exposure index. Thus, the concentration of isoflurane

was measured in the ambient atmosphere in 11 operating theatres at 5 hospitals in Italy and in the urine of 45 of the exposed health care personnel (anaesthetists, surgeons, and nurses). A significant correlation was found between the concentrations of isoflurane in urine and air (r=0.90) (120). In a follow-up study, the measurements were extended to 362 subjects, using personal passive samplers and covering 190 operating theatres in 41 hospitals. Again, significant correlations were found between urine and air levels (121).

Imberti *et al* investigated isoflurane exposure during different exposure scenarios as described in Table 6, i.e. during open circuit and low flow anaesthesia. The effects of active and passive scavenging were also investigated, ensuing large variations in air concentrations. The study involved anaesthetists and nurses during routine activity. A highly significant correlation was found between breathing zone (time-weighted average, TWA) and urinary concentrations of isoflurane when measured after 3 hours of continuous exposure (119).

Finally, exposure to isoflurane was monitored in 112 operating theatre workers. Urine samples were collected for each subject after the end of the shift on the first and after the last day of a 4-day working week. Time integrated exposure levels during the shift were monitored by means of stationary sampling, with the instrument placed so as to collect the air in a standardised position, close to the patient's head and at the height corresponding to the operator's airway. End of shift urinary isoflurane was 0.7 µg/l (95th percentile 2.6, range 0-4.7) on the first day and 0.8 µg/l (95th percentile 2.0, range 0-5.6) on the last. Atmospheric concentrations in operating theatres ranged from 0.1 to 6.2 ppm isoflurane, with average values of approximately 0.35 ppm. The correlation between atmospheric and biological indicators was not significant. The authors explained this finding in that stationary sampling generally shows higher levels than those that are in fact absorbed by the organism of each individual and that can be measured by diffusive passive sampling (159).

The relationship between isoflurane air concentrations in operating rooms and the corresponding isoflurane concentrations in the exhaled air of the operating personnel at the end of the exposure has also been investigated. Isoflurane was retained in an adsorbent cartridge and after thermal desorption the concentration was estimated by GC. A close relationship was found between the log of exposure dose (expressed as TWA exposure multiplied by exposure time) and the log of concentration in exhaled air (r=0.79). The good correlation between ambient air and exhaled air isoflurane concentrations allowed the biological exposure limits to be calculated. Thus, the biological concentration in mixed exhaled air at end of exposure was 0.52 ppm for exposure to 2 ppm and 1.77 ppm for exposure to 10 ppm (192). No studies on biomonitoring of the isoflurane metabolite TFA were identified.

Sevoflurane
Thirteen men and 23 women occupationally exposed to volatile anaesthetics in paediatric operating rooms were studied during a 2-week period. Sevoflurane

inhalation exposure was monitored by personal passive samplers and post-shift urine samples were collected after 1.75-6 hours morning exposure and analysed by headspace GC-MS. Median sevoflurane air values were 0.13 ppm (range 0.03-18.8 ppm; n=78), urinary sevoflurane levels 0.6 µg/l (not detectable (ND)-18.5 µg/l; n=76) and total urinary HFIP levels 0.49 mg/l (ND-6 830 mg/l; n=75). The low detection limit for urinary sevoflurane (0.03 µg/l), allowed quantitation of all but one sample, whereas the HFIP content was below the detection limit in more than 25% the of urine samples. Urinary sevoflurane correlated well with breathing zone data (r^2=0.697). The correlation was lower for total urinary HFIP that appeared to be influenced also by smoking habits. The biological exposure values corresponding to 0.5 and 2 ppm sevoflurane in air were 1.4 and 3.9 µg/l urine, respectively (calculated by linear regression). For HFIP, these values were 0.82 and 2.66 mg/l urine. In summary, urinary unmodified sevoflurane seemed to be a more sensitive and reliable biomarker of short-term exposure to sevoflurane compared to total urinary HFIP (which appeared to be influenced by physiological and/or genetic individual traits and seemed to provide an estimate of integrated exposure) (3).

Sevoflurane in urine and breathing area were monitored in 124 subjects in 11 operating theatres. Passive personal samplers were collected after 2.5-7 hours of exposure, at the same time as post-shift urinary samples. A static headspace sampler coupled with GC-MS was used for analytical determinations (the limit of detection was 0.1 µg/l urine and 50 ppb). Median (range) post-shift urinary and air values for sevoflurane were 1.2 µg/l (0.1-5.0) and 0.4 ppm (0.05-3.0). Urinary levels closely correlated with air levels (r^2=0.754). The biological exposure value corresponding to exposure to 2 ppm sevoflurane for 8 hours, calculated as means of regression slope and intercept, was 3.6 µg/l urine (2).

Mean individual workplace air exposures to sevoflurane and urinary sevoflurane were monitored in 36 subjects working in two paediatric operating rooms. Air and urinary levels were significantly greater in anaesthetists compared to other groups of hospital staff, with median values of 0.65 ppm (interquartile range 1.36; 95th percentile 4.36) for breathing zone sevoflurane and 2.1 µg/l (interquartile range 2.6; 95th percentile 7.6) for urinary sevoflurane. Log-transformed urinary concentrations appeared closely and significantly related to the breathing zone concentrations for the anaesthetists and the nurses, but the relationship was weaker and non-significant for the surgeons and the auxiliary personnel (81).

Desflurane
No studies on biomonitoring of desflurane or its metabolite TFA were identified.

Conclusion
Both urinary and exhaled concentrations of unmodified isoflurane seem appropriate for biological monitoring.

Unmodified sevoflurane in urine may be used for biological monitoring of occupational exposure, whereas monitoring of urinary HFIP seems less appropriate.

No studies were found for desflurane.

9. Mechanisms of toxicity

Neurotoxicity
High doses of isoflurane, sevoflurane and desflurane induce anaesthesia, which account for the widespread use of these compounds. At supraclinical doses, the fluranes cause respiratory depression and death. The exact mechanisms for the anaesthetic effects remain to be identified (87). General anaesthetics were once thought to be drugs without receptors, but probably volatile anaesthetics exert very specific actions at the molecular level with protein receptors as primary targets. The current paradigm of mechanistic investigations focuses heavily on γ-amino-butyric acid A (GABA$_A$) receptors and anaesthetics as allosteric modulators of ligand-gated ion channels. Furthermore, lipids are crucial in neural structure and function and are therefore viable subjects for further research into general anaesthetic mechanism (reviewed by Hemmings *et al* and Mashour *et al* (100, 163)).

Nephrotoxicity
Nephrotoxicity after anaesthesia with sevoflurane has been hypothesised to occur due to inorganic fluoride liberated during metabolism of the anaesthetic. Nephro-toxicity due to liberation of inorganic fluoride is a clinical concern from the time when anaesthesia with methoxyflurane was shown to cause deterioration in renal function. The resulting plasma peak fluoride concentration due to liberation from methoxyflurane was noticed to correlate with the degree of renal injury, with a critical threshold around 50 µM inorganic fluoride. The general structure of the three fluranes in this document resembles that of methoxyflurane. For sevoflurane, serum inorganic fluoride concentrations reach only about 1/3 to 1/4 of that after anaesthesia with methoxyflurane (reviewed by Reiche and Conzen) (200)). Serum levels of fluoride after sevoflurane anaesthesia may exceed 50 µM in approximate-ly 8% of the cases. However, exposure to sevoflurane at anaesthetic dose levels in a large number of patients has not been associated with impairment of renal function. Furthermore, there is no evidence for exacerbation of pre-existing renal or hepatic dysfunction in adults or children by sevoflurane (reviewed in (55)). Possibly the differences in nephrotoxicity between methoxyflurane and sevo-flurane are based on differences in local kidney activity of cytochrome P450 subtypes, which is 3- to 10-fold higher for methoxyflurane than for sevoflurane. Sevoflurane is metabolised to a much higher extent than isoflurane and desflurane (Table 9). The risk for nephrotoxicity due to high fluoride ions would thus be expected to be much higher for sevoflurane than for isoflurane and desflurane, reviewed in (200)[1].

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[1] Absorbents can degrade sevoflurane and produce the metabolite FDVE, especially in a high respiratory gas temperature as in low-flow technique. In rodent studies, FDVE has been associated with nephrotoxicity, but there is no evidence for nephrotoxicity in surgical patients (200).

Cardiovascular effects

All three fluranes decrease blood pressure as a result of diminished cardiac output and vascular resistance. This is due to direct effects of the anaesthetics on the heart and vascular smooth muscle and to indirect effects of the anaesthetics on the autonomic nervous system. The increase in heart rate may be a compensatory response, to maintain perfusion even if blood pressure decreases. It occurs to a lesser degree with sevoflurane compared to isoflurane and desflurane (211). For sevoflurane it has been shown that with increasing exposure concentrations, cardiac sympathetic nerve traffic decreases whereas parasympathetic traffic remains unchanged. This might in part explain the absence of tachycardia with increasing doses of sevoflurane. In contrast, sympathetic nerve traffic increases with administration of desflurane at higher dose levels, reviewed in (63).

Hepatotoxicity

The proposed mechanisms for hepatotoxicity caused by inhaled halogenated anaesthetics have been reviewed (55, 131, 200). These compounds have been associated with two different forms of hepatotoxicity, metabolic and immune-mediated, both described in detail for halothane.

The metabolic form is the milder of the two. It is clinically detected as a transient elevation of liver enzymes and altered cellular integrity, as observed by electron microscopy. For halothane, the lesion results from intracellular degradation of the compound via its anaerobic and aerobic pathways in combination with local hypoxia caused by an alteration of the hepatic oxygen demand and supply relationship. This form can be observed in about 20% of halothane-treated patients. Laboratory studies indicate that this effect is most pronounced after halothane exposure, and less so after isoflurane and sevoflurane exposure. Further, this type of injury is considered concentration- and/or dose-dependent (200).

The immune-mediated form is potentially life-threatening. It is not caused by the parent compounds, but rather by metabolites produced by cytochrome P450-mediated biotransformation. Metabolism of the halogenated anaesthetics generates reactive intermediates. These intermediates may in turn bind covalently to hepatic proteins, resulting in tissue acetylation by trifluoroacetyl (CF_3CO^-) as the first step in pathogenesis. The second step involves formation of antibodies directed towards these acetylated neo-antigens. Thus, the suggested mechanism for this type of hepatotoxicity is an autoimmune response directed against hepatic proteins that have been altered by the covalent binding of metabolites of the anaesthetics. Immune-mediated hepatotoxicity occurs in a small number of patients, i.e. the incidence for halothane is 1:35 000. Occupational exposure to halothane has also been shown to induce hepatotoxicity. Very few cases of hepatotoxicity have been reported in patients after anaesthesia with isoflurane and desflurane. As investigated at anaesthetic dose levels in a large number of patients, no evidence suggests that sevoflurane impairs liver function. Trifluoroacetyl is formed during the metabolism of isoflurane and desflurane, but not sevoflurane. Further, the

amount of acetylated hepatic proteins correlates with the well-documented relative rates of metabolism of halothane, isoflurane or desflurane (Table 9). The rareness of hepatotoxicity after isoflurane and desflurane anaesthesia is presumably explained by the very limited metabolism of these substances. Sevoflurane is metabolised by a distinctly different pathway compared to the halogenated anaesthetics with a methyl-ethyl structure, i.e. halothane, enflurane, isoflurane and desflurane. This may explain the lack of immune-mediated hepatoxicity following anaesthesia with sevoflurane (30, 55, 131, 200).

Genotoxicity
Some studies indicate that the fluranes cause genotoxic effects. The mechanisms by which the fluranes might induce DNA damage are not well understood. A mechanism for the genotoxic activity could be the direct reaction of the parent anaesthetics with DNA (9). If isoflurane reacts with DNA directly, the most probable alkaline-labile modification is an alkylation at the N7 position of purines. Another mechanism of genotoxicity could be the formation of reactive metabolites. The sevoflurane degradation product formed in the anaesthesia machines, FDVE, is an alkylating agent, and therefore a potential genotoxin (68). No empirical studies were found.

Developmental toxicity
An increased neuronal death has been associated with exposure to isoflurane during the period of brain growth spurt (the period of synaptogenesis) in juvenile rats. This probably stems from induction of widespread neuronal apoptosis. It has been proposed that isoflurane promotes the spontaneous apoptotic neuro-degenerative process that occurs naturally in the normal developing brain (124, 249).

10. Effects in animals and *in vitro* studies

Animal studies described below that are of relevance for dose-effect and dose-response relationships are summarised in Tables 13-15 in Chapter 12. For the single exposure studies, only exposure levels below 0.8 MAC (human) are included in the tables.

10.1 Irritation and sensitisation

Isoflurane
No appropriate investigations of airway irritation were located.

Tissue irritation was investigated by injecting adult Lewis rats (n=6-24, mixed sex) intraperitoneally through the midline into the lower abdomen with isoflurane (0.5, 1.0 or 1.5 ml/kg). Necropsies two weeks later revealed fibrosis of surfaces of liver, spleen, omentum and diaphragm surfaces with rounding and fusion of liver

lobes, and, occasionally, adhesions of these organs (156). The results indicate that isoflurane is irritating at high dose levels.

Sevoflurane
Lewis rats (n=6-24, mixed sex) were injected intraperitoneally through the mid-line into the lower abdomen with 0.5-4 ml/kg sevoflurane, in 0.5 ml increments. At necropsy two weeks later, no visible lesions were detected (156).

No signs of airway irritation, such as coughing, were registered in six horses during determination of the MAC for sevoflurane. Sevoflurane was given through a face mask and the responses of the horses to painful stimuli were examined while increasing or decreasing the inspiratory sevoflurane concentration in 2 000 ppm steps from 26 000 ppm. In reality, the examined inspiratory concentrations varied between 21 000 and 28 000 ppm (4).

The results indicate that sevoflurane is not irritating even at high exposure levels.

Desflurane
No animal studies on irritation and sensitisation were identified.

10.2 Effects of single exposure

10.2.1 Lethality
Reported lethal concentrations for 50% of the exposed animals at single inhalation exposures (LC_{50}) of 3-4 hours duration are given in Table 10. Comparison of LC_{50}s for a 3-hour exposure period indicates that sevoflurane possesses approximately half the acute toxicity of isoflurane, comparable to the difference in MAC-values for these compounds (Table 4). No LC_{50}s were identified for desflurane.

Table 10. Lethal concentrations for 50% of the exposed animals at single inhalation exposure (LC_{50}).

Anaesthetic/ species	Exposure duration (hours)	LC_{50} (ppm)	Reference
Isoflurane			
Rat	0.5	125 200	(138)
Rat/mouse	1	58 000-83 000	(160)
Rat	3	15 300	(209)
Mouse	3	16 800	(209)
Sevoflurane			
Rat	3	28 800	(209)
Mouse	3	28 300	(209)
Desflurane	No data available		

10.2.2 Central nervous system effects
Comparative studies
Amnesic potency of isoflurane, sevoflurane and desflurane were investigated in
adult male Sprague Dawley rats. During exposure to either air or subanaesthetic
concentrations of isoflurane (530, 1 160, 2 010, or 3 020 ppm), sevoflurane (1 050,
2 160, 3 080, or 4 110 ppm) or desflurane (4 400, 10 130, or 20 200 ppm), rats
were trained in the inhibitory avoidance procedure (n=4 - 21). In a device with
two compartments, the rat was placed into the bright compartment. After three
minutes, a door was opened into the dark compartment. Rats instinctively prefer
dark environments, but as the rat stepped into the darkness, a foot shock was
delivered until the animal escaped back into the bright starting compartment.
Learning was considered to have occurred when the animal avoided going back to
the dark-shock compartment for more than 100 consecutive seconds. Memory was
tested 24 hours later in the same device. Anaesthetic effects on pain thresholds
were separately determined by assessment of tail shock pain sensitivity threshold.
For isoflurane, the number of shocks to learning the 100-second criterion was
increased at the two highest exposure levels, but this was not significant. Memory
retention latency was significantly decreased, consistent with amnesia at 2 010
ppm, and even more so at 3 020 ppm isoflurane. The tail flinch threshold was
significantly increased at 2 010 ppm, indicating an analgesic response. For
sevoflurane, there was a significant increase in the trials to criteria at 3 080 ppm.
Memory retention latency was numerically decreased at 2 160 ppm and signifi-
cantly decreased at the two higher exposure levels. The flinch threshold for tail
withdrawal increased significantly from 1 050 ppm. For desflurane, significant
inhibition of task acquisition was observed at 10 130 ppm, increasing further at the
highest exposure level. Retention latency was decreased already at the lowest ex-
posure level, i.e. 4 400 ppm, and further decreases were observed at the two higher
levels. An analgesic response was only significant at the highest concentration.
Amnesic potency and oil:gas partition coefficients generally correlated well (6).

   In the same device, it was investigated whether isoflurane, sevoflurane or
desflurane might enhance aversive memory. Male Sprague Dawley rats (n=22)
were exposed to air, 1 200 ppm isoflurane, 1 100 ppm sevoflurane, or 7 700 ppm
desflurane during inhibitory avoidance training as described above, although the
animals in this study were removed immediately after delivery of one shock in the
dark compartment. Memory was assessed after 24 hours. It took significantly
longer time for the animals to cross over into the dark side of the apparatus when
they had been exposed to sevoflurane during training, whereas no effect of iso-
flurane and desflurane was observed. These data indicate that sevoflurane may
enhance aversive memory formation in the rat (7).

Isoflurane
The analgesic potency of isoflurane was measured in male Sprague Dawley rats
(n=6-9) by means of the latency to withdraw the hind paw when exposed to heat.
After baseline measurements, isoflurane was delivered in a stepwise manner, each

step held at 30-40 minutes. At 1 145 ppm, isoflurane was hyperalgesic (anti-analgesic), as latency to remove the paw in response to heat was decreased compared to controls. At the higher exposure levels of 2 920 and 5 840 ppm, isoflurane was analgesic (255).

The effects of isoflurane on learning of the rabbit nictitating membrane responses were studied. Classical conditioning of the nictitating membrane responses was accomplished by presenting a 400-millisecond tone conditioned stimulus before the presentation of a 100-millisecond shock unconditioned stimulus over 6 daily training sessions. The percentages of conditioned responses were calculated for New Zealand albino rabbits treated with 0, 2 000, 4 000, or 8 000 ppm isoflurane (n=7-13, sex not specified) for 20 minutes. Isoflurane suppressed acquisition of the conditioned response dose-dependently. Animals in the low-exposure group learned the task slower than the controls, 4 000 ppm isoflurane allowed some learning, whereas 8 000 ppm isoflurane completely abolished the acquisition of the conditioned response (70).

Male ddN mice (n=15-20) were trained to escape an aversive electric foot shock as an unconditioned stimulus within 3 seconds after being exposed to light and a buzzer as a conditioned stimulus. Immediately after training, the animals were exposed to isoflurane for 2 hours. After a period of recovery, the animals were retested on the avoidance task (second session). Two experiments were performed. In the first experiment, isoflurane concentrations were 2 960, 6 020, and 12 040 ppm, and the intersession interval was 2 hours and 30 minutes (139). In the second experiment, the interval was 24 hours, and the exposure levels of isoflurane were 2 960 and 12 950 ppm (140). In both studies, performance was increased more during retest if the animals had been exposed to 2 960 ppm iso-flurane immediately after the training session compared to the control condition with no exposure. Performance neither increased nor decreased at the higher exposure levels.

Male Sprague Dawley rats (n=8-16) were trained to fear tone by applying three (three-trial) or one (one-trial) tone-shock pairs while breathing 3 700, 5 700 or 7 500 ppm isoflurane for 30 minutes. Groups of rats were similarly trained to fear context while breathing isoflurane by applying shocks (without tones) in a distinctive environment. The next day, memory of the conditioned stimuli was determined by presenting the tone or context (without shock) and measuring the proportion of time each rat froze, i.e. appeared immobile. Isoflurane provided dose-dependent amnesia for classic fear conditioning with clear effects at exposure levels of 3 700 ppm and above (59).

Sevoflurane
Tight-seal whole-cell recordings were made from CA1 pyramidal cells in hippocampal slices prepared from adult male ddY mice (n=5-6). The effects of 0.05-0.07 and 0.5 mM sevoflurane (corresponding to 900-1 200 and 9 000 ppm, respectively) on the glutamatergic excitatory postsynaptic currents (EPSCs) were investigated. For the lowest dose level, also extracellular recordings of field

excitatory postsynaptic potential (fEPSP) and population spike (PS) were made, and long-term potentiation effects on the fEPSP slope and PS amplitude were analysed. Sevoflurane at 0.5 mM reversibly suppressed the amplitude of EPSCs with an increase in the paired-pulse facilitation ratio, whereas 0.05 mM sevoflurane reversibly increased the EPSC amplitudes without appreciable changes in the ratio. The 0.07 mM sevoflurane dose also showed facilitatory influences on long-term potentiation of PS amplitude but not on long-term potentiation of the fEPSP slope. The observations suggest that sevoflurane at low subanaesthetic concentrations postsynaptically enhances excitatory synaptic transmission, whereas sevoflurane at higher concentrations presynaptically inhibits excitatory synaptic transmission in the hippocampal CA1 region (187).

Desflurane
No studies regarding neurotoxicity in animals were found for desflurane besides the comparative studies described at the beginning of this section.

Conclusions
Several studies have investigated neurofunction after acute exposure to isoflurane at subanaesthetic levels. In rats, pain sensitivity during isoflurane exposure was unaffected at the lowest level tested, i.e. 530 ppm (no observed adverse effect level, NOAEL) (6), but at 1 145 isoflurane exerted antianalgesic effects. At higher exposure levels (at and above 2 920 ppm) isoflurane induced analgesia (255). Cognitive function has been studied at exposure levels ranging from 530 to 8 000 ppm. Learning and memory during avoidance training was unaffected by isoflurane at exposure levels up to 1 160 ppm in rats, whereas performance was impaired at 2 010 ppm (6). At 2 000 ppm, also classical conditioning of the nictitating membrane responses was affected in rabbits (70). Even higher exposure concentrations, have been associated with increased neurodegeneration in foetal guinea pigs and neonatal rats and mice (124, 125, 205), indicating that the developing nervous system may be sensitive to isoflurane exposure. The latter studies are described in Section 10.6.

Sevoflurane induced analgesia and increased memory of an aversive event in rats at the lowest exposure levels studied, i.e. 1 050 and 1 100 ppm, respectively (6, 7). At higher levels, impairment of cognitive function and increased indices of anxiety are apparent (Table 14). Thus, for sevoflurane, a NOAEL for neurofunction effects cannot be identified.

Neurotoxicity of desflurane has been the topic of interest in two studies with single exposure and one short-term study (Section 10.3), all in rats. Exposure levels ranging from 4 400 ppm to 20 200 ppm were associated with impaired neurofunction in tests of cognitive function and anxiety tests (6, 188). No effect on aversive memory was observed at 7 700 ppm, the only level tested for this endpoint (7). Thus, also for desflurane, a NOAEL cannot be identified for nervous system effects.

10.2.3 Nephrotoxicity
Isoflurane
The nephrotoxic potential of a 4-hour anaesthesia with 14 000 ppm isoflurane was investigated in obese and non-obese male Fischer 344 rats (n=7-9; one year of age). Weight-paired rats received either a regular or a high fat diet for 16 weeks and gained 20% respectively 45% in weight. Serum fluoride levels increased significantly to the same extent in both groups of rats 4 hours after anaesthesia compared to pre-anaesthetic levels. Whereas fluoride levels peaked at this time point in non-obese rats, levels continued to increase in obese rats until measured again 24 hours after termination of exposure. At 72 hours, plasma fluoride levels had returned to baseline values, but the urinary excretion rate was still slightly elevated. Urinary osmolality decreased slightly, but significantly, following anaesthesia in both groups, and creatinine and urea nitrogen clearance were decreased in obese rats 1 and 3 days after anaesthesia. Biomarkers of kidney function were measured 24 hours post anaesthesia. In non-obese rats, both aspartate and alanine aminotransferases were increased, but in obese rats only the latter increased (203).

No studies on subanaesthetic exposure levels were identified.

Sevoflurane and desflurane
No studies on nephrotoxicity were identified.

10.2.4 Cardiovascular effects
Comparative studies
Direct myocardial effects of isoflurane, sevoflurane and desflurane were studied on isolated human right atrial trabeculae obtained from patients undergoing coronary bypass surgery. At clinically relevant concentrations, the agents did not modify isometric relaxation (95).

Isoflurane
Fourteen anaesthetised mongrel dogs (sex not specified) received, in random order, 0, 2 500 or 5 000 ppm end-tidal isoflurane in 40% oxygen. Forty minutes of equilibration was allowed for each intervention before measurements of a range of cardiovascular and ventilatory parameters were performed. Gas exchange was assessed by blood gas analysis and by estimating ventilation and perfusion mis-match using the multiple inert gas elimination technique. After administration at each exposure level, dogs were ventilated without isoflurane for at least 30 minutes, while values returned to baseline. Cardiac output and systemic vascular resistance decreased at 5 000 ppm isoflurane but not at 2 500 ppm (195).

The effects of isoflurane on the cardiovascular system were investigated in New Zealand white male rabbits. Electrocardiogram, mean arterial pressure, and heart rate were obtained by telemetry in rabbits in the conscious, unsedated state and at the end of 30 minutes of isoflurane anaesthesia, at exposure levels of 0, 9 000, 18 000, and 27 000 ppm, administered in random order (n=10). Isoflurane increased the heart rate at all exposure levels and decreased systolic, diastolic, and

mean arterial blood pressure at the two highest exposures. No significant changes were observed for plasma norepinephrine and epinephrine levels. The analysis of spectral components of heart rate variability and baroreflex function indicated that isoflurane induced a marked reduction in the low- and high-frequency spectral power of heart rate variability independent of isoflurane concentrations, and reduced baroreflex sensitivity at all exposure levels. In vagotomised anaesthetised rabbits, heart rate and blood pressure values were not different from those obtained in intact anaesthetised animals at equal concentrations of isoflurane (161).

Sevoflurane and desflurane
No studies were found besides the comparative study described at the beginning of this section.

10.2.5 Hepatotoxicity
No studies at subanaesthetic exposure levels were identified.

10.2.6 Lung toxicity
Isoflurane
Lung clearance of the radionuclide technetium-99m hexamethyl propylene amine oxime (Tc-99m HMPAO) was studied in male New Zealand rabbits (n=6). receiving either 0 or 15 000-25 000 ppm isoflurane. Following a 10-minute stabilisation period, rabbits were administered the radionuclide intravenously and dynamic images were acquired for the following 10 minutes. The imaging protocol was repeated 3 days later, apart from the exposure to isoflurane. After each imaging session, lung biopsy was performed in two rabbits from each group. There was no significant difference between control and isoflurane groups with respect to radionuclide lung clearance. Pathological evaluation demonstrated minimal changes in isoflurane exposed animals compared to controls (90).

Sevoflurane
Sixteen pigs (mixed sex) were randomly selected to receive either thiopentone infusion or sevoflurane (n=8) at 4 000 ppm inspiratory concentration in air for 6 hours. Tissue samples from the lungs were obtained at the end of the experiment for histopathological light and electron microscopy. Pulmonary haemodynamics were comparable in both groups. Light microscopy showed no difference between the groups in the amount of alveolar macrophages, red blood cells, or oedema. Electron microscopy showed minor changes such as moderate local swelling of alveolar epithelium in both study groups, compared to naive controls. Alveolar type II cells were ultrastructurally unaltered in both study groups (228). Arterial blood samples were taken 1, 2, 4, and 6 hours after stabilisation of the exposure air concentration. At the end of the exposure period, bronchoalveolar lavage samples were collected. Compared to baseline controls, significant increases in bronchoalveolar lavage leukotriene C_4, thromboxane B_2, and nitrate levels were observed after sevoflurane anaesthesia. Also a significant decrease in total blood leukocyte count was observed (229).

Desflurane
No studies on lung toxicity were identified.

10.2.7 Other studies
Isoflurane
Anaesthetised (pentobarbital), ventilated Sprague Dawley rats (n=7) received either no treatment, lipopolysaccharide only (5 mg/kg, intravenous), or isoflurane (13 000 ppm, inhalation) 15 minutes after administration of lipopolysaccharide. After 4 hours of endotoxaemia, inhalation of isoflurane was found to have attenuated the release of tumour necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β) as compared to the lipopolysaccharide group, while interleukin-6 (IL-6) and interleukin-10 (IL-10) levels were not significantly altered. Nitrite release was significantly increased in the isoflurane group as compared to the lipopolysaccharide group. Thus, inhalation of isoflurane at anaesthetic dose levels decreased the response to inflammatory stimuli by attenuating the systemic release of proinflammatory cytokines (76).

The effects of air or 22 500 ppm isoflurane on cilia beat frequency were investigated in human nasal epithelial brushings from healthy adult and non-smoking patients (n=6). The epithelial brushings were obtained by passing a bronchoscopy brush over the inferior nasal turbinates. Cilia beat frequency was observed before and 1, 2, and 3 hours after initiation of exposure. No difference was observed at 1 hour, but cilia beat frequency of the samples exposed to isoflurane significantly decreased (by approximately 10%) at 2 and 3 hours, compared to exposure to air alone. The cilia beat frequency had returned to baseline values after 60 minutes of air washout after exposure (198). With the same experimental set-up, recovery after air washout was investigated after exposure to 22 500 ppm isoflurane for 1 hour. Cilia beat frequency had recovered completely after 60, but not 30 minutes (199).

Female New Zealand white rabbits (n=10) were exposed to 25 000 ppm iso-flurane in oxygen for 30 minutes. Blood samples were obtained before anaesthetic induction, and at 1, 10, 30, 60, 120 minutes and 24, 48 and 72 hours after endo-tracheal intubation. Serum corticosterone and serotonin levels increased shortly after administration was initiated, but adenocorticotropic hormone concentrations were largely unaffected. Shortly after isoflurane administration, also serum glucose, aspartate aminotransferase, blood urea nitrogen, and creatinine levels increased. Values were normalised 24 hour later. Alanine aminotransferase and alkaline phosphatase were unaffected throughout the period of monitoring (83).

Sevoflurane
Adult male CBi mice were anaesthetised with 30 000 sevoflurane in oxygen for 40 minutes, and non-treated animals served as controls (n=12). Sevoflurane diminished the number of peripheral blood lymphocytes and splenic B-cell counts, enhancing CD4+ lymphocytes in the spleen. The *in vitro* functionality of macrophages and the mitogen-induced lymphoproliferative response were preserved, while the *in vivo* immune response to sheep red blood cells was enhanced in treated animals.

Microscopic studies revealed conserved architecture of the spleen, thymus, lymph node, liver and kidney, and there were no differences in serum parameters of hepatic and renal functions between treated and control groups (194).

Anaesthetised (pentobarbital), ventilated male Sprague Dawley rats (n=6) received either no treatment, lipopolysaccharide only (5 mg/kg, intravenous), or lipopolysaccharide and sevoflurane inhalation (20 000 ppm) initiated 15 minutes after induction of endotoxaemia. After 4 hours of endotoxaemia, the sevoflurane group showed significantly attenuated plasma levels of TNFα and IL-1β as compared with the lipopolysaccharide only group. Nitrite release from alveolar macrophages was significantly suppressed by sevoflurane. Sevoflurane attenuated the inflammatory response during endotoxaemia *in vivo* (112).

Desflurane
Platelet-rich plasma was exposed to 10 000 ppm of desflurane or clean air and stimulated by platelet agonists adenosine diphosphate and collagen. Response was measured by Born aggregometry and percentage of CD62P (P-selectin)-positive cells. Aggregation in response to both stimulants was significantly reduced in platelets exposed to desflurane. CD62P expression before and after stimulation with receptor agonists was not significantly different in platelets exposed to desflurane as compared to platelets exposed to air. Thus, desflurane impaired platelet aggregation without affecting alpha-degranulation, indicating that the anaesthetic may impair platelet thromboxane receptor signalling (27).

The effects of exposure to 12 000 ppm desflurane for 6 hours on caspase activation, amyloid precursor protein processing and amyloid-ss protein generation were investigated in H4 human neuroglioma cells (H4 naive cells) as well as those overexpressing amyloid precursor protein (H4-APP cells). Desflurane alone did not affect either endpoint, but in combination with mild hypoxia (18% oxygen) it induced caspase-3 activation, altered amyloid precursor protein processing, and increased amyloid-ss protein generation in H4-APP cells (254).

Anaesthetised (pentobarbital), ventilated male Sprague Dawley rats (n=6) received either no treatment, lipopolysaccharide only (5 mg/kg, intravenous), or continuous inhalation of desflurane (60 000 ppm) just before and during endo-toxaemia with lipopolysaccharide. After 4 hours of endotoxaemia, the desflurane group displayed significantly decreased release of the proinflammatory cytokines TNFα and IL-1β in plasma and in bronchoalveolar lavage fluid as compared to the lipopolysaccharide only group. Release of IL-6 was unaffected. Within the lung, the nitric oxide release was notably increased in supernatants of cultured alveolar macrophages from the desflurane group compared to both the other groups. Thus, inhalation of desflurane at anaesthetic dose levels decreased the response to inflammatory stimuli by attenuating the systemic release of proinflammatory cytokines (29).

10.3 Effects of short-term exposure (up to 90 days)

Isoflurane
ICR mice, Sprague Dawley rats, and Hartley guinea pigs were exposed continuously for 35 days to 0, 150, 500, 1 500 ppm isoflurane (n=11-31; mixed sex, equally divided between males and females). Animals were young and in an active phase of growth and were housed under controlled environmental conditions. Anaesthetic exposure took place in exposure chambers, and controls and exposed groups were treated identically, apart from the exposure. Five days prior to exposure, the animals were placed in the exposure chambers. Animals that failed to gain weight in this period were replaced. In the control groups, up to two guinea pigs and two mice failed to survive the 35 days of exposure. All rats survived. Body weights were recorded 7, 14, and 35 days after initiation of exposure. Small, but significant decreases in body weight gain were observed in mice at the two highest exposure levels at all time points. After 35 days of exposure, mice in all exposed groups had gained significantly less weight than controls. Weight gains were decreased to a similar extent in all three groups, i.e. no dependence on dose was observed. Body weight gains were also reduced in guinea pigs after 35 days of exposure at the highest exposure level. Organ weights generally varied with body weights and relative liver weights were unaffected by exposure. Blood was obtained from rats exposed from the middle exposure group; haematocrits, erythrocyte, leukocyte and differential counts were similar to controls. Livers from animals exposed to isoflurane manifested either no or small increases in lesions in the high-exposure group compared with controls (224). A lowest observed adverse effect level (LOAEL) of 150 ppm can be identified from this study although, as also commented by the Deutsche Forschungsgemeinshaft (56), there are some miscalculations of body weight gains, and only body weight gains and no absolute body weights are given.

Swiss Webster mice were exposed to compressed air or to 200, 1 000 or 5 000 ppm of isoflurane for 4 hours/day, 5 days/week for 9 weeks (n=13 each sex). Overall, there were no significant differences in body weights among exposure groups. Small decreases were present during weeks 1 through 3 for females and week 2 for males at the highest exposure level. At all times, differences remained within 10% of body weights in control animals. Following 9 weeks of exposure, organ weights, haematocrits, and serum aspartate aminotransferase levels were similar among exposure groups. Histological evaluation of organs revealed no anaesthetic-related organ toxicity. Levels of hepatic cytochromes, b5 and P450 were similar among exposure groups. Hepatic microsomal metabolism *in vitro* (defluorination) of three volatile halogenated ether anaesthetics (methoxyflurane, enflurane, and isoflurane) did not show any difference among the groups (202).

Groups of 16 male Sprague Dawley rats were subjected to either oxygen or 13 440 ppm isoflurane in oxygen for 2 hours every second day for 2 weeks, yielding a total of six 2-hour exposures. Twenty-four hours after the last exposure, the rats were autopsied. Specimens were taken from the brain, pituitary, lung,

heart, duodenum, pancreas, kidney, and liver. No tissue damage attributable to isoflurane exposure was found nor was weight gain affected (66).

Sevoflurane

Rats (sex not specified) received 0 or 3 000 ppm sevoflurane in oxygen, 4 hours/day for 30 days (n=10). The day after termination of exposure, behaviour was investigated. In the hole-board test, exploratory activity was significantly decreased in sevoflurane exposed animals compared to controls. In the elevated plus maze test, exposed animals spent significantly less time on the open arms and more time on the closed arms. Also learning and memory, assessed in the multiple T maze test, was significantly and adversely affected by sevoflurane, as control rats learned faster than exposed rats and made fewer wrong turns (188).

Cynomolgus monkeys (*Macaca fascicularis*) were assigned to a control group or three treatment groups (4 males and 4 females/group) and anaesthetised with sevoflurane at end-tidal concentrations of 0, 21 600, 32 000, or 40 100 ppm, 3 hours/day, 3 days/week for 8 weeks. Blood was obtained at baseline and 24 hours after the 3rd anaesthesia at weeks 1, 2, 4, 6, and 8 (the exposure protocol used prevented/minimised production and accumulation of FDVE). No cardiovascular or ventilatory support was used, even when indicated. Urine was collected continuously. The animals were killed 24 hours after the last exposure. Blood was assessed by routine haematologic examinations, measurements of serum enzymes, clinical chemistry, and serum electrolytes. Urine was evaluated microscopically and chemically. All animals went through complete post-mortem examinations, including histopathologic examination. Samples from liver and kidney were evaluated by electron microscopy. During exposure, marked hypoventilation was evident and end-tidal concentrations of carbon dioxide were elevated at the highest level. At the highest exposure level, three deaths occurred, and one control male died during the study period. Dose-related increases in serum aspartate and alanine aminotransferase, lactate dehydrogenase, and creatinine kinase were observed in the first week at all exposure levels. Levels stayed elevated for some weeks at the higher exposure levels. At the lowest exposure, levels returned to baseline by week 2. At termination of the study, all serum enzyme concentrations had returned to baseline. Erythrocyte counts were reduced at the two lowest exposure levels and white blood cell counts were lowered at the two highest exposure levels towards the end of the study period. Apart from reduced relative weight of the thymus in exposed monkeys, no gross pathologic, histopathologic, or ultrastructural differences were found with relationship to treatment in any group of monkeys (221).

Adult male mice were anaesthetised with 30 000 ppm sevoflurane in oxygen for 40 minutes once weekly for 3 weeks and untreated animals served as controls (n=6-12). Three days after the latest anaesthetic procedure, the absolute number of both leukocyte and lymphocyte counts were reduced in peripheral blood. Splenic cell composition, macrophage function, and the mitogen-induced lymphopro-lipherative response were preserved. The *in vivo* humoral response to sheep red

blood cells was augmented when assessed on day 8 and 9 after the last anaesthetic procedure. Sevoflurane-treated animals showed no evidence of histological changes or alteration in hepatic or renal function (71).

Desflurane
Rats (sex not specified) received 0 or 6 000 ppm desflurane in oxygen, 4 hours/ day for 30 days (n=10). The day after termination of exposure, behaviour was investigated. In the hole-board test, exploratory activity was significantly decreased in exposed compared to controls. In the elevated plus maze test, exposed animals spent significantly less time on the open arms and more time on the closed arms. Also learning and memory, assessed in the multiple T maze test, was affected adversely by desflurane, as control rats learned faster than exposed rats and made fewer wrong turns (188).

Conclusion
Body weight was unaffected by exposure to even high levels of isoflurane, when exposure was limited to shorter periods of time during the week. At continuous exposure, body weight gain decreased in mice from the lowest dose level tested, i.e. 150 ppm, and in guinea pigs from 1 500 ppm. No significant histopathological effects were observed in any study at any dose level tested.

   Rats exposed to sevoflurane (3 000 ppm) or desflurane (6 000 ppm), 4 hours/ day for 30 days, displayed decreased activity and anxiogenic behaviour in the elevated plus maze test and learning and memory was impaired, the day after termination of exposure.

10.4 Mutagenicity and genotoxicity

Table 11 gives an overview of *in vitro* and animal studies investigating the mutagenicity and genotoxicity of isoflurane, sevoflurane, and desflurane.

Isoflurane
Isoflurane (100-300 000 ppm) was tested for mutagenic activity in *Salmonella typhimurium* strains (TA1535 and TA100) in the presence or absence of liver homogenates prepared from rats treated with the enzyme inducer Aroclor 1254. The investigators also collected urine samples from patients 24 hours before and 24 hours after anaesthesia with isoflurane. The samples were tested in the presence and absence of metabolic activation and β-glucuronidase. The latter was added to hydrolyse glucuronides, thereby freeing possibly mutagenic, conjugated metabolites. Neither isoflurane nor urine from patients anaesthetised with iso-flurane was mutagenic (20). No information was provided on the duration of anaesthesia with isoflurane or the anaesthetic doses administered to the patients.

   Isoflurane (11 600, 50 000, 116 000 ppm) was not mutagenic in the Ames assay with *Salmonella typhimurium* strains (TA98 and TA100) in the presence or absence of liver homogenate (236).

The mutagenic effect of isoflurane was investigated using the sex-linked recessive lethal assay in *Drosophila melanogaster*. Male wild-type flies were exposed to vapour concentrations of 10 000 or 20 000 ppm for 1 hour. Following treatment, male flies were mated with untreated virgin females of the Basc strain and the rate of sex-linked recessive lethals was determined in the F2 generation. Isoflurane was not found to be mutagenic at the tested doses (144).

The same test was used to study the mutagenic effect of isoflurane in combination with nitrous oxide. Male wild-type flies were exposed to 20 000 ppm isoflurane in combination with 75% nitrous oxide for 1 hour and mated with untreated Basc females. When the rate of lethal mutations was assessed in the F2 generation, isoflurane was not mutagenic in the presence of nitrous oxide (22).

An *in vitro* sister chromatid exchange (SCE) assay was carried out in Chinese hamster ovary cells after exposure to 11 300 and 113 000 ppm isoflurane for 1 hour. Isoflurane did not increase the number of SCEs, neither in the absence nor in the presence of a metabolic activating system (241).

Chinese hamster lung cells were exposed *in vitro* to varying doses of isoflurane from 5 000 to 40 000 ppm mixed with oxygen, 5% carbon dioxide, and nitrogen for 1 hour. Isoflurane did not increase the number of SCEs compared to the controls exposed to room air and 5% carbon dioxide (233).

Human lymphocytes were isolated from 8 male volunteers and exposed to different concentrations of isoflurane (0, 0.3, 0.6 and 1.2 mM) for 72 hours. Isoflurane exposure increased the number of SCEs compared to the control at all concentrations. The effect increased with dose (110).

Human lymphocytes were exposed *in vitro* to isoflurane (0.1, 1, 10 mM in 1% dimethyl sulphoxide) for 10 or 30 minutes. DNA strand breaks as well as alkali-labile sites were measured as total comet length (i.e. increase in DNA migration). Isoflurane increased DNA migration in a dose-dependent manner (significantly different from controls starting at 1 mM isoflurane). Lymphocytes exposed to isoflurane at 1 mM repaired completely within 60 minutes (123).

DNA damage was evaluated by the comet assay in lymphocytes, spleen, bone marrow, brain, liver, and lung of male rats (n=50) exposed for 30 or 60 minutes to a 10 000 ppm isoflurane atmosphere with or without alcohol administration (administrated by gastric intubation at 4 g/kg body weight as a 50% solution). DNA damage in lymphocytes, bone marrow, and all the investigated organ tissues of rats exposed to isoflurane were found to increase time-dependently. Isoflurane administered in combination with alcohol led to additional DNA damage (137).

Human lymphocytes were isolated and exposed to isoflurane (1 or 10 mM) at 4 ℃ or 37 ℃ for 10 or 30 minutes. Isoflurane exposure (10 mM) at 4 ℃ increased the number of DNA strand breaks significantly compared to the control. Exposure to isoflurane at 37 ℃ induced DNA damage to a lesser extent than at 4 ℃. Visual inspection of the graphs indicated that the effect was not time-dependent (227).

Isoflurane was tested for its ability to induce micronuclei formation in the rat kidney. Twenty-four hours after removal of the left kidney, the male rats were treated with folic acid to increase the proliferative activity of kidney cells induced

by nephrectomy. Two days after folic acid administration, the rats were exposed to a single peroral dose of isoflurane (4 mmol/kg). A significant increase in the frequency of micronucleated cells was detected in rats 2 days after the exposure to isoflurane (206).

Sevoflurane
Sevoflurane was tested for mutagenic activity with *Salmonella typhimurium* (TA1535 and TA100). In one experiment, bacteria were exposed for 8 hours to vapour containing 1 000-300 000 ppm sevoflurane. In another experiment, the bacteria were exposed to 1, 10 or 30 µl liquid sevoflurane of unknown concentration. The tests were run in both in the absence and presence of metabolic activation system prepared from the liver of enzyme-induced rats. Sevoflurane was not mutagenic in either of the experiments (21).

Isolated human lymphocytes were exposed to sevoflurane (1 or 10 mM) at 4 ºC or 37 ºC for 10 or 30 minutes. Sevoflurane did not increase the number of DNA strand breaks under any of the exposure conditions (227).

Sevoflurane was tested for its ability to induce micronuclei formation in the rat kidney. Twenty-four hours after the removal of the left kidney, the rats were treated with folic acid to increase the proliferative activity of kidney cells induced by nephrectomy. Two days after folic acid administration, the rats were exposed to a single peroral dose of sevoflurane (4 mmol/kg). A significant increase in the frequency of micronucleated cells was detected 2 days after exposure (206).

Desflurane
The only study identified on genotoxic effects of desflurane demonstrated an increase in DNA strand breaks in human lymphocytes exposed to desflurane *in vitro* (0.1, 1 and 10 mM) after 5, 10, 30 and 60 minutes compared to controls. The greatest damage was seen after the 5-minute exposure, probably due to DNA repair occurring after the longer exposures (129). It was not specified whether the increase in DNA strand breaks occurred at all doses.

Summary and conclusion
No mutagenic effect of isoflurane has been found in the Ames test (20, 236) or in *Drosophila melanogaster* (22, 144). No increases in SCEs were observed in Chinese hamster ovary and Chinese hamster lung cell exposed to isoflurane (233, 241). However, increased frequencies of SCEs (110) and DNA strand breaks (123, 227) were observed in human lymphocytes exposed to isoflurane *in vitro*. In rats, increases in DNA strand breaks in blood cells and tissues (137) and in micronucleus formation in the kidney (206) were observed.

No mutagenic effects of sevoflurane were observed neither in the Ames test (21), nor with respect to DNA strand breaks in human lymphocytes exposed *in vitro* (227). In contrast, the number of micronucleated cells was increased in rat kidneys following exposure *in vivo* (206).

Table 11. Genotoxicity tests with isoflurane, sevoflurane and desflurane.

Anaesthetic/ Test system	Test concentrations	Comment/Effect	Reference
Isoflurane			
Human lymphocytes exposed *in vitro* 72 h	0.3, 0.6, 1.2 mM	Dose-dependent increase in SCEs at all concentrations.	(110)
Comet assay, human lymphocytes exposed *in vitro* 10 or 30 min	0.1, 1, 10 mM in 1% DMSO	Dose-dependent increase in DNA strand breaks at 1 and 10 mM after both 10 and 30 min of exposure.	(123)
Comet assay, human lymphocytes exposed *in vitro* 10 or 30 min	1, 10 mM	Increase in DNA strand breaks. The effect was not time-dependent.	(227)
Comet assay, rats exposed by inhalation 30 or 60 min	10 000 ppm	Time-dependent increase in DNA strand breaks in lymphocytes, bone marrow, spleen, brain, liver and lung.	(137)
Rats, single-exposed orally	4 mmol/kg	Increase in the frequency of micro-nucleated cells in the kidney.	(206)
Chinese hamster ovary cells exposed *in vitro* 1 h	11 300, 113 000 ppm	No effect on SCEs.	(241)
Chinese hamster lung cells exposed *in vitro* 1 h	5 000, 10 000, 20 000, 40 000 ppm	No effect on SCEs.	(233)
Sex-linked recessive lethal assay, *Drosophila melanogaster*	10 000, 20 000 ppm	No effect on the frequency of lethal mutations.	(144)
Sex-linked recessive lethal assay, *Drosophila melanogaster*	20 000 ppm	No effect on the frequency of lethal mutations. Co-exposure to 75% N_2O.	(22)
Ames test, *Salmonella typhimurium* strains (TA1535 and TA100)	100-300 000 ppm	No effect on gene mutation in presence or absence of metabolic activation.	(20)
Ames test, *Salmonella typhimurium* strains (TA100 and TA98)	11 600, 50 000, 116 000 ppm	No effect on gene mutation in presence or absence of metabolic activation.	(236)
Sevoflurane			
Comet assay, human lymphocytes exposed *in vitro* 10 or 30 min	1, 10 mM	No effect on DNA strand breaks.	(227)
Rats, single-exposed orally	4 mmol/kg	Increase in the frequency of micro-nucleated cells in the kidney.	(206)
Ames test, *Salmonella typhimurium* strains (TA1535 and TA100)	1 000-300 000 ppm	No effect on gene mutation in presence or absence of metabolic activation.	(21)
Desflurane			
Comet assay, human lymphocytes exposed *in vitro* 5, 10, 30 or 60 min	0.1, 1, 10 mM in 1% DMSO	Dose-dependent increase in DNA strand breaks. Greatest damage after the 5-min exposure.	(129)

DMSO: dimethyl sulphoxide, N_2O: nitrous oxide, SCE: sister chromatid exchange.

Only one study of the genotoxic effects of desflurane has been identified. The study showed an increased frequency of DNA strand breaks in human lymphocytes exposed *in vitro* (129).

In conclusion, recent studies on human lymphocytes exposed *in vitro* and rats exposed *in vivo* suggest that isoflurane possesses genotoxic potential. Due to the limited number of studies, no conclusion on the genotoxicity of sevoflurane and desflurane can be made.

10.5 Effects of long-term exposure and carcinogenicity

10.5.1 Carcinogenicity
Isoflurane
The carcinogenic potential of isoflurane has been investigated in three animal studies.

Mice (mixed sex) were exposed to either air (n=181), 1 000 ppm isoflurane (n ≤ 167), or 4 000 ppm isoflurane (n=165), for 4 hours/day, 5 days/week. After 78 weeks of exposure, the animals were left untreated for 3 weeks and then killed. There were no statistical differences among the groups regarding the number of mice with a particular tumour at a specific site, the ratio of benign to malignant tumours, or the time to tumour appearance. However, body weights were decreased in mice exposed to isoflurane at both exposure levels, 1-5% and 5-8%, respectively (no statistical analysis of body weights was provided) (23).

In another study, mice (a total of about 700 animals, mixed sex) were exposed for 2-hour periods to 375, 1 500 or 6 000 ppm isoflurane or air during pregnancy days 11, 13, 15, and 17. On the 5th day after delivery, exposure of the pups was resumed (3 times weekly) for a total of 24 exposures after delivery, altogether 8 weeks. There was no increase in the number of neoplastic lesions in isoflurane exposed offspring compared to control mice at 9 and 15 months after delivery (65).

The number of neoplasms in mice (mixed sex) exposed to isoflurane during foetal life and after birth was investigated by Corbett (53). Pregnant mice were exposed to either 1 000 ppm isoflurane on gestation days 12, 14, and 16 or to 5 000 ppm isoflurane on gestation days 12, 14, 16, and 18 (n=20). The offspring from both groups were further exposed to 1 000 ppm isoflurane from postnatal day 5 to 30. Each exposure period lasted 2 hours. A control group of pregnant mice and their offspring were exposed to room air. The number of tumours was evaluated in the offspring at ages 3, 6, and 9 months. An increased number (not significant) of lung adenomas was found in the isoflurane-exposed offspring compared to controls. The offspring was examined for hepatic neoplasms at the age of 15 months. Male offspring from the low-exposure and high-exposure groups of pregnant mice had 17% (5/30) and 27% (10/37) hepatic neoplasms, respectively. The number of hepatic neoplasms in the treated mice was significantly greater than in controls (53). In a later paper with Corbett as a co-author (65), it was pointed out that the Corbett study (53) is problematic for several reasons: uncertainty of exposure conditions for control mice, risk of

contamination of food and lack of blinding of the pathological samples to the evaluator. The many uncertainties of the above study make it difficult to conclude as to the potential carcinogenicity of isoflurane based on this study.

In summary, one study showed no carcinogenic effects of isoflurane after about 1.5 years of exposure to 1 000 and 4 000 ppm of isoflurane (4 hours/day, 5 days/week). Further, there was no evidence of carcinogenicity in 9 and 15 months old mice exposed to isoflurane prenatally as well as after delivery. In a third paper, increased numbers of mice with adenomas in the liver were observed. However, that study was not properly designed and there was a lack of experimental detail.

Sevoflurane and desflurane
No reports on carcinogenicity were identified.

10.5.2 Other effects of long-term exposure
The hepatic and renal effects of chronic inhalation of isoflurane were studied in rats (191). Male Fischer 344 rats were continuously exposed to 20 ppm isoflurane or air for 30 weeks (n=12). Urinary fluoride excretion was increased during exposure to isoflurane. Chronic isoflurane exposure did neither lead to hepatocellular necrosis nor to changes in serum alanine aminotransferase activity, liver size, hepatic microsomal cytochrome P450 content, or hepatic fat content. No evidence of toxic damage to the renal tubules was observed.

10.6 Reproductive and developmental studies

Isoflurane: Fertility and preimplantation development
The effects of non-toxic doses of isoflurane on fertility and development were examined in *Drosophila melanogaster.* Male and female fruit flies were exposed to isoflurane at 0, 5 000, 10 000, 20 000, 40 000, 50 000, or 70 000 ppm. Exposed males were mated with untreated females and exposed females were mated with untreated males. Isoflurane exposure exerted no effect on fertility, as there were no significant differences of brood or total numbers of male and female offspring among the different treatment groups (143).

The epididymal spermatozoa of (C57B1/C3H)F1 mice were examined for morphologic abnormalities following exposure to air, 1 000, or 10 000 ppm isoflurane for 4 hours/day on 5 consecutive days (n=5). Examination 28 days after initiation of exposure revealed no significant increases in the percentages of abnormal spermatozoa in isoflurane exposed compared to controls (154). As the spermatogenic cycle in mice takes 56 days and male mice began exposure only 33 days before investigation of epididymal spermatozoa, effects on the earlier stages of spermatogenesis would not have been detected in this study.

The effects on fertility and reproductive wastage were evaluated in Swiss Webster mice after exposure to isoflurane. Untreated female mice were mated with males exposed to air, 1 000 or 4 000 ppm (n=15-24) isoflurane, 4 hours/day for 6 weeks before mating. In a second experiment, male (n=55) and female mice (n=110) were distributed to three groups exposed to air, 1 000, or 4 000 ppm iso-

flurane, 4 hours/day for 2 weeks before mating and during mating and pregnancy. Body weights and male fertility were unaffected by exposures. When uterine examinations were performed on day 18 of gestation, no significant effects on pregnancy rate or number of implantations were observed in either experiment. The mice exposed to 4 000 ppm isoflurane appeared lightly anaesthetised (166). Effects on the earliest stages of spermatogenesis would not have been detected, as male mice were exposed to isoflurane for only 42 days before mating.

Rabbits were exposed to 13 000 ppm isoflurane for 4 hours/day for 5 consecutive days (n=7-8). Semen was collected on days 12, 19, 26, 33, and 41 after exposure, i.e. analysis was performed throughout a seminiferous tubule cycle. Following the last semen collection, testicular biopsies were stained and examined by light microscopy. The spermatogenic epithelium was classified as: normal, hypospermatogenic, maturation arrest or total or partial atrophy. In exposed animals, sperm concentration was significantly reduced by approximately two thirds on assessment days 26, 33, and 41 compared to preanaesthetic values. Sperm cell motility decreased somewhat during the period, from 36% to 75% immotile cells, and was significantly different from the pre-exposure value on day 41. Histologically, varying degrees of injury to spermatogenic cells were observed in exposed animals when assessed at day 41, compared to controls. Sertoli and Leydig cells appeared normal (45). The reported observations (described in detail below, see sevoflurane heading) should be interpreted in the light of the variations observed also in control animals and the lack of proper data presentation. For example, not only exposed but also control animals presented with a depressive trend with respect to sperm concentration. Thus, the adverse effects cannot be entirely attributed to the isoflurane exposure.

The effects of clinical concentrations of isoflurane on *in vitro* fertilisation were evaluated in a mouse model. Mouse oocytes were exposed to either air, mixtures of oxygen + nitrous oxide, or oxygen + nitrous oxide + 5 000 ppm isoflurane just prior to insemination. After insemination, development of the zygotes was assessed. The fertilisation (2-cell) rate and early embryonic growth rate (i.e. 4-cell and morula rates) were similar in the three groups (155).

Preimplantation mouse embryos were exposed to isoflurane to investigate effects on embryo development. Two-cell embryos were exposed for 30 minutes *in vitro* to 15 000, 30 000 or 50 000 ppm isoflurane, 5-6 hours, 3-4 hours, or 0-1 hour before the onset of their first cleavage (n=31-51). Also the effects of 50 000 ppm isoflurane on 4-cell embryos exposed about 2 hours after the first cleavage and on morula stage embryos were examined. When 2-cell embryos were exposed for 3-4 hours or 0-1 hour before the expected onset of cleavage, 30 000 and 50 000 ppm isoflurane inhibited development to the blastocyst stage. The development of embryos exposed to isoflurane at the 4-cell or morula stage was unaffected (235). In another study, 2-cell mouse embryos were exposed to 15 000 ppm isoflurane *in vitro*. Isoflurane exposure reduced the number of mouse embryos developing into the blastocyst stage (47).

The effects of sera from patients given various anaesthetics were investigated on *in vitro* mouse preimplantation embryo development. Patients electing different operative procedures were anaesthetised with nitrous oxide combined with either isoflurane, fentanyl or morphine (n=8). When sera collected one hour after anaesthetic induction was added to 2-cell mouse embryos, the number of 2-cell embryos that developed into the blastocyst stage was significantly reduced in the isoflurane group as compared to that of preanaesthesia sera. No detrimental effects were revealed from sera of patients given nitrous oxide in combination with fentanyl or morphine. At least 15 embryos were cultured for each serum sample (n=8) (164).

Isoflurane: Developmental toxicity
The carcinogenic effect of exposure to isoflurane prenatally was investigated by Eger *et al* (65). No evidence for transplacental carcinogenesis was found in 9- and 15-month old mice exposed to isoflurane prenatally as well as after delivery (described in Section 10.5).

Eggs from *Drosophila melanogaster* were exposed continuously to 1 000 or 2 000 ppm of isoflurane during development. A dose-dependent increase in the duration of metamorphosis and a decrease in the number of flies were seen. No morphological abnormalities were observed (145).

Developmental toxicity was examined in Swiss Webster mice exposed to 60, 600, or 6 000 ppm isoflurane, 4 hours/day on gestation days 6-15 (n=23-27). Uterine contents were examined for external, internal and skeletal abnormalities on day 18 of pregnancy. No adverse effects on litter size and sex ratio were demonstrated following exposure of dams to isoflurane at any exposure level. However, exposure to 6 000 ppm isoflurane resulted in significantly decreased foetal weight, decreased skeletal ossification, and increased incidences of minor hydronephrosis, increased renal pelvic cavitation, and cleft palate. At this exposure level, dams were lightly anaesthetised and maternal weight gain was decreased by approximately 10% (167). Approximately half of the decrease in maternal weight gain was due to significantly lower foetal weight combined with fewer offspring per dam. Reduced ossification, minor hydronephrosis, and increased renal pelvic cavitation may be indicative of retarded foetal maturation, and the incidence of cleft palate generally increases when pregnant mice are stressed during gestation.

Both male (n=54) and female Swiss Webster mice (n=110) were exposed to air, 1 000, or 4 000 ppm isoflurane 4 hours/day for 2 weeks before mating and during mating and pregnancy. When uterine examinations were performed on day 18 of gestation, the number of implantations, resorptions, live foetuses, viability, foetal weight, and sex ratio showed no differences between exposed and control groups. One third of the pregnant dams were allowed to deliver and rear their offspring until postnatal day 28. Birth weights and survival of exposed offspring did not depart significantly from controls. No maternal toxicity was observed but at the highest concentration light anaesthesia was observed (166).

The reproductive and teratogenic effects of isoflurane were studied in pregnant Sprague Dawley rats. Rats were exposed to either air or 3 500 ppm isoflurane for 24 hours on day 8 of pregnancy (n=30-40). Exposure did not affect the number of implantations, resorptions, live foetuses, sex ratio, or mean foetal body weight. No increases in visceral or skeletal abnormalities were found. Exposed dams were slightly sedated during exposure and their body weights were significantly lower on days 12, 14, and 16 of pregnancy compared to controls (78).

The neurotoxic effects of anaesthesia were studied in Hartley guinea pigs in which gestation lasts 59-72 days and brain development is mostly a prenatal phenomenon. Pregnant guinea pigs were exposed for 4 hours to 0 or 5 500 ppm isoflurane during three different gestational periods: gestation days 20-25, 35-40, or more than 50 days. Both control and isoflurane exposed guinea pigs were intubated under fentanyl anaesthesia, and in controls, anaesthesia was maintained with fentanyl. Therefore a "true" control group was added, which did not undergo treatment at all. During exposure, maternal homeostasis was maintained within control values. Two hours after cessation of exposure, several relevant regions in foetal brains were immunochemically stained for caspase-3 as a proxy for late apoptosis. There were no significant differences in staining between the fentanyl-treated controls and the true controls, although the level of caspase-3 positive neurons was somewhat lower in true controls. In foetuses from animals treated with isoflurane on gestation days 35-40, the number of caspase-3 positive neurons was increased 3-6 fold compared to true controls in several brain regions (205). The outcome of statistical comparisons with fentanyl-treated controls was not provided. Only total numbers of pregnant animals in each stage were stated, not numbers of pregnant animals in each exposure group. Furthermore, all offspring from all mothers were assessed, which increases the risk for litter effects.

Pregnant Sprague Dawley rats were exposed 6 hours/day on gestation days 8-10, 11-13, or 14-16 to 10 500 ppm isoflurane or air (n=21-50). The dams were lightly anaesthetised and their weight gains were significantly reduced compared to controls. At laparotomy on gestation day 21, foetal weights were reduced in offspring of rats exposed to isoflurane on gestation days 8-10 and 14-16. Otherwise no reproductive indices, including malformation rate, were altered in any of the exposed groups (168).

Pregnant Sprague Dawley rats were anaesthetised with 13 000 ppm isoflurane or carrier gas (30% oxygen, balanced with nitrogen) for 6 hours at gestational day 21. A pilot study (n=4) showed that at this exposure level the dams breathed spontaneously and no significant changes were observed for arterial blood gas and mean arterial blood pressure as compared to the controls. Some dams underwent laparotomy 2 or 18 hours after termination of exposure (n=5-6), other dams delivered their offspring naturally (n=7-8). In the latter offspring, lactational weight gain was similar in control and exposed offspring. In all groups of offspring, apoptosis was quantified in the hippocampus and cortex. Spontaneous apoptosis was significantly decreased 2 hours after isoflurane exposure in the hippocampal CA1 region and in the retrosplenial cortex, observed as an 80-87%

decrease in the density of caspase-3 and TUNEL (terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling) positive cells. No differences were evident 18 hours after treatment or at postnatal day 5. When the naturally delivered pups were examined in the Morris water maze at ages 5 and 17 weeks, the only observed difference was a significant increase in the percentage of time spent in the probe quadrant for isoflurane exposed compared to control juvenile offspring (157). The latter finding is usually interpreted as a sign of increased cognitive performance.

Ten-week-old pregnant Jcl:ICR mice were exposed to 0 or 15 000-20 000 ppm isoflurane for 8 hours on day 7 of gestation (data were analysed with the pups (n=66-72) rather than the litter (n=4-5) as the statistical unit, thereby introducing the risk for litter effects). On gestation day 18, foetal weight and the number of ossified sacrococcygeal vertebrae were significantly reduced in exposed offspring compared to controls (96).

Pregnant albino rats were exposed to 17 000 ppm isoflurane on gestation days 1-5, 6-10, 11-15, or 15-20 for 1 hour/day (n=11-19; n was not stated for the late exposure group) to determine effects on development. Eleven control dams and 10 dams exposed on gestation days 1-5 were sacrificed on day 14 of gestation. The remaining dams were sacrificed on day 20 of gestation, apart from the dams exposed during late pregnancy that were allowed to deliver and carry their litters through weaning. Maternal weight gain was reduced in the late exposure group. Otherwise dams were unaffected by exposure, but for signs of sedation. Offspring from the earliest treatment group exhibited somewhat depressed body weights, and dams in the late exposure group delivered and weaned fewer pups than the controls. Otherwise, progeny body weight, gestational parameters, foetal viability, survival, and malformation rate were similar to the control values (132).

Pregnant New Zealand rabbits were exposed on gestation days 6-9, 10-14, and 15-18 to 23 000 ppm isoflurane for 1 hour/day (n=15/group). All animals were sacrificed on gestation day 29. Maternal food consumption and weight gain were similar in all groups. No adverse reproductive effects were observed, and no evidence of teratogenic activity was obtained (132).

Isoflurane: Juvenile studies
Isoflurane was administered to young Sprague Dawley rats at 7 500, 10 000 or 15 000 ppm for 6 hours at the age of 1, 3, 7, 10, or 14 days (n=4‑10). Control offspring were exposed to air. Young rats went through perfusion fixation 18 hours after anaesthesia and brain slices were stained with silver to assess apoptotic neurodegeneration. Isoflurane treated animals exhibited dose-dependent neuro-degeneration in the laterodorsal and anteroventral thalamic nuclei at postnatal days 1 and 3, significantly greater than in the corresponding controls at the highest exposure level. The involvement of the intrinsic apoptotic pathway was investigated by measuring the cytosolic levels of anti-apoptotic protein (bcl-x_L) and cytochrome c in brain extracts from the cortex and the anterior thalamus in 7- and 14-day old pups. In the same tissues, the involvement of the extrinsic apoptotic pathway was

investigated by measuring the expression of Fas (a member of the TNFα superfamily). Values in exposed pups did not differ from those of control pups (249). Another study used a similar exposure protocol, but limited the age at exposure to 7 days post partum. After anaesthesia, some of the young rats went through perfusion fixation and 50 μm brain sections were stained with silver or processed to reveal caspase-3. In control animals, both silver and caspase-3 staining revealed a sparsely scattered pattern of baseline physiological cell death. Isoflurane treated animals exhibited dose-dependent neurodegeneration. The most vulnerable brain regions were the laterodorsal and anteroventral thalamic nuclei, where even the lowest concentration caused a significant increase in neuronal degeneration (16- and 9-fold, respectively). The parietal cortex was also affected in an apparently dose-dependent manner, although the damage was significantly greater than in controls only at the highest exposure level. Long-term electro-physiological function was examined in hippocampal slices from pups exposed at postnatal day 7, prepared at postnatal days 29-33. Baseline excitatory postsynaptic potentials in isoflurane treated pups did not differ from controls. Long-term potentiation was less robust in treated animals, but this difference did not show significant when compared to controls (124).

Infant mice were exposed to 7 500, 15 000, or 20 000 ppm isoflurane for 4, 2, or 1 hours, respectively. Group sizes ranged from 6 to 16 pups. At each condition, control and experimental pups were taken from the same litters, but probably only from one or two dams. The number of neuronal profiles undergoing apoptosis in the brains was evaluated quantitatively 5 hours after initiation of exposure by activated caspase-3 immunostaining. Blood glucose values were determined under the same conditions. Isoflurane was associated with significant increases of 178-477% in neuroapoptosis in the caudate putamen, compared with the control condition. Blood glucose determinations indicated that hypoglycaemia was not a potential cause of the brain damage (125).

These studies were partly replicated *in vitro*. Organotypic hippocampal slices were prepared from rat pups on postnatal days 4, 7, and 14 and cultured 7 days *in vitro*. The slices were exposed to 15 000 ppm isoflurane in fresh gas (21% oxygen, 5% carbon dioxide, 69% nitrogen) or fresh gas only for 1, 3, or 5 hours. Hippo-campal CA1, CA3, and dentate gyrus neuronal survival were assessed 3 days later (n ≥ 12 slices/condition and brain structure). Isoflurane significantly increased cell death in slices exposed to isoflurane for 5 hours. No significant effect was evident after 1 or 3 hours of exposure (244).

Newborn Sprague Dawley rats were removed from their mother 2-4 hours after birth. The pups were placed on a heated pad and were administered either normobaric oxygen/carbon dioxide for 10 minutes or 20 000 ppm isoflurane for 3 minutes followed by 10 000 ppm isoflurane for 7 minutes, in normobaric oxygen/carbon dioxide (group size not indicated). Neonatal anaesthesia did not affect body temperature and all animals fed at least once within 4 hours of being returned to their mothers. There was no effect of isoflurane on body weights at any time point postexposure. When reflex development was investigated three

times a week from postnatal day 2 to 15, isoflurane exposed offspring performed significantly slower in surface righting, and were able to hang onto the wood bar with their forepaws for significantly shorter times than control offspring; performance in negative geotaxis and cliff aversion was similar in the two groups. On postnatal day 25, isoflurane offspring displayed significantly more foot slips while traversing a balance beam during 5 out of 6 trials. Learning in both the Morris water maze (postnatal days 45-46) and the radial arm maze (postnatal days 58-72) was impaired in isoflurane exposed compared to control offspring. When the study was terminated at postnatal day 80, hippocampal volume and neuron number were decreased by approximately 20% in exposed offspring compared to controls (208). It should be mentioned, that none of the juvenile studies describes the allocation of pups from different dams to the different exposure groups, why litter effects cannot be excluded.

Isoflurane: Myometrial contraction
The effects of 6 000, 12 000 and 18 000 ppm isoflurane on isolated human uterine muscle were evaluated. Specimens were obtained from 15 non-gravid and 4 gravid uteri removed at elective operations (n=6-7 strips per dose level). Each muscle strip was placed in a freshly prepared bath solution and was attached to an isometric force transducer. After 40-60 minutes of spontaneous contractions, the anaesthetic was added to the muscle-bath system. Direct measurements of isoflurane content in the bath solution showed that equilibration of the water with isoflurane vapour was essentially complete within 3-4 minutes. After exposure for at least 30 minutes, the anaesthetic was discontinued and a control pattern comparable to the initial control pattern reappeared. Myometrial response was evaluated by measuring the mean resting tension, developed tension, and frequency of contractions, with contractility defined as the area between the resting and developed tensions per unit time. Gravid and non-gravid muscle strips showed similar, and progressive depression of contractility with increasing dose level, significantly different from control values at the lowest and the highest dose level (*post hoc* analysis corrected for multiple comparisons). The mean frequency of contractions decreased with increasing dose, but was only significantly different from controls at the lowest dose level. Significant reductions in developed tension occurred at the highest dose level (175).

The effect was also investigated in myometrial strips from 11 non-gravid women undergoing elective abdominal hysterectomy (age range 29-57, mean 39 years). Following 45 to 60 minutes of equilibration and spontaneous uterine activity, 22 strips were exposed to 5 000 ppm isoflurane. Eight strips were unexposed and served as controls. Frequency of contractions and uterine activity decreased significantly and the mean interval between contractions increased (41).

Uterine specimens were obtained at the time of operation during hysterectomy and caesarean section from premenopausal women aged 18-54 years. Non-gravid uterine strips began to contract spontaneously, whereas gravid strips were initially stimulated by adding oxytocin. After 30-60 minutes, isoflurane was administered

for 30 minutes and then discontinued for at least 30 minutes until a comparable control pattern reappeared. Then the uterine strips were exposed to the next higher concentration. Each strip were exposed at two to four dose levels of 5 000, 10 000, 20 000 and 30 000 ppm (n=6). Contractility of both gravid and non-gravid uterine muscle was significantly depressed at all dose levels. At 20 000 ppm, the effect was stronger in gravid than non-gravid strips (231).

Isoflurane: Other reproductive effects
Four-day-old chick embryos were exposed to 25 000 or 50 000 ppm of isoflurane vapour (n=5). Dorsal aortic blood velocity was measured with a pulsed-Doppler velocity meter. Neither cardiac stroke volume nor embryonic heart rate were affected by isoflurane (245).

The antiproliferative potential of isoflurane was determined *in vitro*, in a C6 glioma cell line, following exposure for 48 hours over a concentration range of 0-2.0 mM, estimated 48 hours after exposure. The drug concentration that inhibited C6 glioma proliferative action by 50% was approximately 1.0 mM. Antiproliferative action was not associated with anaesthetic-induced cytotoxicity. Flow cytometric analysis of growth-arrested cell populations revealed no specific accumulation in any cell cycle phase, as observed for e.g. the teratogen valproate (186).

In preterm pregnant sheep, maternal and foetal cardiovascular data, amniotic pressure, uterine blood flow, and foetal brain oxygenation were monitored before, during, and after isoflurane anaesthesia at mid-gestation, i.e. gestation day 90 (n=11). Anaesthesia was induced with thiopental after sedation with midazolam, and thereafter maintained with 15 000 ppm isoflurane in oxygen for 4 hours. Iso-flurane produced moderate foetal hypotension and bradycardia, but markers of systemic oxygenation did not change from baseline. An initial increase in foetal oxygen saturation was observed, followed by a gradual decline to baseline. Within the foetal brain, oxygenated haemoglobin changed by less than 10% (non-significant) and deoxygenated haemoglobin and total haemoglobin varied by less than 5%, although foetal mean arterial pressure decreased (170).

Sevoflurane
Rabbits were exposed to 23 000 ppm sevoflurane 4 hours/day on 5 consecutive days (n=7-8). Semen was collected on day 12, 19, 26, 33, and 41 after the first day of exposure, i.e. analysis was performed throughout a seminiferous tubule cycle. Following the last semen collection, testicular biopsies were stained and examined by light microscopy. The spermatogenic epithelium was classified as: normal, hypospermatogenic, maturation arrest or total or partial atrophy. From the 12th day after initiation of exposure, sperm concentration was decreased by 50% compared to the pre-exposure value, significantly so from the 19th to the 41st day. Furthermore, exposed rabbits displayed complete absence of motile sperm on day 33, with significantly decreased values also on day 26. On day 41, motility had returned to pre-exposure values. Histologically, varying degrees of injury to spermatogenic cells were observed in exposed animals on day 41, whereas Sertoli

and Leydig cells appeared normal (45). However, these observations should be interpreted in the light of the variation observed in the control animals. In these animals, sperm concentration values decreased progressively throughout the assessment period, and had decreased by 32% on day 41 compared to pre-exposure. On day 26, the value was significantly different from the pre-exposure value. It can therefore not be excluded that the pre-exposure value in the exposed animals was a one-time high. If this is the case, the lower values in exposed animals may not be related to exposure, but are rather chance findings. Regarding motility values, controls presented with 0% immotile sperm cells pre-exposure, increasing to 17% at termination of the study. In the sevoflurane group, the corresponding pre-exposure value was much higher, i.e. 52% immotile sperms. Furthermore, data were presented as means without specification of variation, and the results of the histological assessment were presented as selected microphoto-graphs, and abnormal findings from animals exposed to sevoflurane as totals together with findings from animals exposed to isoflurane; study described above in this section ("Isoflurane: Fertility and preimplantation development"). Thus no formal summing up or statistical analysis of the differences between the control and exposed groups was performed.

Anaesthesia was induced with propofol in 10 pregnant goats. Following the induction, the animals inhaled 27 000 and 41 000 ppm end-tidal concentration of sevoflurane each for 30 minutes, and then recovered. Compared to pre-anaesthetic values, sevoflurane caused minimal change in maternal haemodynamics. Foetal blood pressure decreased significantly during the last minutes of exposure to 27 000 ppm and remained low during exposure to 41 000 ppm sevoflurane. The uterine contractions disappeared throughout sevoflurane inhalation, but recurred within 15 minutes after cessation of exposure (218).

The antiproliferative potential of sevoflurane was determined *in vitro* in a C6 glioma cell line, following a single exposure over a concentration range of 0-2.0 mM for 48 hours. Within this exposure range, the concentration of sevoflurane that inhibited C6 glioma proliferative action by 50% could not be determined. Antiproliferative action was not associated with anaesthetic-induced cytotoxicity. Flow cytometric analysis of growth-arrested cell populations revealed no specific accumulation in any cell cycle phase, as observed for e.g. the teratogen valproate (186).

During elective caesarean section, a small sample of myometrium was isolated from 12 healthy women, and the effects of 2 010, 5 025, 10 050, 15 075, 20 100, 30 150, 50 250, and 70 350 ppm sevoflurane were investigated after spontaneous contractions had appeared after approximately 90 minutes (n=6/dose level). Following a 10-minute exposure period, the mean values for three consecutive contractions were measured and expressed as a percentage of the preceding control measurements. Exposure was then discontinued for 10 minutes washout, after which exposure at the next dose level was initiated. Sevoflurane dose-dependently depressed uterine contractility (defined as the area under the force-time curve); significantly different from the control condition at 5 025 ppm and

above. Developed tension was similarly depressed, and differed significantly between the groups at concentrations of 10 050 ppm and greater. At the two highest dose levels (50 250 and 70 350 ppm), the frequency of spontaneous contractions were significantly increased and uterine activity was virtually abolished (234).

Desflurane
No animal or *in vitro* studies evaluating reproductive effects of desflurane were found in the literature, apart from the two studies on effects on myometrial contractions described below.

Comparative studies on myometrial contraction
Uterine specimens were obtained from normal full-term pregnant women undergoing elective lower-segment caesarean delivery. Longitudinal muscle strips were mounted to isometric force transducers and a resting tension was placed (n=11-15). The isometric tension was recorded during exposure to isoflurane (6 500-39 000 ppm), sevoflurane (10 000-60 000 ppm), or desflurane (30 000-180 000 ppm). The three anaesthetics produced a dose-dependent depression in contractility, although the response in exposed strips did not differ significantly from control specimens at the lowest dose levels. Contractility was significantly decreased at 13 000 ppm isoflurane and above, and for sevoflurane and desflurane at and above 20 000 and 60 000 ppm, respectively (250).

A small segment of myometrium was excised from the lower uterine segment of 20 non-labouring term parturients undergoing caesarean section. The study protocol consisted of a 60-minute period of spontaneous contractions, control recording with oxytocin for 10 minutes, washout for 10 minutes, administration of sevoflurane (11 500, 23 000, and 46 000 ppm; n=10) or desflurane (28 500, 57 000, and 114 000 ppm; n=10) (3 times per 15-minute period), response to oxytocin (10-minute period), a further washout interval (10-minute period) and subsequent control recording with oxytocin without anaesthetics. The frequency and amplitude of contractions induced with oxytocin decreased significantly at all dose levels. The duration increased at the two lowest dose levels, but decreased at the highest dose level. At the middle dose level, i.e. 1 MAC, desflurane inhibited the amplitude less than sevoflurane. The duration of contractions at the highest dose level decreased in both groups (248). A similar protocol was used in longitudinal myometrial strips from non-pregnant and pregnant (gestation days 19-20) Wistar rats (n=10 per compound). Both agents inhibited the duration, amplitude, and frequency of induced contractions in a dose-dependent manner, and the responses differed significantly from the control condition at all dose levels (57). When the response of pregnant rat myometrium was compared with that of non-pregnant rats in a similar experimental protocol (n=10 condition/ compound), both agents inhibited the duration, amplitude, and frequency of induced contractions in a dose-dependent manner, except at the lowest dose level in the non-pregnant group. Isolated strips of pregnant rat myometrium were more sensitive to the inhibitory effects of both agents than strips from non-pregnant rats (89).

Longitudinal smooth muscle layers were obtained from 24 pregnant Sprague Dawley rats, and cut into small strips. Muscle tissue was contracted with oxytocin, and isoflurane or sevoflurane (10 000, 20 000, or 30 000 ppm) were introduced into the tissue bath (n=6). Both volatile anaesthetics inhibited muscle contraction dose-dependently concomitant with a decrease in intracellular free Ca^{2+}; significantly so from the lowest dose level. When the anaesthetic concentrations were expressed as multiples of their MACs, the inhibitory potencies of the two agents were similar (247).

Conclusions

Isoflurane is the flurane best studied for reproductive effects. Regarding fertility, isoflurane was not associated with effects on male fertility in two studies in mice at exposure levels ranging from 1 000 to 10 000 ppm (154, 166). One study in rabbits demonstrated decreased sperm counts starting 26 days after initiation of exposure to 13 000 ppm isoflurane. In the latter study, control animals presented with significant variation in spermatogenic parameters, therefore the reported findings may not pertain to isoflurane exposure (45). None of the studies covered the full spermatogenic cycle.

Two studies showed decreased development of 2-cell mouse embryos into the blastocyst stage after exposure to supraanaesthetic doses of isoflurane *in vitro* (47, 235), whereas one study at anaesthetic dose level did not (155). In an *in vivo* mouse study, female fertility was unaffected after exposure to up to 4 000 ppm isoflurane, 4 hours/day for 2 weeks before and during mating (166).

The effects of isoflurane exposure on foetal development were investigated in several studies, in mice, rats, and rabbits. Isoflurane reduced offspring body weights at exposure levels at and above 6 000 ppm. One study showed significantly increased incidences of minor anomalies (minor hydronephrosis, increased renal pelvic cavitation) and cleft palate in mice exposed prenatally to 6 000 ppm isoflurane, 4 hours/day during organogenesis. These offspring also displayed decreased foetal weight and delayed ossification (167). At 4 000 ppm and below, the studies consistently showed no effect of isoflurane (78, 166, 167).

Exposure to isoflurane during neonatal life has been associated with increased apoptosis in neural tissue in rodents (125, 208, 244). The developmental stage of the nervous system in juvenile rodents corresponds roughly to that of the nervous system in the human foetus during the third term. These results indicate that, potentially, the foetal nervous system in humans may be sensitive to isoflurane exposure. It should be mentioned, that other recent results demonstrate that brief exposure to other anaesthetics, e.g. ketamine and midazolam also triggers neuro-apoptosis in the developing mouse brain (125). In contrast, one study demonstrated decreased spontaneous apoptosis in term rat offspring exposed *in utero* to 13 000 ppm isoflurane for 6 hours. Exposed offspring demonstrated indications of increased cognitive performance later in life (157).

For sevoflurane, the only study on fertility in male rabbits, showed decreased quality of semen after exposure to near MAC-values of sevoflurane (45).

However, in this study, control animals presented with significant variation in spermatogenic parameters, why the reported findings may not pertain to sevoflurane exposure.

Finally, as investigated *in vitro* on myometrial strips, all 3 fluranes significantly inhibit muscle contraction, also at subanaesthetic dose levels around 0.5 MAC. The NOAEL for this effect has not been determined (41, 175, 231, 248, 250).

11. Observations in man

Isoflurane, sevoflurane and desflurane are used as anaesthetics. In comparison to most other compounds in the work environment, there is therefore relatively much knowledge as to the effects of single exposure to high dose levels in humans, i.e. side-effects. However, for most of these side-effects, the NOAELs are not known. The most common side-effects are listed in Appendix 2, where also the incidence of each effect is stated. In addition to the central nervous system, fluranes at anaesthetic levels have been shown to affect most organ systems. Most concern has concentrated around the potential for hepatotoxicity and nephrotoxicity. Hepatitis may occur in less than 1/1 000 after isoflurane anaesthesia, and in between 1/1 000 and 1/10 000 after sevoflurane and desflurane anaesthesia. Nephrotoxicity is only a concern for sevoflurane, with an incidence less than 1/10 000 patients. Other side-effects include leukocytosis in patients undergoing anaesthesia with desflurane ($\geq 1/10$) and malign hyperthermia, which appears in between 1/1 00 and 1/1 000 patients after desflurane anaesthesia.

The effects of isoflurane, sevoflurane and desflurane have been investigated in a number of experimental studies with a single acute exposure at subanaesthetic concentrations. These are described below together with a few cross-sectional studies that investigated the effects of occupational exposure to mainly isoflurane. The studies that are of relevance for dose-effect and dose-response relationships are summarised in Tables 16-19 in Chapter 12.

11.1 Irritation and sensitisation

11.1.1 Irritation
Isoflurane
None of 8 anaesthetists (gender not specified) experienced discomfort, and all subjects found inhaling isoflurane acceptable or pleasant and suffered no nausea after exposure to 1 150, 2 300, and 4 600 ppm of isoflurane in 100% oxygen for at least 20 minutes at least one week apart (178). In contrast, 6 of 12 volunteers (mixed gender) breathing 4 000 ppm isoflurane found the odour unpleasant, 2 found it neutral, and 4 found it pleasant. Two rated the exposure unacceptable, whereas the remainder found it acceptable (172).

Elicitation of airway reflexes by nasal exposure to isoflurane was investigated in 13 female patients anaesthetised with flunitrazepam, pentazocine, and nitrous oxide. The patients were intubated by a saline-filled double-cuffed endotracheal

tube, and spontaneous respiration resumed while the patients breathed 50% nitrous oxide in oxygen. Changes in breathing pattern were measured with a pneumotachograph while changes in laryngeal and tracheal wall tension were assessed by measuring changes in the proximal cuff pressure and the distal cuff pressure, respectively. In 8 of 13 patients, the dose-response relationship was determined by administering 10 000, 30 000, or 50 000 ppm isoflurane to the nostril. Nasal insufflation with 10 000 and 30 000 ppm isoflurane did not elicit responses. In all 13 patients, nasal insufflation at a concentration of 50 000 ppm prolonged mean (±SD) expiratory time (from 2.2 ± 0.5 to 3.9 ± 1.7 seconds). In 6 patients, expiratory time increased by more than 5 seconds, during which there was a small but consistent increase in laryngeal wall tension. The prolongation in expiratory time possibly occurs due to activation of reflexogenic receptors in the nose, indicating airway irritation at high exposure levels (181).

Sevoflurane
No studies regarding irritation were found for sevoflurane besides the comparative studies described below.

Desflurane
Six unpremedicated young, adult males going through day-case arthroscopy of the knee were administered desflurane (and 60% nitrous oxide in oxygen) at an inspired concentration of 30 000 ppm, increased by 3% every 3-6 breaths as tolerated, up to a maximum of 120 000 ppm. Of the 6 patients, 5 developed laryngospasm, breath-holding, coughing, and increased secretions. Hiccups and bronchospasm occurred in 1 patient (34).

MAC for desflurane in oxygen was determined to 72 500 ppm in 18-30-year old unpremedicated male patients, and to 60 000 ppm in the 31-65-year age group. Signs of minor airway irritation were apparent, i.e. more than half coughed during induction, and the odour was most commonly described as "burning" or "soap in the nose" (197).

Comparative studies
The effect of breathing 1 200 ppm isoflurane or 6 000 ppm desflurane for 3 minutes on the incidence of adverse airway events was investigated. Twenty-five volunteers known to develop adverse airway events in response to surgical levels of desflurane or isoflurane took part in the study. Smokers were excluded. Adverse airway events were identified both clinically (cough) and by recording diagrams of inspiration and expiration, allowing identification of e.g. breath holding. None of the volunteers displayed adverse airway events during the 3 minutes of breathing 1 200 ppm isoflurane or 6 000 ppm desflurane in 100% oxygen (86).

Airway irritation was studied in 11 male volunteers after inhalation of 11 500 and 23 000 ppm isoflurane and 17 100 and 34 200 ppm sevoflurane for 15 seconds. The order of presentation of the four exposures was random and the interval between inhalations was at least three minutes. Inhalation of both agents

decreased tidal volume, increased respiratory frequency, and decreased functional residual capacity as measured by plethysmography. Activation of the cough reflex was also registered. Sevoflurane did not elicit cough, and overall the effects and subjective rating of airway irritation were relatively stronger for isoflurane (58).

The pungency and tolerability of isoflurane, sevoflurane, and desflurane were compared in a randomised, double-blind study. Eighty-one unpremedicated male patients inhaled 23 000 ppm isoflurane, 40 000 ppm sevoflurane or 120 000 ppm desflurane for 60 seconds from an anaesthetic breathing circuit via a mask (n=27). One sevoflurane patient coughed, but completed the study, whereas 11 isoflurane patients and 20 desflurane patients coughed, objected verbally, or removed the mask forcefully before the end of the study. The irritability grading at 2 MAC was: desflurane > isoflurane > sevoflurane (230).

Conclusion
At anaesthetic dose levels, especially desflurane but also isoflurane irritates the airways. When the irritative effect of isoflurane and desflurane were evaluated at subanaesthetic dose levels (1 200 ppm isoflurane, 6 000 ppm desflurane), no adverse airway events were observed during the 3 minutes of exposure. Since sevoflurane displays lesser irritative effects than isoflurane and desflurane, the above studies indicate that irritative effects of the fluranes would not be expected to occur at the much lower exposure levels present in the workplace.

11.1.2 Sensitisation
There are a few case reports of allergic reactions to isoflurane and sevoflurane. No studies concerning allergic reactions to desflurane have been identified. Contact dermatitis has been reported in anaesthetists exposed to isoflurane (42, 74).

Three cases of occupational asthma and allergy to sevoflurane and isoflurane have been described in anaesthetic staff after more than 3 years of occupational exposure. Evidence for asthmatic reactions to isoflurane and sevoflurane was evaluated giving sequentially increasing exposures to isoflurane or sevoflurane on separate days. Sequential exposures of 3 (2 500 ppm), 5 (2 500 ppm), and 7-10 breaths (3 700-5 000 ppm) were given at 10-minute intervals, provided that the forced expiratory volume in one second (FEV_1) was within 15% of baseline. In one case, sevoflurane exposure increased peak flow variability, decreased FEV_1 (by 32%), and increased the sensitivity to metacholine. The patient subsequently had an anaphylactic reaction to sevoflurane during general anaesthesia. Challenge testing of the second case was negative for both isoflurane and sevoflurane, but the latter resulted in a late asthmatic reaction with increased metacholine sensitivity. The third case developed a skin rash after exposure to isoflurane together with an increase in metacholine sensitivity, but no effect was observed for FEV_1 (238).

Anaphylactic reactions have been described in a patient exposed to isoflurane (220) and in an anaesthetic nurse during general sevoflurane anaesthesia after 4 years of occupational exposure to isoflurane and sevoflurane (238).

11.2 Effects of single and short-term exposure

11.2.1 Central nervous system
Isoflurane, sevoflurane, and desflurane are all used as anaesthetic agents, i.e. they induce hypnosis and amnesia by acting on the central nervous system (193). Thus, all three agents cause sedation and analgesia at anaesthetic dose levels, with MAC-values of 12 000, 21 000 ppm, and 60 000 respectively (Table 4). Recovery from surgical anaesthesia generally occurs within 15-30 minutes, assessed as time from cessation of anaesthesia to response to command and to correct statement of place and date (67). Recovery is fastest after desflurane anaesthesia, followed by sevoflurane and then isoflurane, although the differences in terms of minutes are small (92).

Isoflurane
Peak velocity of saccadic eye movements was studied in 6 volunteers (gender not specified) exposed to 600 and 1 200 ppm of isoflurane for 25 minutes. Saccades were generated by having the subjects follow the movements of an illuminated target, which was presented on a linear array of light-emitting diodes. Mean saccadic peak velocity decreased significantly after exposure to 600 ppm iso-flurane for 15 minutes, and decreased further when the concentration was doubled, as compared with exposure to air (79).

Fourteen healthy volunteers (7 women, 7 men) inhaled vehicle (100% oxygen) and 1 000 and 2 000 ppm isoflurane for 40 minutes each, across separate sessions. Four psychomotor tests were applied (i.e. tests of digit-symbol substitution, logical reasoning, coordination, and auditory reaction time) together with ratings of drug effects. Feeling of sleepiness, tingling, and feeling "high" increased dose-dependently, as did mean inhalant drug effects score, but ratings differed significantly from the control condition at the highest exposure level only. This also characterised the outcome of the psychomotor tests. For the digit-symbol sub-stitution test, performance decreased significantly already at the lowest exposure level (24).

Levels of consciousness and auditory evoked responses were evaluated in 8 healthy male volunteers (anaesthetists) exposed to 0, 1 150, 2 300, and 4 600 ppm of isoflurane in 100% oxygen, at random sessions (total duration of gas exposure per session was 21 minutes) separated by at least one week. Experiments were constructed to allow blinding of both subjects and testers. The volunteers' ability to react on verbal commands and remember lists of words were tested after 10 minutes of exposure. Auditory evoked responses were recorded from electrodes at the subjects' vertex and occipital bone. At the two lowest exposure levels, the eyelash reflex was present in all volunteers. Response to commands was impaired at 1 150 ppm in 3 subjects and at 2 300 ppm in all subjects. None of the subjects responded at the 4 600 ppm level, at which also 5 displayed absence of the eyelash reflex. The amplitudes of the auditory evoked responses decreased and latencies increased progressively with increasing anaesthetic concentration. Differences in auditory evoked responses were small, but inspection of the individual waveforms

showed clear differences as the concentration varied between 0 and 4 600 ppm. One hour after the experiment the subjects were interviewed about their memory of the experiment. At the lowest exposure level, some of the subjects showed impairment of recall of command, even if they apparently reacted normally during the session. Both recall and recognition of neutral words was lost at 2 300 ppm and higher, but 4 of the subjects remembered having heard a "shock" word at 2 300 ppm. No clinically significantly changes (> 10%) occurred in either heart rate or arterial pressure at any exposure level (178, 179).

Ten healthy male volunteers received isoflurane in increasing concentrations for 15 minutes at each exposure level (end-tidal concentrations of 1 900, 3 500 and 5 100 ppm in oxygen). Learning during anaesthesia was tested 1 hour after the end of anaesthesia by means of a category-example task by eliciting whether intra-anaesthetic suggestions influenced subsequent verbal behaviour. Thus, examples from specific categories (e.g. colours, fruits, musical instruments) were presented to the anaesthetised volunteers, who were tested for their memory of these examples after recovering from anaesthesia. Already at the lowest exposure level did isoflurane decrease the percentage of explicitly remembered words, i.e. words the volunteers were aware of hearing during anaesthesia, and a further decrease was observed as exposure levels increased (48).

Effects of subanaesthetic isoflurane concentrations on suppression of memory and responsiveness were evaluated in 17 healthy male volunteers. Each volunteer was studied at four end-tidal concentrations of isoflurane, consecutively 1 920, 3 840, 5 760, and again 1 920 ppm. After 15 minutes of equilibration at each end-tidal concentration, volunteers were tested for voluntary response to command and were presented with verbal information to be recalled after the study. Memory was evaluated the day after exposure in the 12 volunteers completing the study. Both voluntary response and memory decreased in a dose-related manner. At the lowest exposure level, all volunteers responded purposefully, and some responded even at the highest concentration. Memory decreased more steeply than voluntary response by increasing concentrations of isoflurane, and was prevented by 5 760 ppm (60).

A blind, randomised, cross-over trial was conducted to determine the degree of psychomotor/cognitive impairment and the recovery profile produced by exposure to 100% oxygen placebo, 2 000 or 4 000 ppm isoflurane in 4 female and 6 male healthy volunteers. Performance was assessed by the logical reasoning test that measures higher mental processes, recall of lists of words, auditory reaction time, and the digit-symbol substitution test, where the subject with a pencil replaces a number with a corresponding symbol. Fifteen minutes into exposure, both concentrations of isoflurane significantly decreased performance in the logical reasoning test and the digit-symbol substitution test. Only the highest exposure level was associated with significantly decreased performance in the free recall memory test and increased auditory reaction time during exposure. The subjects returned to control-level functioning 5 minutes after cessations of drug inhalation. However, isoflurane induced delayed amnesia, as the mean number of words

correctly recalled 60 minutes after cessation of exposure was decreased in both exposure groups compared to controls (252).

Thirteen voluntary subjects (5 women, 8 men) performed an incidental encoding task for words presented auditorily during the inhalation of 2 600 ppm isoflurane. After termination of isoflurane administration, a 1-minute distractor task and self-assessment of wakefulness were performed. Then free recall of words was assessed together with recording of the event-related potential, by electroencephalography (EEG), during a syllable completion task and during a passive listening task. Eleven non-medicated control subjects (9 women, 2 men) were tested in a similar manner. When memory was assessed, wakefulness of the two groups did not differ significantly. Controls remembered significantly more words in the recall test and showed robust early and late event-related potential repetition effect. The isoflurane group displayed no free recall and showed no event-related potential repetition effect (243).

The subjective, psychomotor, and memory effects of isoflurane were evaluated in a double-blind trial of 9 volunteers (mixed gender) exposed to 0, 3 000 and 6 000 ppm isoflurane. Isoflurane odour was detected by more than half of the volunteers. Isoflurane significantly increased ratings of e.g. confusion and sedation and decreased ratings of body and mind control and impaired immediate and delayed free recall. Isoflurane lengthened auditory reaction time and lowered performance in the eye-hand coordination test. Five minutes after the inhalation period ceased, psychomotor performance returned to baseline levels (251). The statistical analysis does not indicate whether these changes in response differed significantly from the lower to the higher exposure level, but data indicate that isoflurane effects were concentration-related.

Twelve volunteers (mixed gender) breathed 4 000 ppm isoflurane for 20 minutes. Physical and mental tests were performed 35 minutes after initiation of drug administration. Choice reaction time, ability to tap two areas on a board, and ability to perform mathematical problems were significantly impaired by isoflurane inhalation as compared to oxygen inhalation. Performance returned promptly to the baseline after the discontinuation of exposure (172).

In summary, the effects of subanaesthetic doses of isoflurane, ranging from 600 to 6 000 ppm on the central nervous system have been investigated. The lowest test concentration of 600 ppm resulted in conspicuous but reversible neuro-behavioral effects.

Sevoflurane
Volunteers (mixed gender) were presented with a series of 36 emotional and neutral slides while under 0, 1 000, 2 000, or 2 500 ppm sevoflurane in 100% oxygen for at least 20 minutes (n=5-9). Pictures were presented in random order for 6 seconds each and subjects registered their emotional arousal reaction ratings to each slide. Subjects exposed to sevoflurane rated significantly more slides as neutral (rather than emotional) at all exposure levels when compared to placebo. When tested one week later, sevoflurane dose-dependently reduced free recall,

significantly so at the two highest exposure levels. Recall was reduced by 49% when the slides were presented at 2 000 ppm. As measured with a recognition test, a memory boost was evident for emotionally arousing items in the placebo and the 1 000 ppm groups, but was not observed for the 2 000 and 2 500 ppm groups. Although no additional effect on recall was observed when the exposure level was increased from 2 000 to 2 500 ppm, memory performance rates were lowered. In a second experiment, brain activity changes of 11 additional volunteers exposed to 2 500 ppm sevoflurane were assessed with positron emission tomography (PET). Structural equation modeling of PET data revealed that sevoflurane suppressed the effective connectivity between amygdala and hippocampus (8).

Fourteen healthy volunteers (mixed gender) inhaled vehicle (100% oxygen) and 2 000 and 4 000 ppm sevoflurane, for 40 minutes each, across separate sessions. Four psychomotor tests were applied (tests of digit-symbol substitution, logical reasoning, coordination, and auditory reaction time) together with ratings of drug effects. In the digit-symbol substitution test, performance decreased significantly already at the lowest exposure level. Feeling of sleepiness, tingling, and feeling "high" as well as mean inhalant drug effects score increased dose-dependently, but only at the highest exposure level did ratings differ significantly from the control condition. This also characterised the outcome of the psychomotor tests (24).

The effects 0, 2 000, 4 000, and 8 000 ppm sevoflurane in oxygen were studied in 16 light and 16 moderate drinkers (volunteers of mixed gender; average number of weekly drinks 0.7 and 13.9, respectively). During each of four sessions, subjects inhaled a concentration of sevoflurane or 100% oxygen (placebo) for 10 minutes each. Subjective and psychomotor testing commenced 5 minutes into each sampling trial. Psychomotor performance (the digit-symbol substitution test) decreased significantly in moderate drinkers at all three sevoflurane concentration, in light drinkers only at the two highest exposure levels. A number of subjective effects reported during inhalation of sevoflurane were significantly lower in the moderate than in the light drinking group at 4 000 and 8 000 ppm. Generally, only light drinkers reported increased subjective effects at these exposure levels compared to the placebo condition. However, on a drug-effect scale, ratings of the extent the participants currently felt a drug effect were similar in the two groups and differed from the placebo situation at all three exposure levels (253).

Global and regional cerebral blood flows following exposure to escalating concentrations of sevoflurane were estimated by PET in 9 healthy human volunteers (mixed gender). PET scans were performed during baseline and after inhalation of 4 000, 7 000 and 20 000 ppm sevoflurane for at least 10 minutes. Exposure continued for at least 3 min during the PET scanning procedure. Cardio-vascular and respiratory parameters were monitored and EEG and bispectral index were registered. Cardiorespiratory parameters and global cerebral blood flow were unaffected by exposures. Sevoflurane decreased the bispectral index values dose-dependently and changed EEG signals, significantly so at all exposure levels. Regional blood flow increased in some and decreased in other brain areas, already

from the lowest exposure level. Some of the most profound changes in regional cerebral blood flow were observed in structures related to pain processing (216).

Regional cerebral blood flow was measured by functional magnetic resonance imaging in 16 healthy volunteers (mixed gender). Subjects inhaled 5 000 ppm sevoflurane in two cycles of 25 minutes, separated by a control interval of 25 minutes. The cerebral blood flow was measured before, during inhalation of sevoflurane and after termination of anaesthesia. During each assessment, the volunteers were presented with activation tasks: a flickering checkerboard pattern on a screen (visual task), a tone relayed over headphones (auditory task) and instruction to press a button in response to a visual cue (visually cued motor task). All subjects were awake during exposure, although some reported an inability to perform the motor tasks despite understanding the visual cue. Allover, sevoflurane decreased cerebral blood flow by 6.5% (not stated whether the decrease was significant) but in none of 11 regions of interest related to visual, auditory, and motor activation tasks did blood flow decrease significantly from pre-exposure values. However, the regional blood flow during activation tasks increased significantly less during exposure to sevoflurane as compared to the control condition, in the primary and secondary visual cortices, thalamus, hippocampus, and supplementary motor area (196).

Sevoflurane anaesthesia may be associated with epileptiform activity. Thus, sevoflurane may produce epileptiform discharges as observed with EEG as well as clinical seizures. Apparently this effect is dose-dependent and appears only at surgical levels of anaesthesia (55, 122).

Desflurane
No studies evaluating the neurotoxic effect, apart from anaesthesia, were identified.

11.2.2 Cardiovascular system
The studies described below are central for several reviews of the cardiovascular effects of isoflurane, sevoflurane and desflurane (50, 55, 63). The fluranes affect a number of cardiovascular parameters at anaesthetic dose levels. As the depth of anaesthesia increases after induction, haemodynamic variables generally decrease. All three compounds exert hypotensive effects, starting at the lowest tested concentration, i.e. 7 000 ppm for isoflurane, 10 000 ppm for sevoflurane and 30 000 ppm for desflurane (for details see below).

Isoflurane
Isoflurane was administered to 7 healthy, young (20-31 years) randomly selected male volunteers. Haemodynamic and neural measurements were carried out at conscious baseline. After induction of anaesthesia, measurements were repeated 15 minutes after end-tidal isoflurane concentrations of 7 000, 14 000, and 21 000 ppm. With increasing anaesthetic concentration, heart rate increased, and mean arterial and forearm venous pressures decreased progressively from the lowest

exposure level. Efferent muscle sympathetic nerve activity recorded by micro-neurography was decreased at the lowest exposure level (62).

Sevoflurane
Sevoflurane was administered to 12 healthy, young (19-32 years) randomly selected male volunteers. Haemodynamic and neural measurements and blood sampling were carried out at conscious baseline. After induction of anaesthesia, measurements were repeated 10 minutes after end-tidal sevoflurane concentrations of 10 000, 20 000, and 30 000 ppm. With increasing anaesthetic concentration, mean arterial pressure decreased progressively from the lowest exposure level. No changes were observed in heart rate, central venous pressure, plasma nor-epinephrine or in efferent muscle sympathetic nerve activity recorded by micro-neurography (64).

Desflurane
Desflurane was administered to 9 healthy, young (19-32 years) randomly selected male volunteers. Haemodynamic and neural measurements and blood sampling were carried out at conscious baseline. After induction of anaesthesia, measure-ments were repeated 10 minutes after end-tidal desflurane concentrations of 30 000, 60 000, and 90 000 ppm. With increasing anaesthetic concentration, mean arterial and central venous pressures decreased progressively from the lowest exposure level. Heart rate was unchanged by exposure. Efferent muscle sym-pathetic nerve activity recorded by microneurography was increased at the two highest levels and plasma norepinephrine at the highest concentration (64). In a study of almost similar design (exposure levels 36 000, 72 500, and 109 000 ppm; n=7), mean arterial and forearm vascular resistance also decreased progressively already from the lowest exposure level, and heart rate was increased at the highest level (62).

11.2.3 Liver
As described in Chapter 9, halogenated anaesthetics have been associated with two different forms of hepatotoxicity, metabolic and immune-mediated hepato-toxicity, respectively. The latter occurs in a small number of patients. The incidence for halothane is 1:35 000, and occupational exposure to halothane has also been shown to induce hepatotoxicity. The incidences of hepatotoxicity after anaesthesia with isoflurane and desflurane are presumably much lower, due to the very limited metabolism of these substances. In a few case reports, hepatitis after isoflurane and desflurane anaesthesia have been described (26, 32, 44, 162, 214, 219). Also, anti-trifluoroacetyl antibodies were detected in the sera of 3 patients diagnosed with isoflurane hepatitis (184). However, in 1987 a panel of experts reviewed 45 cases of liver injury following isoflurane anaesthesia. They concluded that current evidence did not indicate a reasonable likelihood of an association between the use of isoflurane and the occurrence of postoperative hepatic dys-function (225).

As investigated at anaesthetic dose levels in a large number of patients, no findings indicate that sevoflurane impairs liver function (55, 131, 200). Abott Laboratories evaluated liver function tests from a total of 3 684 patients from 22 controlled, comparative clinical studies with 2 069 patients receiving sevoflurane, and 1 615 patients receiving either isoflurane, enflurane, halothane or propofol. No clinically significant adverse effects on liver function were observed with sevoflurane or other anaesthetics (described by Delgado-Herrera *et al* (55)). The lack of liver effects of sevoflurane is probably due to its distinctly different metabolic pathway compared to the other halogenated anaesthetics (200).

Isoflurane
Hepatocellular integrity was monitored in 20 patients (mixed gender) who received long-term sedation (38 hours, ranging from 24-127 hours) with 1 000-6 000 ppm isoflurane. Blood samples were collected before, 24 hours after the start of the sedation, and 0, 24, 72, 120, and 172 hours after termination of anaesthesia. The activities of alanine aminotransferase and aspartate amino-transferase did not change appreciably, but glutathione transferase B_1 concentration decreased significantly at all times studied. The enzyme activity/ concentration was similar in isoflurane patients and in 20 patients who received long-term sedation with low dose midazolam (114).

Repeated anaesthetic exposure to isoflurane in 66% oxygen and nitrous oxide was investigated with respect to effects on liver function in 11 children (gender not specified) presenting daily for radiotherapy. The average number of exposures were 24 (range 10-39) and total anaesthetic time per exposure varied between 15 and 30 minutes. Isoflurane concentrations ranged from 20 000 to 50 000 ppm. Liver function was assessed by determining serum total bilirubin, aspartate amino transferase, gamma glutamyl transferase, and alkaline phosphatase before the start of treatment and every 5 days thereafter. There was no measurable change in any of the monitored biomarkers (126).

Isoflurane and sevoflurane
Sixty patients had their liver function assessed after repeated exposure within 30 to 180 days to isoflurane (30 patients) or sevoflurane (30 patients), in 67% nitrous oxide and oxygen. Approximately 4 MAC-hours were administered to each patient, roughly corresponding to 46 000 ppm-hours isoflurane and 68 000 ppm-hours sevoflurane. Serum values were measured before and 1, 3, 7, and 14 days after surgery. Aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase increased with peaks 7 days after surgery, and the number of patients with abnormal values was larger in the isoflurane than in the sevo-flurane group. None of the variables showed differences between the first and second anaesthesia, indicating that repeated exposure impounded no additional risk (183). Another study of 10 patients undergoing sevoflurane anaesthesia twice within 30-90 days also demonstrated changes indicative of mild liver and kidney injury, but repeated exposure did not enhance these changes (182).

Desflurane
No studies on liver effects were identified.

11.2.4 Kidney
Isoflurane and sevoflurane
Sixty patients were studied for kidney function after repeated exposures within 30 to 180 days to isoflurane or sevoflurane (30 patients per group, mixed gender) for approximately 4 MAC-hours in 67% nitrous oxide and oxygen. This corresponds roughly to 46 000 ppm-hours isoflurane and 68 000 ppm-hours sevoflurane. Urinary qualitative analyses were performed 1, 3, and 7 days after surgery. Blood urea nitrogen and creatinine were within the normal range after anaesthesia in either group. Renal excretion of protein and glucose was increased 1 and 3 days after anaesthesia with no difference between the anaesthetics. None of the variables differed between the first and the second anaesthesia (183)

Sevoflurane
Mazze *et al* (2000) retrospectively evaluated pooled renal laboratory data from 22 different clinical trials that compared sevoflurane with three other widely used anaesthetics. The trials examined postoperative changes in serum creatinine and blood urea nitrogen levels from a total of 3 436 adult surgical patients administered either sevoflurane (n=1 941) or a control drug (isoflurane, enflurane, or propofol) as the maintenance anaesthetic (n=1 495). The incidences of increased serum creatinine and blood urea nitrogen concentrations were similar among patients administered sevoflurane and those administered control drugs. The data for changes in serum creatinine and blood urea nitrogen indicate that, for exposures of less than 80 000 ppm-hours, sevoflurane is not associated with an increased risk of renal toxicity compared with other commonly used anaesthetics (169).

In an earlier review, results from five studies of more than 750 individuals administered sevoflurane indicated that serum fluoride exceeded 50 µM in 7.7% of the cases. However, no evidence links sevoflurane with impaired renal function, as investigated at anaesthetic dose levels in this large number of patients. Furthermore, there is no evidence for exacerbation of pre-existing hepatic dysfunction in adults or children (55).

Regarding smoking and exposure to sevoflurane, effects on kidney function and fluoride in serum were investigated in 25 non-smoking and 25 smoking (> 10 cigarettes/day) women (19-68 years), undergoing gynaecological elective surgery under standardised anaesthesia with 27 000 ppm sevoflurane for 45 minutes. Glomerular and tubular function was assessed by measuring serum and urine tumour-associated trypsin inhibitor, β_2-microglobulin, and serum creatinine for 48 hours after sevoflurane inhalation. There were no differences between the two study groups with regard to serum fluoride or any other measures. Serum fluoride increased significantly in both groups: in non-smokers, from a baseline of 1.0-11 µmol/l (median, 1.6 µmol/l) to a maximum of 8.2-40 µmol/l (26 µmol/l) and, in smokers, from a baseline of 0.5-5.2 µmol/l (1.7 µmol/l) to a maximum of 19-71 µmol/l (25 µmol/l). Serum fluoride remained elevated for the entire sampling

period. In all 5 women (1 non-smoker and 4 smokers) with a maximum serum fluoride of 40 μmol/l or higher and an area under the serum fluoride concentration-time curve of 500 μmol/h/l or higher, serum tumour-associated trypsin inhibitor increased above the pathological concentration of 3.0 nmol/l, whereas only 6 of the 45 patients with serum fluoride below 40 μmol/l had serum tumour-associated trypsin inhibitor above 3.0 nmol/l. β_2-Microglobulin increased significantly (> 1 mg/l) in 2 of the 5 patients with high serum fluoride relative to 2 of the 45 patients with serum fluoride below 40 μmol/l. None of the patients developed clinically detectable renal dysfunction (153).

Desflurane
No studies on kidney effects were identified.

11.2.5 Other effects of single and short-term exposure
In vivo nasal mucociliary clearance was evaluated before and after exposure to isoflurane, sevoflurane and desflurane in a prospective, randomised, double blind study. Patients scheduled for ear and neck surgery under general anaesthesia were randomised into three groups (n=20). Each group received 11 500 ppm isoflurane, 20 000 ppm sevoflurane or 60 000 ppm desflurane during maintenance of anaesthesia, induced intravenously with propofol and remifentanil. A saccharin granule was placed on the inferior nasal concha (or turbinate), 1 cm behind the anterior end. Mucociliary clearance time was assessed by the time it took the patient to recognise sweet taste, taken to represent transport of saccharin to the oropharynx. The time was recorded before and after anaesthesia. The mean clearance values were similar for the three anaesthetics as were values pre and post anaesthesia for each gas (133).

Systemic redox balance was investigated in patients undergoing laparohysterectomy (n=20) under anaesthesia maintained with 20 000 ppm sevoflurane or 60 000 ppm desflurane (induced with 3 mg/kg sodium thiopental). Anaesthesia was maintained for at least 60 minutes. Systemic redox balance was evaluated by measuring blood concentration of glutathione, plasma antioxidant capacity (Trolox equivalent antioxidant capacity), and lipid peroxidation products (malondialdehyde and 4-hydroxynonenal protein adducts). Blood were sampled prior to anaesthesia, after 60 minutes, and 24 hours postoperatively. At 60 minutes, anaesthesia with sevoflurane was associated with significantly decreased plasma glutathione and general antioxidant capacity. A similar pattern was observed for desflurane, although values did not differ significantly from preanaesthesia values. Both anaesthetics seemed to shift the redox balance slightly towards oxidation (49).

11.3 Effects of long-term exposure

Isoflurane

Two groups of health professionals were studied (gender not specified). Eighty subjects were judged to be occupationally at potential hepatotoxic risk due to exposure to isoflurane (mean 0.16 ppm, SD 0.12) and nitrous oxide (30 ppm, SD 29) for up to 6 hours daily, 30-36 hours a week, for a period varying from 2 to 27 years. Ninety-two subjects with similar job titles but who were at no known hepatotoxic risk comprised the comparison group. Medical history with attention to liver and blood pathology and alcohol intake was collected. The environmental concentrations of nitrous oxide and isoflurane were measured in the breathing zone during a 2-hour period by personal samplers, and nitrous oxide and iso-flurane were measured in urine collected at the end of the work shift. Blood samples were drawn on the same day. Serum alanine aminotransferase and aspartate aminotransferase and erythrocyte mean corpuscular volume did not differ significantly between exposed and control subjects. Alcohol consumption, but not anaesthetic exposure, was significantly correlated to an increase in aspartate aminotransferase (77).

Neurofunction was investigated in 112 operating theatre workers exposed to anaesthetic gases (nitrous oxide and isoflurane) and 135 non-exposed hospital workers from 10 Italian hospitals (mixed gender, mostly women). The exposed and control subjects were comparable for both basic intellectual abilities and subjective stress levels. Before and after the shift on the first and the last day of a 4-day working week, three different tests were administered: a complex reaction time test, a questionnaire for neuropsychological symptoms, and a test of visuo-spatial conceptualisation ability. Urine samples were collected for each subject after the end of the shift, and time integrated exposure levels during the shift were monitored by means of stationary sampling. In the exposed population, the end of shift urinary isoflurane level was 0.7 µg/l (95th percentile 2.6, range 0-4.7) on the first day, and 0.8 µg/l (95th percentile 2.0, range 0-5.6) on the last. Atmospheric concentrations in operating theatres ranged from 0.1 to 6.2 ppm isoflurane, with an average value of approximately 0.35 ppm. No statistical differences were observed between exposed and control subjects for neuropsychological tests and symptoms. No dose-effect relationships were indicated by the exposure indicators combined with the test results (159).

Sevoflurane

A single study evaluated the renal effects by occupational exposure to sevoflurane (232). Exposure of 61 operating room staff members to nitrous oxide and sevo-flurane was monitored by personal samplers and analysis of urine. In urine, also total urinary proteins, N-acetyl-β-D-glucosaminidase, and glutamine synthetase were measured. The latter was also measured in 43 control hospital staff, not occupationally exposed to anaesthetics. The mean (range) environmental sevo-flurane level was 0.28 (0-1.88) ppm, hereof 0.41 (0.02-1.88) ppm in operating areas using open anaesthetic circuits, and 0.18 (0-1.4) ppm in areas with semi-

closed anaesthetic circuits. Subjects working in areas with open circuits excreted significantly more sevoflurane in urine than subjects working in areas with semi-closed circuits. Total urinary protein was slightly, but significantly, increased in exposed subjects compared to controls although all values were within the normal range. Total urinary protein did not correlate with indices of anaesthetic dose. For persons working with open, but not semi-closed anaesthetic circuits, positive correlations were observed for exposure to both nitrous oxide and sevoflurane with N-acetyl-β-D-glucosaminidase and glutamine synthetase. No significant correlation between length of exposure and urinary indices were observed. The authors concluded that no relevant effect on the kidney was present at the levels of exposure studied (232). Judging from the depiction of correlations of anaesthetic dose with urine biological measures, the reported positive correlations may well depend heavily on one single high value.

Isoflurane, sevoflurane and desflurane
The effect of chronic exposure (more than 5 years) to anaesthetic gases on balance control was tested in 53 operating room personnel (nurses and auxiliary nurses) and compared to 53 non-exposed individuals of the same occupational groups and of similar age, employment duration and postural demand (gender not specified). The subjects were not exposed to anaesthetic gases or alcohol during 48 hours before the test session. Exposed workers showed a poorer quality of balance control, especially when tested with their eyes closed. The exposure was not described in detail, but was reportedly mainly nitrous oxide (59 ppm, range 10-100 ppm). The total concentration of isoflurane, sevoflurane and desflurane altogether was about ten times lower, i.e. approximately 6 ppm (246).

11.4 Genotoxic effects

The studies described below are summarised in Table 12.

Isoflurane
The frequency of SCEs in peripheral lymphocytes from 27 non-smoking operating room personnel exposed to isoflurane and nitrous oxide was compared to 27 non-smoking controls (mixed gender). Occupational exposure was recorded in the breathing zone of the anaesthetists using a direct reading instrument. The 8-hour TWA of nitrous oxide was 11.8 ppm and that of isoflurane was 0.5 ppm. The mean frequency (±SD) of SCEs was increased significantly in exposed personnel (9.0 ± 1.3) as compared to control personnel (8.0 ± 1.4). Because the personnel were exposed to both isoflurane and nitrous oxide, it is unclear, which anaesthetic caused the increase in SCEs (111).

Genetic damage was measured as SCEs and micronuclei in lymphocytes of peripheral blood of operating room personnel (mixed gender) exposed to 12.8 ppm nitrous oxide and 5.3 ppm isoflurane (8-hour TWA). The mean frequency of SCEs was significantly higher (10.2 ± 1.9) in exposed personnel (n=10) than in controls (7.4 ± 2.4) (n=10). The proportion of micronuclei in exposed personnel

was also higher than in controls, but not significantly so. Because the personnel were exposed to both isoflurane and nitrous oxide, it is unclear, which anaesthetic caused the increase in SCEs (107).

The mutagenicity of isoflurane and nitrous oxide was investigated by the SCE test using peripheral blood lymphocytes from 30 patients (mixed gender) before and after anaesthesia. There was no increase in SCEs in the patients immediately after completion of anaesthesia or on the following day when compared to the SCEs before anaesthesia. However, an increase in SCEs compared with pre-anaesthetic values was observed in a subgroup of 11 cigarette smokers (115).

The mutagenicity of isoflurane and nitrous oxide was investigated by the SCE test using peripheral blood lymphocytes from 63 cigarette smoking patients (mixed gender) before and after anaesthesia. There was no increase in SCEs in the patients immediately after completion of anaesthesia or on the following day when compared to the frequency of SCEs before anaesthesia (116).

DNA single strand breaks were investigated in peripheral lymphocytes of patients (mixed gender) before and after 180 minutes exposure to a mixture of isoflurane, nitrous oxide, and oxygen. The frequency of DNA single breaks was increased immediately after anaesthesia. No increase in strand breaks could be detected the following day (201).

DNA single strand breaks using the alkaline single cell gel electrophoresis technique (SCGE-comet assay) were investigated in peripheral lymphocytes of 12 patients (mixed gender), before and after anaesthesia and in a control group. Twelve patients, aged 22-66 years old, were anaesthetised for elective abdominal surgery with isoflurane in oxygen for 120-162 minutes (mean 133.2 minutes). Venous blood samples were obtained from the patients before the induction of anaesthesia, at 60 and 120 minutes of anaesthesia, and on the 1st, 3rd, and 5th day after anaesthesia. The number of undamaged nuclei was almost the same in controls and in patients before anaesthesia. However, significant differences were observed in the proportion of undamaged, intermediate, and tailed nuclei of patients at 60 and 120 minutes of anaesthesia and on the day following anaesthesia. DNA damage started to normalise on the 3rd day after anaesthesia, and was almost identical with that of controls after 5 days (212).

The genotoxic activity of isoflurane was evaluated by the comet assay by examining peripheral blood lymphocytes of patients (mixed gender) before, during and after anaesthesia as compared to an unexposed control group. Significant differences were observed in the proportions of undamaged, intermediate, and tailed nuclei of patients after 60 and 120 minutes of anaesthesia to 10 000-15 000 ppm isoflurane (n=12) and on the following day, although DNA damage began to return to normal levels on the 3rd day following anaesthesia and was almost identical with those of controls on the 5th day (127).

Sevoflurane

The genotoxicity of sevoflurane was studied by evaluating the DNA damage, apoptosis, DNA repair enzyme activity, and glutathione content in peripheral lymphocytes from 20 patients (mixed gender) before, 15 minutes after anaesthesia, and 24 hours after surgery. Lymphocytes isolated 15 minutes after anaesthesia showed an increase in oxidised purine and pyrimidine bases without DNA strand break formation. DNA strand breaks occurred on the 1st post-operative day, associated with an enhancement of DNA repair activity and a decrease in glutathione. Formation of strand breaks could be the consequence of DNA repair activity. Twenty-four hours after surgery, most of the oxidised DNA bases were repaired (9).

The incidence of SCEs in peripheral lymphocytes of anaesthetists (n=25, mixed gender) exposed to anaesthetics was investigated before and after a 2-month leave. There was a significant difference in SCE values of the anaesthetists compared with the non-exposed physicians (11.9 ± 4.4 versus 4.2 ± 1.1). After a 2-month leave from the operating room, the SCE values of the anaesthetists were significantly lower compared with those taken before the leave (4.8 ± 1.8 and 11.9 ± 4.4, respectively). The concentrations of sevoflurane and nitrous oxide in the operating rooms were 8.9 ± 5.6 ppm and 119 ± 39 ppm, respectively, as determined by infrared spectrophotometry measured close to the working anaesthetists. Because the anaesthetists were exposed to a mixture of anaesthetics it is not possible to decide which anaesthetics caused the effects (72).

The formation of SCEs was investigated in lymphocytes of 40 children (mixed gender) before and after anaesthesia induced by inhalation of up to 80 000 ppm sevoflurane and maintained at 25 000-30 000 ppm in oxygen/nitrous oxide (65%/35%). The combined sevoflurane and nitrous oxide anaesthesia did not induce SCE in T-lymphocytes of children (142).

Significant differences were observed in the proportions of undamaged, intermediate, and tailed nuclei of patients (mixed gender) after 60 and 120 minutes of anaesthesia to 10 000-15 000 ppm sevoflurane (n=12) and on the following day. DNA damage began to return to normal levels after the 3rd day following anaesthesia and was almost identical with those of controls on the 5th day (127).

Desflurane

Only one study of the genotoxic effects of desflurane in humans has been identified. The frequency of SCEs was evaluated in peripheral blood lymphocytes of 15 female patients anaesthetised with 50 000-60 000 ppm desflurane in an oxygen/air mixture. Blood samples were taken before anaesthesia and 60 and 120 minutes after the initiation of anaesthesia. In addition, postoperative blood samples were taken on the 1st, 3rd, 7th, and 12th day. The number of SCEs per cell was increased 60 and 120 minutes, and 1, 3, and 7 days after initiation of anaesthesia. On day 12, the number of SCEs per cell had returned to the level preceding anaesthesia (5).

Table 12. Genotoxic effects in peripheral lymphocytes from humans exposed to the fluranes.

Anaesthetic/ co-exposure	Exposure condition	Effect	Reference
Isoflurane			
N$_2$O	Occupational	Increased SCEs.	(107)
N$_2$O	Occupational	Increased SCEs.	(111)
N$_2$O	Patients	No effect on SCEs.	(115)
N$_2$O	Patients	No effect on SCEs.	(116)
N$_2$O	Patients	Increased DNA strand breaks.	(201)
	Patients	Increased DNA strand breaks.	(212)
	Patients	Increased DNA strand breaks.	(127)
Sevoflurane			
N$_2$O	Occupational	Increased SCEs.	(72)
N$_2$O	Patients (children)	No effect on SCEs.	(142)
	Patients	Increased DNA strand breaks.	(127)
	Patients	Increase in oxidised DNA bases without DNA strand breaks (15 min after anaesthesia). Increase in DNA strand breaks, repair activity, number of apoptotic cells and decrease in GSH level (24 h after anaesthesia).	(9)
Desflurane			
	Patients	Increased SCEs.	(5)

GSH: glutathione, N$_2$O: nitrous oxide, SCE: sister chromatid exchange.

Summary and conclusion

For isoflurane, four SCE studies evaluating the genotoxic effects in humans were identified. The two studies performed in patients were negative (115, 116), whereas the two studies in occupationally exposed showed increased frequencies of SCEs (107, 111). Studies evaluating genotoxicity by the comet assay in lymphocytes from patients anaesthetised with isoflurane all showed increased DNA damage immediately after anaesthesia (127, 201, 212). In all the studies, the DNA damage was repaired within 1-5 days after the end of anaesthesia. As DNA repair is regulated genetically (85), isoflurane induced DNA damage could be hypothesised to persist longer in persons with DNA repair defects.

One study of personnel occupationally exposed to both sevoflurane and nitrous oxide showed an increased number of SCEs (72), while a study of patients exposed to sevoflurane and nitrous oxide showed no effect (142). Two studies of patients exposed only to sevoflurane showed increased levels of DNA strand breaks (9, 127).

Only one study of genotoxicity of desflurane in humans has been identified. The study showed increased number of SCEs in patients exposed to desflurane (5).

All three studies on occupational exposure to isoflurane and sevoflurane demonstrated increased frequencies of SCEs. However, it should be noted that in these as well as in several of the studies in patients, co-exposure to nitrous oxide

occurred. It is therefore not clear which agent caused the genotoxic effects. However, SCEs have not been clearly associated with exposure to nitrous oxide (174). The isolated effect of the three fluranes has only been studied in patients. All these studies showed genotoxicity (increases in DNA strand breaks or SCEs) after surgery and at anaesthetic dose levels. We note that the observed increases in SCEs or DNA strand breaks could be due to premedication. Together, these studies give rise to some concern regarding the genotoxic potential of the fluranes.

Genotoxicity studies on human lymphocytes exposed *in vitro* to the fluranes have been described in Section 10.4. All three studies on isoflurane and the one on desflurane were positive (increases in SCEs and DNA strand breaks) whereas the only one on sevoflurane was negative (no increases in DNA strand breaks).

11.5 Carcinogenic effects

No studies evaluating the carcinogenic effects of the specific fluranes have been identified. One explanation to the lack of studies is that isoflurane and in particular sevoflurane and desflurane are new anaesthetics (introduced in the middle of the 1980s and 1990s), and cancer generally takes a long time to develop. Thus, it is not yet possible to evaluate carcinogenic effects in epidemiological studies. Furthermore, since health care personnel are exposed to a mixture of anaesthetics and other potential genotoxic agents such as X-rays, the effect of exposure to a single anaesthetic may be difficult to deduce from epidemiological studies.

11.6 Reproductive and developmental effects

Following uptake, the fluranes are rapidly distributed in the body. It has been shown that isoflurane and sevoflurane pass almost unimpeded from maternal to foetal blood (Section 7.2). This has not been investigated for desflurane, but, due to the structural similarities of the three compounds, desflurane probably also passes from maternal to foetal blood.

Isoflurane
The effect of anaesthesia on pregnancy outcome after gamete intrafallopian transfer during assisted reproduction was investigated in a multi-centre survey. US fertility programs were invited to contribute information for women who underwent this procedure in 1993-1994 concerning age, the use of propofol, nitrous oxide, midazolam, or a potent inhaled anaesthetic agent, pregnancy, and delivery rate. Seven medical centres participated and contributed data from 455 women. No significant differences were found for the clinical pregnancy or delivery rates between those women who received propofol, nitrous oxide, midazolam, or isoflurane during the procedure and those who did not (25).

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Sevoflurane and desflurane
Fifty healthy women undergoing caesarean section and requesting general anaesthesia were randomly assigned to receive either 10 000 ppm sevoflurane or 30 000 ppm desflurane in combination with 50% nitrous oxide in oxygen. Twenty-five healthy women requesting regional anaesthesia received epidural anaesthesia with ropivacaine. The three groups of women displayed similar characteristics with respect to age, weight, height, parity, and gestational age. No significant differences were detected on neonatal outcomes assessed as Apgar scores 1 and 5 minutes after delivery, and by neurological adaptive capacity score (20 criteria in 5 areas: active tone, adaptive capacity, general assessment, passive tone and primary reflexes) 2 and 24 hours after delivery (128).

Anaesthetic gases in general
Several epidemiological studies have investigated the association between occupational exposure to anaesthetic gases in general and reproductive failure in women. These are described below. The results indicate that such exposure may increase the risk for spontaneous abortions and the incidence of malformations in the children. However, in these studies, hospital staff and other occupational groups were exposed to a mixture of anaesthetic gases, and no information on duration and actual levels of exposure were available. In addition, all studies were retrospective with the risk of recall bias, if couples with a reproductive failure will be motivated (by guilt or concern) to remember more adverse exposures (and outcomes) than couples with normal pregnancies (237).

In a retrospective survey in the Netherlands (189, 207), referred in (99), time to pregnancy was investigated in 427 pregnant women employed in anaesthesia. These were compared to 1 010 pregnant women employed in departments of orthopaedics, gynaecology, or surgery. Time to pregnancy was not affected in operation chamber assistants. The concentrations of several anaesthetic gases were measured and the maximal concentration measured for isoflurane was 120 mg/m^3 (15.6 ppm). However, the composition of the anaesthetic gas mixtures and the level and duration of exposure were not reported.

The outcome of a meta-analysis on studies on spontaneous abortion indicates an increased risk after occupational exposure to anaesthetic gases (28). The analysis was based on epidemiological studies published during the period 1984-1992, estimating the risk of spontaneous abortion. Only peer-reviewed studies were included. Several comparisons (n=24) between exposed and unexposed women were included, obtained from 17 retrospective follow-up studies and 2 case-control studies. Occupational exposure to anaesthetic gases was mostly assessed through postal questionnaires to the mothers, or less often, by interviews. None of the studies included ambient gas sampling. Exposed women included nurses, physicians, technicians, dental assistants, veterinarians and veterinary assistants. Unexposed reference groups generally included women from the same occupational groups, but unexposed to anaesthetic gases. Spontaneous abortions were generally reported by respondents without further validation. Altogether,

the follow-up studies in the meta-analysis included 15 268 exposed pregnancies and 80 368 control (unexposed) pregnancies. The overall relative risk was 1.48 (95% confidence interval (CI) 1.4-1.58). The relative risk varied according to occupation, ranging from 1.18 among physicians to 2.45 in veterinarians and veterinary assistants. To test whether the quality of the studies influenced this result, the validity of the reviewed papers was rated on the basis of three criteria: appropriateness of the unexposed comparison group, control for non-occupational confounding variables, and response rate. The estimate of risk increased to 1.9 (95% CI, 1.72-2.09) when analysis was restricted to the six comparisons which were rated the most rigorous. Year of publication of the study did not influence the relative risk (28). This meta-analysis included studies published in 1984-1992. Isoflurane has been used from the early 1980s, whereas desflurane and sevoflurane are relatively newer (introduced in Denmark in 1994 and 1996, respectively). It must therefore be assumed that of the three fluranes described in this report, only isoflurane figures as an anaesthetic agent in this meta-analysis, together with other anaesthetic agents.

Female anaesthetists (n=231) practicing primarily paediatric anaesthesia were hypothesised to experience increased exposure to trace anaesthetic agents due to increased waste spillage (because of the use of mask inductions and uncuffed tracheal tubes), and thereby a greater risk of obstetric complications, compared to female anaesthetists (n=1 275) performing primarily adult anaesthesia. Question-naires were mailed to all female members of Society for Pediatric Anesthesia and to an equal number of randomly selected female members of American Society of Anesthesiologists. Subjects were explained the purpose of the study and asked to answer questions regarding their former pregnancy outcomes, work history and personal habits. Paediatric anaesthetists were defined as those having >75% paediatric practice. Significantly more paediatric anaesthetists experienced more than 40 hours/week of operating room exposure during their pregnancies than did non-paediatric anaesthetists (79.2% versus 70.6%). Analysis was performed on a total of 1 506 pregnancies. No differences were observed with respect to preterm labour or delivery, birth weight, or birth defects. However, the prevalence of spontaneous abortion was significantly higher among paediatric than among non-paediatric anaesthetists (80). The actual study period is not stated for this study, and no information is given as to exposure to specific anaesthetic gases.

A retrospective study of 6 330 personnel exposed to anaesthetic gases in operating and recovery rooms in Ontario hospitals, and 2 200 non-exposed hospital staff were conducted by questionnaire during the period 1981-1985. A total of 10 232 pregnancies were reported by the exposed women or spouses of exposed men. The number of unexposed pregnancies was 3 656. Logistic regression analysis, with age and smoking standardised, showed that children of women in the exposed group had significantly more congenital abnormalities (odds ratio (OR) 2.24, 95% CI 1.69-2.97). This was also true for wives of male hospital staff (OR 1.46, 95% CI 1.04-2.05) (88). The concentration of the

anaesthetic gases is unknown in this study, and the study period at the most includes only a few years of exposure to isoflurane (used from 1984 in Denmark).

The probably most recent study with respect to time-period of exposure, concerns women working in specified hospital departments for at least 2 months in the period 1990-1997. Questionnaires were mailed to 5 546 women. A total of 427 women were defined as exposed, as they were working during the first months of their last pregnancy. This group was composed of anaesthetists and assistants and operating room nurses. As referents, 1 010 nurses not exposed to anaesthetics or cytostatics were included. ORs corrected for differences in occupational factors indicated that exposure to anaesthetic gases increase the risk for giving birth to a child with malformation (OR 1.8, 95% CI 1.0-4.1). Exposure to anaesthetic gases is presumably highest during the induction phase and during tonsillectomy. Specific risks for these tasks were calculated, resulting in ORs of 1.6 (95% CI 0.9-2.5) and 1.9 (95% CI 1.1-3-6), respectively. The risk of prolonged time to pregnancy, spontaneous abortion, and premature birth were similar in the two groups (207). No information regarding exposure to specific anaesthetic gases was provided.

12. Dose-effect and dose-response relationships

Effects of inhalation exposures to isoflurane, sevoflurane and desflurane in animals are listed in Tables 13-15 and in humans in Tables 16-19. Acute, high exposures are included in the tables if levels were below 0.8 of the human MAC-value, i.e. 9 600 ppm for isoflurane, 16 800 ppm for sevoflurane, and 48 000 ppm for desflurane, as these concentrations are associated with pronounced acute effects in humans (178, 179). Genotoxicity studies in animals and humans are listed in Tables 11-12 (Sections 10.4 and 11.4).

12.1 Animal and *in vitro* studies

Isoflurane
Single exposures to 530 or 1 160 ppm isoflurane for 45 minutes were without effects on cognitive function and pain sensitivity in rats (6). However, anti-analgesic effects of isoflurane were observed in rats exposed to 1 145 ppm for 30-40 minutes, based on a group size of 3 animals serving as their own controls (255). Thus, the acute NOAEL and LOAEL in rats are 530 and 1 145 ppm, respectively. Several studies in rats or rabbits have showed decreased cognitive function and analgesic effects at exposure levels of 2 000 ppm and above (Table 13).

Several studies have addressed the effects of repeated exposures to isoflurane on body weight, reproduction and development, and liver function and histology at exposure levels ranging from 150 ppm and up. The effects of 35 days of continuous exposure to 150, 500, and 1 500 ppm isoflurane were investigated in mice, guinea pigs, and rats. Mice exhibited reduced body weight gain to a similar extent

in all three exposed groups, at the two highest exposure levels already from day 7. Guinea pigs gained less weight at the highest exposure level, whereas rat weights were unaffected by exposure. At the highest exposure level, a tendency towards an increase in the number of animals with degenerative liver lesions (not significant) was observed in all species (224). The study seems well designed and performed although some miscalculations of body weight gains are apparent from the tables, and only body weight gains, not absolute body weights were stated. In another short-term animal study (4 hours/day for 5 days/week, 9 weeks), body weight was unaffected until 5 000 ppm in mice (202).

Male fertility in mice seemed unaffected at exposure levels ranging from 1 000 to 10 000 ppm (154, 166). One study in rabbits reported decreased sperm counts after exposure to 13 000 ppm isoflurane, but interpretation is hampered by control group fluctuations (45). None of the fertility studies investigated the full spermatogenic cycle. At 4 000 ppm and below, developmental toxicity studies consistently showed no effect of isoflurane (78, 166, 167). Exposure to 6 000 ppm, 4 hours/day during organogenesis, reduced foetal weight and maternal weight gain, decreased ossification, and was associated with increased incidence of minor malformations often observed during retarded development and increased maternal stress (167). In rabbits, no maternal or foetal effects were observed after exposure to 23 000 ppm isoflurane, 1 hour/day, during early gestation (132).

A long-term study in mice did not report detectable toxicity to the liver or the kidneys after continuous exposure to 20 ppm isoflurane for 30 weeks (191). Exposure to 1 000 or 4 000 ppm isoflurane, 4 hours/day, 5 days/week for 78 weeks was not associated with carcinogenicity. However, body weights were decreased at both exposure levels, by 1-5% and 5-8%, respectively (no statistical analysis was provided for these data) (23).

Studies evaluating genotoxic effects of isoflurane in human lymphocytes exposed *in vitro* (110, 123, 227) and in rats exposed *in vivo* (137, 206) were positive, whereas tests in bacteria, *Drosophila melanogaster* and Chinese hamster cells were negative (20, 22, 144, 233, 236, 241).

Altogether, 150 ppm can be regarded as an overall LOAEL for isoflurane.

Sevoflurane
Analgesia was evident in rats after 45 minutes of exposure to 1 050 ppm (acute LOAEL) and above, and exposure to 1 100 ppm sevoflurane for 45 minutes caused enhanced aversive memory formation. Cognitive function was not affected in rats exposed at 1 050 and 2 160 ppm, whereas memory impairment was observed at 3 080 ppm and above (6, 7). Activity level and cognition were affected in rats exposed for 30 days, 4 hours/day to 3 000 ppm sevoflurane, the lowest exposure level tested (188). At anaesthetic dose levels (21 600-40 100 ppm, 8 weeks), an exposure dependent reversible increase in the levels of liver enzymes in plasma was observed in cynomolgus monkeys. Apart from reduced blood cell counts and relative thymic weight in exposed animals, no exposure-related gross

pathological, histopathological or ultrastructural differences were found. Three monkeys in the high exposure group died (221) (Table 14).

No studies on long-term exposure were identified.

Considering genotoxicity studies, no increased frequency of DNA strand breaks was observed in human lymphocytes exposed *in vitro* (227) and no effect on gene mutation was observed in bacteria (21) whereas rats exposed *in vivo* (orally) had an increased frequency of micronucleated cells in the kidney (206).

Desflurane

Only three studies at subanaesthetic dose levels of desflurane were identified, all in rats (Table 15). Impaired cognitive function was observed after 45 minutes of exposure to 4 400 ppm desflurane (acute LOAEL). Effects increased further at higher exposure levels, i.e. 10 130 and 20 200 ppm (6). Activity level and cognition were altered in rats exposed to 6 000 ppm desflurane for 30 days, 4 hours/day (measured after 1 day of no exposure) (188).

No studies on long-term exposure were identified.

In the only study evaluating genotoxic effects, a dose-dependent increase in DNA strand breaks was observed in human lymphocytes exposed *in vitro* (129).

12.2 Human studies

Tables 16-19 summarise dose-effect relationships in humans after inhalation exposure to isoflurane, sevoflurane and desflurane.

Isoflurane

In a range of studies, neurofunction in humans under experimental conditions has been investigated. At the lowest tested level, exposure to 600 ppm isoflurane for 25 minutes was associated with decreased saccadic peak velocity (acute LOAEL) (79). Exposure to 1 000 ppm isoflurane for 40 minutes was associated with decreased performance in the digit-symbol substitution test (24). Most other studies investigating neurobehaviour above this exposure level also demonstrated effects of isoflurane (Table 16).

Chronic occupational exposure to low levels of isoflurane did not affect liver function (0.16 ppm, sampled in breathing zone) (77) or neurofunction investigated by several tests (0.35 ppm, stationary sampler) (159). Two studies demonstrated increased frequencies of SCEs in peripheral lymphocytes from health care personnel exposed to 0.5 and 5.3 ppm isoflurane (8-hour TWAs, personal and stationary sampling, respectively) (107, 111). However, the personnel in both studies were concomitantly exposed to 12-13 ppm nitrous oxide (Table 19). It is therefore difficult to ascribe the effect to isoflurane, although SCEs have not been clearly associated with exposure to nitrous oxide either (174). In patients, combined isoflurane and nitrous oxide anaesthesia did not affect the frequency of SCEs (115, 116). However, increased frequencies of DNA strand breaks were seen, also without co-exposure to nitrous oxide (127, 201, 212).

Sevoflurane

Effects on emotional memory and psychomotor performance after acute exposure to sevoflurane were demonstrated in three studies (8, 24, 253). At 1 000 ppm (the lowest exposure level studied) for 20 minutes, sevoflurane suppressed the emotional response (exposed volunteers rated significantly more pictures as neutral than emotional) (acute LOAEL). Additional effects were observed at 2 000 ppm and above, e.g. impaired memory function (8). Psychomotor effects were shown at 2 000 ppm and these effects increased further at higher exposure levels (24, 253). Schlünzen *et al* reported increased regional cerebral blood flow in patients exposed to as low as 0.2 MAC sevoflurane (approximately 4 000 ppm=light sedation). Some of the most profound changes were seen in regions related to pain processing (216) (Table 17).

SCEs were increased in anaesthetists exposed to sevoflurane (8.9 ± 5.6 ppm, mean of stationary sampling close to subjects in 10 rooms) and nitrous oxide (119 ± 39 ppm) compared to non-exposed personnel. The SCE frequencies were significantly lower after 2 months of leave from the operating room than before the leave (72) (Table 19). As co-exposure to nitrous oxide occurred, it is difficult to ascribe the effect to sevoflurane, although SCEs have not been clearly associated with exposure to nitrous oxide either (174). In patients, anaesthetic doses of sevoflurane were not associated with increased frequencies of SCE in presence of nitrous oxide (142), but increased frequencies in DNA strand breaks have been observed in absence of nitrous oxide (9, 127).

Desflurane

Only three human studies with isolated exposure to desflurane were identified (Table 18). At the lowest exposure level tested, exposure to 6 000 ppm desflurane for 3 minutes was not associated with adverse airway events (86). Exposure to 30 000 and 36 000 ppm desflurane for 10 minutes decreased vascular pressure and resistance, respectively (62, 64).

In the only genotoxicity study available, patients anaesthetised with desflurane displayed an increased frequency of SCEs (5).

Isoflurane, sevoflurane and desflurane

Impaired balance control was demonstrated 48 hours after occupational exposure to 59 ppm nitrous oxide and "10 times lower concentration" of isoflurane, sevoflurane, and desflurane (246) (Table 19). Because of the poorly described exposure situation, it is not clear which anaesthetic caused the effect.

Table 13. Dose-effect relationships for experimental animals after inhalation exposure to isoflurane (for single exposures, only levels below 0.8 MAC (human) are included).

Isoflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	References
Single exposure					
530	Rat	10-12	45 min (minimum)	No effect on learning, memory and pain sensitivity.	(6)
1 145	Rat	3	30-40 min	Antianalgesic effect.	(255)
1 160	Rat	10-15	45 min (minimum)	No effect on learning, memory and pain sensitivity.	(6)
1 200	Rat	22	45 min (minimum)	No effect on aversive memory.	(7)
2 000	Rabbit	7	20 min	Slower acquisition of conditioned response.	(70)
2 010	Rat	9-11	45 min (minimum)	Analgesic response and decreased memory. Trials to learning non-significantly increased.	(6)
2 500	Dog	14	40 min	No effects on a range of cardiovascular and ventilatory parameters.	(195)
2 920	Rat	3	Delivered in stepwise manner at 1 145, 2 920 and 5 840 ppm, each step held for 30-40 min.	Analgesic effect (seen also at 5 840 ppm).	(255)
2 960	Mouse	15-20	2 h	Improved performance when memory of avoidance training was assessed.	(139, 140)
3 020	Rat	9-11	45 min (minimum)	Analgesic response. Memory decreased even further compared to at 2 010 ppm. Trials to learning non-significantly increased.	(6)
3 500	Rat	30-40	24 h on gestation day 8	Developmental endpoints unaffected. Dams slightly sedated with lower body weights on gestation day 12-16.	(78)
3 700	Rat	8-16	30 min	Amnesia (seen also at 5 700 and 7 500 in a dose-dependent manner).	(59)
4 000	Rabbit	13	20 min	Suppressed acquisition of conditioned response.	(70)
5 000	Dog	14	40 min	Decrease in cardiac output and in vascular resistance. Other cardiovascular and ventilatory parameters unaffected.	(195)

Table 13. Dose-effect relationships for experimental animals after inhalation exposure to isoflurane (for single exposures, only levels below 0.8 MAC (human) are included).

Isoflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	References
6 020	Mouse	15-20	2 h	Performance similar to controls' when memory of avoidance training was assessed.	(139)
7 500	Infant rat	4-10	6 h	Neurodegeneration similar in control and exposed pups.	(249)
7 500	Infant rat	4-10	6 h	Increase in neuronal degeneration in the laterodorsal and anteroventral thalamic nuclei.	(124)
7 500	Infant mouse	16	4 h	Neuroapoptosis increased by 325% in the caudate putamen.	(125)
8 000	Rabbit	13	20 min	Abolished acquisition of conditioned response.	(70)
9 000	Rabbit	10	30 min	Increased heart rate. No effect on systolic, diastolic, and mean arterial pressure.	(161)
12 000	Human			MAC.	(69)
14 000-14 200	Rat			MAC.	(31, 66)
13 100-17 700	Mouse			MAC.	(222)
Short-term exposure					
60	Mouse	26	4 h/day on gestation days 6-15	Body weights unaffected in dams and offspring, no teratogenic effects.	(167)
150	Mouse	31	35 days, continuous exposure	Decreased body weight gain, liver histology unaffected.	(224)
150	Rat	16	35 days, continuous exposure	No significant effect on body weight gain, liver histology unaffected.	(224)
150	Guinea pig	14	35 days, continuous exposure	No significant effect on body weight gain, liver histology unaffected.	(224)

Table 13. Dose-effect relationships for experimental animals after inhalation exposure to isoflurane (for single exposures, only levels below 0.8 MAC (human) are included).

Isoflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	References
200	Mouse	26	4 h/day, 5 days/week, 9 weeks	No effect at necropsy, including several measures on liver function.	(202)
375	Mouse	50 and 120 at 9 and 15 months	2 h/day on gestation days 11, 13, 15, and 17, and from 5 days after birth, every 2nd day for a total of 24 exposures	No carcinogenic effect in offspring at 9 and 15 months of age.	(65)
500	Mouse	31	35 days, continuous exposure	Decreased body weight, liver histology unaffected.	(224)
500	Rat	16	35 days, continuous exposure	No significant effect on body weight gain, liver histology, or blood cell counts.	(224)
500	Guinea pig	11	35 days, continuous exposure	No significant effect on body weight gain or liver histology.	(224)
600	Mouse	27	4 h/day on gestation days 6-15	Body weights unaffected in dams and offspring, no teratogenic effects.	(167)
1 000	Mouse	26	4 h/day, 5 days/week, 9 weeks	No effect at necropsy, including several measures on liver function.	(202)
1 000	Mouse	15	4 h/day for 6 weeks before mating	Male fertility and body weight unaffected.	(166)
1 000	Mouse	15 (male) 32 (female)	4 h/day for 2 weeks before mating and during mating and pregnancy	No effects on copulatory or pregnancy rate, implantations, resorptions, or viability of foetuses; postnatal survival and weight gain of offspring unaffected.	(166)

Table 13. Dose-effect relationships for experimental animals after inhalation exposure to isoflurane (for single exposures, only levels below 0.8 MAC (human) are included).

Isoflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	References
1 000	Mouse	5	4 h/day, 5 days	No effect on epididymal spermatozoa.	(154)
1 500	Mouse	78 and 107 at 9 and 15 months	2 h/day on gestation days 11, 13, 15, and 17, and from 5 days after birth, every 2nd day for a total of 24 exposures	No carcinogenic effect in offspring at 9 and 15 months of age.	(65)
1 500	Mouse	29	35 days, continuous exposure	Decreased body weight gain. Non-significant increase in liver lesions, otherwise, no abnormalities found at necropsy.	(224)
1 500	Rat	16	35 days, continuous exposure	No significant effect on body weight gain. Non-significant increase in liver lesions, otherwise, no abnormalities found at necropsy.	(224)
1 500	Guinea pig	16	35 days, continuous exposure	Decreased body weight gain. Non-significant increase in liver lesions, otherwise, no abnormalities found at necropsy.	(224)
4 000	Mouse	15	4 h/day for 6 weeks before mating	Male fertility and body weight unaffected.	(166)
4 000	Mouse	15 (male) 32 (female)	4 h/day for 2 weeks before mating and during mating and pregnancy	Dams appeared lightly anaesthetised. No effects on copulatory or pregnancy rate, implantations, resorptions, or viability of foetuses; postnatal survival and weight gain of offspring unaffected.	(166)
5 000	Mouse	26	4 h/day, 5 days/week, 9 weeks	Body weight non-significantly reduced during week 2 in males, and week 1-3 in females. No effect at necropsy, including several measures on liver function.	(202)
6 000	Mouse	117 and 205 at 9 and 15 months	2 h/day on gestation days 11, 13, 15, and 17, and from 5 days after birth, every 2nd day for a total of 24 exposures	No carcinogenic effect in offspring at 9 and 15 months of age.	(65)

Table 13. Dose-effect relationships for experimental animals after inhalation exposure to isoflurane (for single exposures, only levels below 0.8 MAC (human) are included).

Isoflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	References
6 000	Mouse	23	4 h/day on gestation days 6-15	Reduced foetal weight and reduced maternal weight gain, decreased ossification, increased minor malformations.	(167)
10 000	Mouse	5	4 h/day, 5 days	No effect on epididymal spermatozoa.	(154)
10 500	Rat	21-25	6 h/day on gestation days 8-10, 11-13, or 14-16	Reduced foetal weights, development otherwise unaffected. Dams lightly anaesthetised with reduced weight gains.	(168)
13 000	Rabbit	7-8	4 h/day, 5 days	Reduced sperm concentration, some histological findings related to spermatogenic cells (results difficult to interpret, see p 43).	(45)
13 440	Rat	16	2 h every 2nd day for 2 weeks	No effects at autopsy.	(66)
17 000	Rat	11-19	1 h/day on gestation days 1-5, 6-10, 11-15, or 15-20	Depressed offspring weights in early groups, reduced litter size in late group. Maternal weight gain reduced in late exposure group.	(132)
23 000	Rabbit	15	1 h/day on gestation days 6-9, 10-14, or 15-18	No developmental or maternal endpoints affected.	(132)
Long-term exposure					
20	Rat	12	30 weeks, continuous exposure	No hepatic or renal toxicity.	(191)
1 000	Mouse	167	4 h/day, 5 days/week, 78 weeks	No carcinogenicity. Organ weights unaffected, body weights reduced by 1-5% (body weights were not analysed statistically).	(23)
4 000	Mouse	165	4 h/day, 5 days/week, 78 weeks	No carcinogenicity. Organ weights unaffected, body weights reduced by 5-8% (body weights were not analysed statistically).	(23)

CO_2: carbon dioxide, MAC: minimum alveolar concentration of anaesthetic that produces immobility in 50% of humans exposed to a supramaximal painful/noxious stimulus.

Table 14. Dose-effect relationships for experimental animals after inhalation exposure to sevoflurane (for single exposures, only levels below 0.8 MAC (human) are included.

Sevoflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	Reference
Single exposure					
1 050	Rat	11-21	45 min (minimum)	Analgesia, no effect on cognition.	(6)
1 100	Rat	22	45 min (minimum)	Increased memory of aversive event.	(7)
2 160	Rat	7-12	45 min (minimum)	Analgesia, no effect on cognition.	(6)
3 080	Rat	7-10	45 min (minimum)	Analgesia, learning impaired, memory retention latency decreased.	(6)
4 000	Pig	7-8	6 h	No effect on pulmonary haemodynamics or in lung tissue samples, evaluated by light and electron microscopy. In bronchoalveolar lavage, leukotriene C_4, thromboxane B_2, and nitrate levels were increased. Decreased total blood leukocyte count.	(228, 229)
4 110	Rat	8-10	45 min (minimum)	Analgesia, decreased memory retention latency.	(6)
21 000	Human			MAC.	(69)
25 000	Rat			MAC.	(66)
25 000	Mouse			MAC.	(194)
Short-term exposure					
3 000	Rat	10	4 h/day, 30 days	The day after termination of exposure: Decreased activity and time on open arms in elevated plus maze, impaired learning and memory.	(188)
21 600	Cynomolgus monkey	8	3 h/day, 3 days/week, 8 weeks	1st week: Increased serum aspartate and alanine aminotransferase, creatinine kinase and lactate dehydrogenase; values normalised at autopsy. At autopsy, reduced erythrocyte counts and relative weight of thymus. Autopsy otherwise normal.	(221)

Table 14. Dose-effect relationships for experimental animals after inhalation exposure to sevoflurane (for single exposures, only levels below 0.8 MAC (human) are included.

Sevoflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	Reference
23 000	Rabbit	7-8	4 h/day, 5 days	Reduced sperm concentration, some histological findings related to spermatogenic cells (results difficult to interpret, see pp 49-50).	(45)
32 000	Cynomolgus monkey	8	3 h/day, 3 days/week, 8 weeks	Week 1-2: Increased serum aspartate and alanine aminotransferase, creatinine kinase and lactate dehydrogenase; values normalised at autopsy. At autopsy, reduced erythrocyte and white blood cell counts, and reduced relative weight of thymus. Autopsy otherwise normal.	(221)
40 100	Cynomolgus monkey	8	3 h/day, 3 days/week, 8 weeks	First half of study: Increased serum aspartate and alanine aminotransferase, creatinine kinase and lactate dehydrogenase; values normalised at autopsy. End-tidal CO_2 was increased at exposure. At autopsy, reduced white blood cell counts and relative weight of thymus. Autopsy otherwise normal. 3 animals died.	(221)

MAC: minimum alveolar concentration of anaesthetic that produces immobility in 50% of humans exposed to a supramaximal painful/noxious stimulus.

Table 15. Dose-effect relationships for experimental animals after inhalation exposure to desflurane (for single exposures, only levels below 0.8 MAC (human) are included).

Desflurane level (ppm)	Species	No. of animals/group	Exposure duration	Effects	References
Single exposure					
4 400	Rat	10-13	45 min (minimum)	Impaired memory. No effect on learning and pain sensitivity.	(6)
7 700	Rat	22	45 min (minimum)	No effect on aversive memory.	(7)
10 130	Rat	12-15	45 min (minimum)	Decreased learning, memory impaired further. Pain sensitivity unaffected.	(6)
20 200	Rat	10-12	45 min (minimum)	Further impairment of memory and learning. Decreased pain sensitivity.	(6)
60 000	Human			MAC.	(69)
72 100	Rat			MAC.	(31)
65 500-91 200	Mouse			MAC.	(222)
Short-term exposure					
6 000	Rat	10	4 h/day, 30 days	The day after termination of exposure: decreased activity and time on open arms in elevated plus maze, impaired learning and memory.	(188)

MAC: minimum alveolar concentration of anaesthetic that produces immobility in 50% of humans exposed to a supramaximal painful/noxious stimulus.

Table 16. Dose-effect relationships in humans after inhalation exposure to isoflurane (for single exposure studies, levels below 0.8 MAC are included.

Isoflurane level (ppm)	Exposure duration	No. of exposed	Effect	Reference
Single exposure				
600	25 min	6	Decreased saccadic peak velocity after 15 min.	(79)
1 000	40 min	14	Decreased performance in the digit-symbol substitution test.	(24)
1 150	10 min	8	Response to commands impaired in 3/8. Heart rate or arterial pressure unchanged.	(178, 179)
1 200	3 min	25	No adverse airway events.	(86)
1 200	25 min	6	Decreased saccadic peak velocity after 5 min.	(79)
1 900	15 min	10	Impaired learning (slight).	(48)
1 920	15 min	17	No effect on voluntary response.	(60)
2 000	15 min	10	Decreased performance in the logical reasoning test and digit-symbol substitution test.	(252)
2 000	40 min	14	Decreased performance in a number of psychomotor tests.	(24)
2 300	10 min	8	Response to commands impaired in all. Recall and recognition of neutral words was lost, but half remembered having heard a "shock" word. Heart rate and arterial pressure unchanged.	(178, 179)
2 600	5 min	13	Impaired free recall and no event-related potential repetition effect.	(243)
3 500	15 min	10	Further impairment of learning (explicit learning decreased).	(48)
3 840	15 min	17	Suppressed memory. It was estimated that memory was suppressed by 50% at 2 560 ppm and that voluntary response was prevented in 50% at 4 860 ppm.	(60)
4 000	15 min	10	Increased auditory reaction time and decreased performance in logical reasoning test, digit-symbol substitution test and free recall memory test.	(252)
4 000	20 min	12	Increased choice reaction time, decreased ability to tap two areas on a board, and decreased ability to solve mathematical problems.	(172)

Table 16. Dose-effect relationships in humans after inhalation exposure to isoflurane (for single exposure studies, levels below 0.8 MAC are included.

Isoflurane level (ppm)	Exposure duration	No. of exposed	Effect	Reference
4 600	10 min	8	No response to verbal commands. 5/8 persons displayed absent eyelash reflex. Heart rate or arterial pressure unchanged.	(178, 179)
5 100	15 min	10	Learning abolished (explicit learning suppressed entirely).	(48)
5 760	15 min	17	Some subjects responded to verbal commands, memory completely abolished.	(60)
6 000	15 min	9	Increased auditory reaction time and decreased performance in the digit-symbol substitution test, eye-hand coordination test and impaired free recall.	(251)
7 000	15 min	7	Increased heart rate, decreased mean arterial and forearm venous pressures, decreased efferent muscle sympathetic nerve activity.	(62)
12 000			MAC.	(69)
Short-term exposure				
1 000-6 000	38 h continuous exposure (range 24-127)	40 patients (various surgery)	Biomarkers of liver function (glutathione transferase, alanine and aspartate aminotransferase) similar to patients anaesthetised with midazolam.	(114)
20 000-50 000	15-30 min daily for 24 days (range 10-39)	11 children (undergoing radiotherapy)	Biomarkers of liver function (total bilirubin, aspartate aminotransferase, γ-glutamyl transferase and alkaline phosphatase) not impaired compared to pre-exposure.	(126)

MAC: minimum alveolar concentration of anaesthetic that produces immobility in 50% of subjects exposed to a supramaximal painful/noxious stimulus.

Table 17. Dose-effect relationships in humans after single inhalation exposures to sevoflurane (only levels below 0.8 MAC are included).

Sevoflurane level (ppm)	Exposure duration	No. of exposed	Effect	Reference
1 000	20 min (minimum)	5-9	Suppressed emotional response (exposed volunteers rated significantly more pictures as neutral than emotional).	(8)
2 000	20 min (minimum)	5-9	Increased rating of pictures as neutral rather than emotional, free recall decreased, memory boost abolished for emotionally arousing items.	(8)
2 000	40 min	14	Decreased performance in the digit-symbol substitution test. No effect on other psychomotor functions.	(24)
2 000	5 min	16	Decreased performance in the digit-symbol substitution test (moderate drinkers only). Participants reported drug effect.	(253)
2 500	20 min (minimum)	5-9	Increased rating of pictures as neutral rather than emotional, free recall decreased, memory boost abolished for emotionally arousing items.	(8)
4 000	5 min	16	Further decreased performance in the digit-symbol substitution test (light and moderate drinkers). Drug effect increased further.	(253)
4 000	40 min	14	Impaired performance in different psychomotor tests.	(24)
4 000	10 min (minimum)	9	Regional cerebral blood flow increased in some and decreased in other brain areas. Some of the most profound changes were in regions related to pain processing. Bispectral index values decreased dose-dependently and EEG signals changed significantly.	(216)
5 000	25 min	16	Regional blood flow in some brain areas increased less during exposure than in the control condition during activation tasks.	(196)
7 000	10 min (minimum)	9	Increased regional blood flow in some and decreased in other brain areas. Some of the most profound changes were in regions related to pain processing. Bispectral index values decreased dose-dependently and EEG signals changed significantly.	(216)
8 000	5 min	16	Further decreased performance in the digit-symbol substitution test. Drug effect increased further.	(253)

Table 17. Dose-effect relationships in humans after single inhalation exposures to sevoflurane (only levels below 0.8 MAC are included).

Sevoflurane level (ppm)	Exposure duration	No. of exposed	Effect	Reference
10 000	10 min	12	Decreased mean arterial pressure. No change in heart rate, central venous pressure, plasma norepinephrine or in efferent muscle sympathetic nerve activity recorded by microneuro-graphy.	(64)
21 000			MAC.	(69)

EEG: electroencephalography, MAC: minimum alveolar concentration of anaesthetic that produces immobility in 50% of subjects exposed to a supramaximal painful/noxious stimulus, N_2O: nitrous oxide, PET: positron emission tomography, SCE: sister chromatid exchange.

Table 18. Dose-effect relationships in humans after single inhalation exposure to desflurane (only levels below 0.8 MAC are included).

Desflurane level (ppm)	Exposure duration	No. of exposed	Effect	Reference
6 000	3 min	25	No adverse airway events.	(86)
30 000	10 min	9	Decreased mean arterial and central venous pressures. Unchanged heart rate, efferent muscle sympathetic nerve activity, and plasma norepinephrine.	(64)
36 000	10 min	7	Decreased mean arterial and forearm vascular resistance, no change in heart rate.	(62)
60 000			MAC.	(69)

MAC: minimum alveolar concentration of anaesthetic that produces immobility in 50% of subjects exposed to a supramaximal painful/noxious stimulus.

Table 19. Dose-effect relationships in humans after occupational exposure via inhalation to isoflurane, sevoflurane and desflurane.

Mean level of flurane (ppm)	Mean level of N$_2$O (ppm)	Sampling	No. of exposed	Effect	Reference
Isoflurane					
0.16 ± 0.12 [a]	30 ± 29	Personal, breathing zone	80	No hepatic changes as evaluated by serum alanine aminotransferase and aspartate aminotransferase.	(77)
0.35 (0.1-6.2)	23.2 (3-183)	Stationary	112	No effect on neurofunction.	(159)
0.5 (8-h TWA)	11.8	Personal, breathing zone	27	Increased frequency of SCEs in peripheral lymphocytes.	(111)
5.3 ± 12.8 [a] (8-h TWA)	12.8	Stationary	10	Increased frequency of SCEs in peripheral lymphocytes.	(107)
Sevoflurane					
0.28 (0-1.88)	31.3 (0.9-112)	Personal, breathing zone	61	Slight increase in total urinary protein, without dose-response (all values within normal range). Other indices of kidney function unaffected.	(232)
8.9 ± 5.6 [a]	119 ± 39	Stationary, close to working anaesthetists	25	Increased levels of SCEs in exposed anaesthetists. After 2 months leave from the operating room, the levels of SCEs were significantly lower compared to those taken before the leave.	(72)
Isoflurane, sevoflurane and desflurane					
~6 (total of all the fluranes)	59 (10-100)	Not specified	53	Impaired balance control.	(246)

[a] Standard deviation.
N$_2$O: nitrous oxide, SCE: sister chromatid exchange, TWA: time-weighted average.

13. Previous evaluations by national and international bodies

In 1987, the *International Agency for Research on Cancer (IARC)* concluded that volatile anaesthetics (unspecified) are not classifiable as to their carcinogenicity to humans (118).

In 1993, the *German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area* evaluated isoflurane, based on an extensive review on the literature published at the time, i.e. human experience at anaesthetic doses and findings in animal studies (acute, subchronic, chronic, and reproductive toxicity, and genotoxicity and carcinogenicity). Although the acute anaesthetic effects of isoflurane are well established, the committee found the knowledge of the effects of isoflurane insufficient for the establishment of a MAK (Maximale Arbeitsplatzkonzentration) value, especially concerning chronic exposure to subanaesthetic dose levels (56).

The *Swedish Criteria Group for Occupational Standards* evaluated sevoflurane and desflurane in 1998. They concluded that there was no scientific information on either compound that could be used as a basis for identifying a critical effect relevant to occupational exposures (160).

In 1998, the *Dutch Expert Committee on Occupational Standards (DECOS)* evaluated isoflurane. At that time, data on reproductive effects were lacking. However, as the toxicity profile for isoflurane resembled that of enflurane, the committee concluded that the mode of action is similar for isoflurane and enflurane. Given the similarity in chemical structure between these two compounds, the committee recommended the same value for a health based occupational exposure limit for isoflurane as for enflurane, i.e. 20 ppm (98). DECOS also evaluated the effects of isoflurane on reproduction, in 2002. They concluded that lack of appropriate human and animal data precluded the assessment of the effect of isoflurane on fertility and lactation. Lack of appropriate human data precluded the assessment of the effect of isoflurane on foetal development, but sufficient animal data showed that no classification for effects on development was indicated (99).

In 2000, the *Norwegian Labour Inspection Authority* concluded that the documentation on the health risks concerning exposure to isoflurane, sevoflurane, and desflurane was too sparse to identify a critical effect (12-14). It was concluded that isoflurane is teratogenic at high doses based on the animal studies by Mazze *et al* 1985 (167) and Warren *et al* 1992 (235).

The *National Institute of Occupational Safety and Health (NIOSH)* in the Unites States presently announces intentions to review and evaluate toxicity data for isoflurane, sevoflurane and desflurane (180).

A list of occupational exposure limits is presented in Appendix 1.

14. Evaluation of human health risks

14.1 Assessment of health risks

Isoflurane, sevoflurane, and desflurane are all used as inhalation anaesthetics. Thus, all three agents cause sedation and analgesia at anaesthetic dose levels, with MAC-values in humans of 12 000, 21 000, and 60 000 ppm, respectively. The fluranes are used separately or in combination with other anaesthetics, mostly nitrous oxide. Human data for dermal uptake are lacking, but the potential for systemic uptake of fluranes via the skin seems low.

Due to the anaesthetic nature of the three fluranes, neurological effects would be expected to occur also at subanaesthetic exposures of short duration. This is confirmed by experimental studies of neurofunction in human volunteers. There is also relatively much knowledge as to other effects of single exposure to high dose levels in humans. However, for most such effects NOAELs are unknown. Apart from the central nervous system effects, concern has mostly concentrated around the potential for hepatotoxic, nephrotoxic, and reproductive and developmental effects. Hepatitis may occur in less than 1/1 000 patients after isoflurane anaesthesia, and in 1/1 000-1/10 000 patients for sevoflurane and desflurane. Nephrotoxicity is only a concern for sevoflurane, with an incidence less than 1/10 000 patients.

Several studies indicate that occupational exposure to anaesthetic gases in general (including agents such as nitrous oxide, halothane, diethylether and also isoflurane) may increase the risk for spontaneous abortion and the incidence of malformations. However, the studies are weak and due to mixed exposure to anaesthetic gases the causative agent and exposure levels are not known. For isoflurane, animal studies at 4 000 ppm and below consistently showed no such effects. At subanaesthetic dose levels around 0.5 MAC, all three fluranes significantly inhibited myometrial muscle contraction (*in vitro*). Results from studies on rodents exposed neonatally at anaesthetic levels indicate that the foetal nervous system in humans may be sensitive to isoflurane. No NOAELs can be identified for these outcomes.

A few long-term exposure studies have demonstrated genotoxic (increased frequency of SCEs) effects in subjects occupationally exposed to fluranes. The genotoxicity of the three fluranes has also been studied in patients at anaesthetic concentrations, with some positive findings. Together, these studies give rise to some concern regarding the genotoxic potential of the fluranes. However, in these studies, co-exposure to other anaesthetics, especially nitrous oxide or patient premedication, hampers the interpretation.

The few animal studies on carcinogenicity do not indicate that isoflurane is carcinogenic.

Irritative effects are not to be expected at present exposure levels at the workplace, although especially desflurane, but also isoflurane, irritates the airways at anaesthetic exposure levels. Sevoflurane seems to irritate the airways to a lesser extent.

A few cases of contact dermatitis, occupational asthma and allergy to isoflurane and sevoflurane have been described.

Isoflurane, sevoflurane, and desflurane
A study in nurses and auxiliary nurses exposed mainly to nitrous oxide (59 ppm) but also the three fluranes (approximately 6 ppm) demonstrated impaired balance control following more than 5 years of occupational exposure. As the subjects had not been exposed to anaesthetics or ethanol during 48 hours before the test, the impairment does not reflect an acute effect. Because of the poorly described exposure situation, it is not clear which anaesthetic caused the effect.

Isoflurane
In human volunteers, exposure to 600 ppm isoflurane (LOAEL, lowest dose tested) for 25 minutes decreased saccadic eye movements and exposure to 1 000 ppm for 40 minutes resulted in decreased performance in the digit-symbol sub-stitution test. In rats, neither analgesia nor effects on cognitive function were observed after a single exposure to 530 ppm (NOAEL), however, antianalgesia was observed at 1 145 ppm.

Long-term occupational exposure to 0.16 and 0.35 ppm isoflurane were not associated with effects on liver function or neurofunction, respectively. Also, no effects on liver function were observed in a short-term study in children, exposed at anaesthetic dose levels for short periods for several days. In rats, no detectable effects on the liver or kidneys were observed after continuous exposure to 20 ppm isoflurane for 30 weeks. In rats, mice and guinea pigs, 35 days of continuous exposure to 150 and 500 ppm did not affect the liver, but at 1 500 ppm, liver lesions were non-significantly increased in all three species. Body weight gains were reduced to a similar extent at and above 150 ppm (overall LOAEL) in mice, and in guinea pigs only at 1 500 ppm.

At anaesthetic concentrations, isoflurane affected male fertility in rabbits in one study, whereas studies in mice showed no such effect. The rabbit study is hampered e.g. by a depressive trend with respect to sperm concentration also in the controls. Thus, the adverse effects cannot be entirely attributed to the isoflurane exposure. The design of the mice studies did not allow detection of effects on the earlier stages of spermatogenesis.

Isoflurane anaesthesia during gestation does not seem to interfere with foetal development in animals. However, exposures at or above 6 000 ppm during organogenesis are associated with developmental effects in rats and mice.

Exposure during neonatal life at anaesthetic dose levels has been associated with increased apoptosis in rodent neural tissue. The developmental stage of the nervous system in juvenile mice and rats is comparable to that of the nervous system in the human foetus during the 3rd term. These results indicate that also the nervous system of the human foetus may be sensitive to isoflurane.

Increased SCEs were observed in humans after chronic exposure to 0.5 and 5.3 ppm isoflurane. In both studies, nitrous oxide was the dominating anaesthetic agent, thus it is not possible to assess the potential impact of isoflurane although

SCEs have not been clearly associated with exposure to nitrous oxide either. Isoflurane did not induce SCEs in patients at anaesthetic concentrations. The isolated effect of isoflurane has been studied in patients after anaesthesia and in human lymphocytes exposed *in vitro* to isoflurane. These studies have all shown genotoxicity (increases in DNA single strand breaks or SCEs). However, there are also negative *in vitro/in vivo* studies (bacterial tests, *Drosophila* tests as well as Chinese hamster cell line tests were negative).

Sevoflurane
At the lowest dose studied, 1 000 ppm for 20 minutes, sevoflurane suppressed the emotional response (exposed human volunteers rated significantly more pictures as neutral than emotional) (LOAEL). In rats, analgesia and enhanced aversive memory formation were observed after exposure to 45 minutes at 1 050 and 1 100 ppm, respectively (lowest levels tested).

Increased SCEs in anaesthetists exposed to 8.9 ppm sevoflurane was reported in one study, while a study of patients exposed to sevoflurane showed no effect on this endpoint. In both these human studies, the subjects had also been exposed to nitrous oxide. Thus the responsible agent cannot be identified. The isolated effect of sevoflurane has been studied in patients after anaesthesia and *in vitro*. The studies of patients showed genotoxicity, whereas sevoflurane did not induce genotoxicity in human lymphocytes *in vitro*.

One study in rabbits indicated that sevoflurane may have the potential to affect male fertility at near MAC dose levels. The interpretation of the study is hampered by a depressive trend with respect to sperm concentration also in the controls. Thus, the observed decrease may have been caused by other factors than sevo-flurane.

Desflurane
Neurofunction was not tested at subanaesthetic desflurane concentrations in humans. No sign of airway irritation was observed at the lowest exposure level tested (6 000 ppm for 3 minutes). At much higher, but still subanaesthetic concentrations (30 000 ppm), vascular resistance decreased. In rats, cognitive function was impaired at 4 400 ppm, the lowest dose tested. Effects of chronic exposure have not been studied for desflurane in animals. Desflurane induced SCEs in patients at anaesthetic concentrations and a dose-dependent increase in DNA strand breaks was observed in human lymphocytes exposed *in vitro* to desflurane.

14.2 Groups at extra risk

No groups at extra risk have been identified. In epidemiological studies, occupational exposure to anaesthetic gases in general has been associated with increased risk of spontaneous abortions and increased incidence of malformations, but the studies are weak and the causative agent is not identified.

14.3 Scientific basis for an occupational exposure limit

The critical effect for acute exposure to isoflurane, sevoflurane and desflurane is neurotoxicity. A few long-term exposure studies have demonstrated neurotoxic (impaired balance control) and genotoxic (increased frequency of SCEs) effects in subjects occupationally exposed to fluranes. The genotoxicity of the three fluranes has also been studied in patients at anaesthetic concentrations, with some positive findings. Together, these studies give rise to some concern regarding the genotoxic potential of the fluranes. However, in these studies, co-exposure to other anaesthetics, especially nitrous oxide or patient premedication, hampers the interpretation. Thus, the data are not sufficient to identify a critical effect for long-term exposure to any of the three fluranes.

Isoflurane
Considering acute effects in humans, decreased eye movements were reported at 600 ppm (LOAEL). In addition, a decreased performance in the digit-symbol substitution test was observed after exposure to 1 000 ppm. No acute NOAEL has been identified in humans.

Regarding animal studies, continuous exposure to 150 ppm for 35 days was associated with depressed body weight gain in mice (overall LOAEL).

Developmental toxicity was observed in mice exposed to 6 000 ppm during gestation. At 4 000 ppm and below, no developmental toxicity of isoflurane was observed. Results from studies on rodents exposed neonatally at anaesthetic levels indicate that the foetal nervous system in humans may be sensitive to isoflurane.

The few animal studies on carcinogenicity do not indicate that isoflurane is carcinogenic.

Sevoflurane
An acute effect on neuropsychological function in humans was observed at 1 000 ppm. Sevoflurane caused analgesia at 1 050 ppm in rats. No NOAELs can be identified.

Desflurane
Data for desflurane are very scarce. In rats, impaired cognitive function was observed at acute exposure to 4 400 ppm. No NOAELs can be identified.

15. Research needs

Because data on the newer agents, sevoflurane and desflurane are limited, research in the health effects of these agents are needed to gain more knowledge about adverse health effects.

- For desflurane, data for most organ systems/toxicological effects are lacking. This is to some extent also true for sevoflurane.
- Due to the anaesthetic nature of the three fluranes, neurological effects would be a concern. However, there is a lack of long-term studies on neurological effects, in animals as well as humans.
- Male fertility. One study indicates that isoflurane and sevoflurane may affect male semen production and motility, but interpretation is difficult due to great variation in values from the concurrent control group (45). In addition, no study has yet investigated the full spermatogenic cycle.
- The carcinogenic effect of isoflurane has only been investigated in one species. Additional studies on the carcinogenicity of isoflurane in another species would gain further insight into the carcinogenic effects of isoflurane. Studies of the long-term effects of desflurane and sevoflurane should be established to obtain knowledge about carcinogenicity. The genotoxic potential of the fluranes should be established.
- Developmental neurotoxicity, after exposure throughout gestation. Also, NOAELs for apoptosis in nerve cells after juvenile exposure should be established.

16. Summary

Isoflurane, sevoflurane and desflurane (fluranes) are halogenated ethers. They are stable, clear, and colourless volatile liquids at room temperature. None of the fluranes occur naturally. The sole use of fluranes is as anaesthetic agents after vapourisation. Isoflurane is the most potent, and 50% of human subjects inhaling 12 000 ppm remain immobile when exposed to a painful stimulus (MAC). MAC-values for sevoflurane and desflurane are 21 000 and 60 000 ppm, respectively. Occupational exposure occurs mainly by inhalation and remains generally below 2 ppm in the most recent assessments. Effects have primarily been studied at anaesthetic dose levels at a single occasion rather than in personnel exposed to low concentrations for long periods. Isoflurane is the most studied of the three compounds, whereas the toxicological database on sevoflurane is limited and the one on desflurane is very poor.

Due to the properties of the three fluranes, i.e. low irritancy and low frequency of side-effects even at the anaesthetic level, neurological effects are considered to be the critical effect for acute exposures.

For *isoflurane,* the acute human lowest observed adverse effect level (LOAEL) is 600 ppm (eye movement). An overall LOAEL can be set at 150 ppm, at which continuous exposure for 35 days was associated with reduced body weight gain in mice.

For *sevoflurane,* an acute effect on neuropsychological function in humans was observed at 1 000 ppm. Sevoflurane also caused analgesia at 1 050 ppm in rats.

Data for *desflurane* are very scarce. In rats, impaired cognitive function was observed at acute exposure to 4 400 ppm.

For the three fluranes, studies on effects of long-term exposure are scarce or non-existing. A few studies of subjects exposed occupationally or during anaesthesia have shown genotoxicity. In some of the studies, co-exposure to nitrous oxide or premedication hampers interpretation. The few animal studies on carcinogenicity do not indicate that isoflurane is carcinogenic.

Irritative effects would not be expected to occur at exposure levels present in the workplace, whereas at anaesthetic dose levels, especially desflurane but also isoflurane irritates the airways. Sevoflurane seems to irritate the airways to a lesser extent.

Only a few cases of contact dermatitis, occupational asthma and allergy to isoflurane and sevoflurane have been described.

Keywords: anaesthetic, desflurane, isoflurane, neurotoxicity, occupational exposure limit, review, risk assessment, sevoflurane, toxicity

17. Summary in Danish

Saber AT and Hougaard KS. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 141. Isoflurane, sevoflurane and desflurane.* Arbete och Hälsa 2009;43(9):1-117.

Isofluran, sevofluran og desfluran (fluraner) er halogenerede ætere. De er stabile, klare og farveløse fordampelige væsker ved stuetemperatur. Ingen af fluranerne forekommer naturligt. Fluraner anvendes udelukkende som anæsteserende stoffer efter fordampning. Isofluran er det mest potente af fluranerne, og 50% af de mennesker, som indånder 12 000 ppm forbliver immobile ved udsættelse for smertefulde stimuli (MAC). MAC-værdierne for sevofluran og desfluran er henholdsvis 21 000 og 60 000 ppm. Erhvervsmæssig eksponering forekommer primært ved indånding og eksponeringsniveauerne ligger generelt under 2 ppm i nyere undersøgelser. Effekterne er fortrinsvis studeret efter enkeltudsættelser for anæstetiske doser frem for efter personales længerevarende eksponering ved lavere doser. Isofluran er den best undersøgte af de tre forbindelser. Den toksikologiske viden om sevofluran og desfluran er henholdsvis begrænset og meget ringe.

Eftersom de tre fluraner selv ved anæstetiske doser er associeret med ringe luftvejsirritation og en lav frekvens af bivirkninger, anses neurologiske effekter som den kritiske effekt ved akutte eksponeringer.

For *isofluran* er der observeret en akut human LOAEL på 600 ppm (øjenbevægelse). En overordnet LOAEL bestemmes til 150 ppm, hvor kontinuert eksponering i 35 dage var forbundet med reduceret kropsvægtsøgning i mus.

For *sevofluran* blev der ved 1 000 ppm observeret en akut påvirkning af neuropsykologiske funktioner i mennesker. Sevofluran forårsagede analgesi (nedsat smertefølsomhed) ved 1 050 ppm i rotter.

Data for *desfluran* er meget begrænsede. Rotter udviste nedsat kognitiv funktion ved akut eksponering ved 4 400 ppm.

For de tre fluraner er studier af effekter af langtidseksponering begrænset eller ikke-eksisterende. Enkelte studier af personer eksponeret erhvervsmæssigt eller ved anæstesi har vist genotoksicitet. I nogle af disse studier besværliggøres fortolkningen af resultaterne af samtidig eksponering for dinitrogenoxid eller premedicinering. De få dyrestudier af carcinogenicitet indikerer ikke at isofluran er carcinogent.

Irritative effekter forventes ikke at forekomme ved de eksponeringsniveauer, som findes på arbejdspladser. Derimod irriterer især desfluran men også isofluran luftvejene ved anæstetiske dosisniveauer. Sevofluran irriterer tilsyneladende luftvejene i mindre grad. Der er blevet beskrevet ganske få tilfælde af kontaktdermatitis, erhvervsbetinget astma og allergi overfor isofluran og sevofluran.

Nøgleord: anæstesimiddel, desfluran, erhvervsmæssig grænseværdi, isofluran, neurotoksicitet, review, risikovurdering, sevofluran, toksicitet

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19. Data bases used in search of literature

A number of reviews on isoflurane, sevoflurane and desflurane formed the basis for the search for literature on the health effects to the agents; Burm, 2003 (35), Byhahn *et al,* 2001 (40) and Delgado-Herrera, 2001 (55). Literature was retrieved from the online databases:

    Medline (April 2008)
    Toxline Special (April 2008)
    DART (January 2007)
    RTECS (July 2007)
    RISKLINE (April 2008)

Application of specific search terms depended on database. CAS-numbers were applied in all searches in all databases, and the options for addition of relevant synonyms for the fluranes were chosen when possible. For example were synonyms added from ChemIDplus when using the TOXNET databases DART and TOXLINE. Studies were included in the document only when written in English, German, Swedish, Norwegian, and Danish.

Terms used in the literature search.

Agent	Effects	Occupational
Anaesthetics	Allergy	Health care
Isoflurane	Asthma	Anaesthetists
Sevoflurane	Cancer	
Desflurane	Carcinogenicity	
26675-46-7	Genotoxicity	
28523-86-6	Mutagenicity	
57041-67-5	Reproductive effects	
	Teratogenicity	

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Appendix 1. Occupational exposure limit values

Occupational exposure limit values for the fluranes in different countries as 8-hour TWAs (numbers in brackets are short-term exposure limits).

Country	Isoflurane		Sevoflurane		Desflurane		Ref.
	ppm	mg/m^3	ppm	mg/m^3	ppm	mg/m^3	
Denmark	-	-	-	-	-	-	(1)
Finland	10 (20)	77 (150)	10 (20)	83 (170)	10 (20)	70 (140)	(2)
Germany (MAK)	-	-	-	-	-	-	(3)
The Netherlands	-	-	-	-	-	-	(4)
Norway	2 (4)	15 (30)	20 (30)	140 (210)	20 (30)	140 (210)	(5)
Sweden	10 (20)	80 (150)	10 (20)	80 (170)	10 (20)	70 (140)	(6)
United Kingdom	50 (-)	383 (-)	-	-	-	-	(7)
US (ACGIH)	-	-	-	-	-	-	(8)
US (NIOSH)	2 C [a]	-	2 C [a]	-	2 C [a]	-	(9)
European Union	-	-	-	-	-	-	(10, 11)

[a] Recommended exposure limit (REL) for exposure to waste anaesthetic gas.
C: ceiling (60 min), TWA: time-weighted average.

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Appendix 2. Side-effects of the fluranes at anaesthetic dose levels

Side-effects of isoflurane, sevoflurane and desflurane at anaesthetic dose levels (150-152).

Organ system	Incidence		
	Isoflurane	Sevoflurane	Desflurane
Heart			
Arrhythmia	>1/100	≥1/1 000 to <1/100	
Tachycardia, bradycardia		≥1/100 to <1/10	≥1/10
Bradycardia (older patients)		≥1/10	
Lung oedema		<1/10 000	
Blood- and lymph system			
Leukocytosis	>1/100	≥1/1 000 to <1/100	≥1/10
Leucopoenia		≥1/1 000 to <1/100	
Haemorrhage			≥1/1 000 to <1/100
Nervous system			
Increased secretions			≥1/100 to <1/10
Headache		≥1/100 to <1/10	≥1/100 to <1/10
Vertigo		≥1/100 to <1/10	≥1/100 to <1/10
Fever		≥1/100 to <1/10	≥1/100 to <1/10
Tremor		≥1/100 to <1/10	
Somnolence		≥1/100 to <1/10	≥1/100 to <1/10
Migraine			<1/10 000
Convulsions		<1/10 000	<1/10 000
Dystonic movements		≥1/10 000 to <1/1 000	
Shivering	>1/100	≥1/100 to <1/10	
Eyes			
Conjunctival hyperaemia			≥1/100 to <1/10
Eye irritation			<1/10 000
Airways, thorax and mediastium			
Coughing (children)		≥1/10	
Coughing	>1/100	≥1/100 to <1/10	≥1/10
Depression of respiration		≥1/100 to <1/10	
Laryngospams/laryngismus	<1/1 000	≥1/100 to <1/10	≥1/100 to <1/10
Bronchospasms	<1/1 000		≥1/100 to <1/10
Asthma, hypoxia		≥1/1 000 to <1/100	≥1/1 000 to <1/100
Dyspnoea			≥1/1 000 to <1/100
Apnoea		≥1/1 000 to <1/100	≥1/100 to <1/10
Pharyngitis			≥1/100 to <1/10
Acute respiratory deficiency syndrome, lung oedema, respiratory failure			<1/10 000
Gastrointestinal tract			
Nausea, vomiting	>1/1 000 to <1/100	≥1/10	≥1/10
Ilius	<1/1 000		
Diarrhoea			<1/10 000
Increased saliva secretion		≥1/100 to <1/10	
Skin and subcutaneous tissue			
Pruritis			≥1/1 000 to <1/100
Skin eruption, urticaria			<1/10 000

Side-effects of isoflurane, sevoflurane and desflurane at anaesthetic dose levels (150-152).

Organ system	Incidence		
	Isoflurane	Sevoflurane	Desflurane
Bones, joints, muscles and connective tissue			
Myalgia			≥1/1 000 to <1/100
Kidneys and the urinary system			
Urine retention, glucosuria		≥1/1 000 to <1/100	
Acute kidney insufficiency		<1/10 000	
Metabolism and nutrition			
Hyperglycaemia		≥1/1 000 to <1/100	
Hyperpotassaemia			≥1/1 000 to <1/100
Increased serum glucose and serum creatinine. Decreased serum cholesterol and alkaline phosphatase	>1/100		
Malign hyperthermia	<1/1 000	≥1/10 000 to <1/1 000	≥1/1 000 to <1/100
Hypothermia		≥1/100 to <1/10	
Affection of the liver	<1/1 000		
Infections and parasitic diseases			
Pneumonia			<1/10 000
Vascular diseases			
Hypotension	>1/100	≥1/10	
Hypertension		≥1/100 to <1/10	≥1/100 to <1/10
Liver and biliary passage			
Hepatitis	<1/1 000	≥1/10 000 to <1/1 000	≥1/10 000 to <1/1 000
Decreased liver function		≥1/10 000 to <1/1 000	
Increased level of liver enzymes	<1/1 000		
Liver necrosis	<1/1 000		<1/10 000
Icterus	<1/1 000		
Psychological effects			
Agitation (children)		≥1/10	
Agitation		≥1/1 000 to <1/100	≥1/1 000 to <1/100
Confusion		≥1/1 000 to <1/100	
Hostility			<1/10 000

Appendix 3. Previous NEG criteria documents

NEG criteria documents published in the scientific serial Arbete and Hälsa (Work and Health):

Substance/Agent	Arbete och Hälsa issue
Acetonitrile	1989:22, 1989:37*
Acid aerosols, inorganic	1992:33, 1993:1*
Acrylonitrile	1985:4
Allyl alcohol	1986:8
Aluminium	1992:45, 1993:1*
Ammonia	1986:31, 2005:13*
Antimony	1998:11*
Arsenic, inorganic	1981:22, 1991:9, 1991:50*
Arsine	1986:41
Asbestos	1982:29
Benomyl	1984:28
Benzene	1981:11
1,2,3-Benzotriazole	2000:24*D
Boric acid, Borax	1980:13
1,3-Butadiene	1994:36*, 1994:42
1-Butanol	1980:20
γ-Butyrolactone	2004:7*D
Cadmium	1981:29, 1992:26, 1993:1*
7/8 Carbon chain aliphatic monoketones	1990:2*D
Carbon monoxide	1980:8
Ceramic Fibres, Refractory	1996:30*, 1998:20
Chlorine, Chlorine dioxide	1980:6
Chloromequat chloride	1984:36
4-Chloro-2-methylphenoxy acetic acid	1981:14
Chlorophenols	1984:46
Chlorotrimethylsilane	2002:2
Chromium	1979:33
Cobalt	1982:16, 1994:39*, 1994:42
Copper	1980:21
Creosote	1988:13, 1988:33*
Cyanoacrylates	1995:25*, 1995:27
Cyclic acid anhydrides	2004:15*D
Cyclohexanone, Cyclopentanone	1985:42
n-Decane	1987:25, 1987:40*
Deodorized kerosene	1985:24
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Diesel exhaust	1993:34, 1993:35*
Diethylamine	1994:23*, 1994:42
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Diethylenetriamine	1994:23*, 1994:42
Diisocyanates	1979:34, 1985:19
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Dimethyldithiocarbamates	1990:26, 1991:2*
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Dimethylformamide	1983:28
Dimethylsulfoxide	1991:37, 1991:50*
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Epichlorohydrin	1981:10
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Substance/Agent	Arbete och Hälsa issue
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Ethylene glycol	1980:14
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Ethylene oxide	1982:7
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2-Ethylhexanoic acid	1994:31*, 1994:42
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Formaldehyde	1978:21, 1982:27, 2003:11*D
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Glutaraldehyde	1997:20*D, 1998:20
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Hydrogen fluoride	1983:7
Hydrogen sulphide	1982:31, 2001:14*D
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Methyl chloroform	1981:12
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Methyl ethyl ketone	1983:25
Methyl formate	1989:29, 1989:37*
Methyl isobutyl ketone	1988:20, 1988:33*
Methyl methacrylate	1991:36*D
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Methyl-tert-butyl ether	1994:22*D
Microbial volatile organic compounds (MVOCs)	2006:13*
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Nitrogen oxides	1983:28
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Oil mist	1985:13
Organic acid anhydrides	1990:48, 1991:2*
Ozone	1986:28
Paper dust	1989:30, 1989:37*
Penicillins	2004:6*
Permethrin	1982:22
Petrol	1984:7

Substance/Agent	Arbete och Hälsa issue
Phenol	1984:33
Phthalate esters	1982:12
Platinum	1997:14*D, 1998:20
Polyethylene,	1998:12*
Polypropylene, Thermal degradation products in the processing of plastics	1998:12*
Polystyrene, Thermal degradation products in the processing of plastics	1998:12*
Polyvinylchloride, Thermal degradation products in the processing of plastics	1998:12*
Polytetrafluoroethylene, Thermal degradation products in the processing of plastics	1998:12*
Propene	1995:7*, 1995:27
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Propylene glycol ethers and their acetates	1990:32*N
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Refined petroleum solvents	1982:21
Refractory Ceramic Fibres	1996:30*
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Styrene	1979:14, 1990:49*, 1991:2
Sulphur dioxide	1984:18
Sulphuric, hydrochloric, nitric and phosphoric acids	2009;43(7)*
Synthetic pyretroids	1982:22
Tetrachloroethane	1996:28*D
Tetrachloroethylene	1979:25, 2003:14*D
Thermal degradation products of plastics	1998:12*
Thiurams	1990:26, 1991:2*
Tin and inorganic tin compounds	2002:10*D
Toluene	1979:5, 1989:3, 1989:37*, 2000:19*
1,1,1-Trichloroethane	1981:12
Trichloroethylene	1979:13, 1991:43, 1991:50*
Triglycidyl isocyanurate	2001:18*
n-Undecane	1987:25, 1987:40*
Vanadium	1982:18
Vinyl acetate	1988:26, 1988:33*
Vinyl chloride	1986:17
Welding gases and fumes	1990:28, 1991:2*
White spirit	1986:1
Wood dust	1987:36
Xylene	1979:35
Zinc	1981:13

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