4 HUMAN HEALTH

4.1.3 Risk characterisation [1]

4.1.3.1 General aspects

Humans may be exposed to chloroform at workplace from the industrial production of chloroform or indirectly in swimming pools and via the environment. The use of chloroform is limited to professional and industrial applications through regulation (see 4.1.1.1), thus no direct consumer use of chloroform and consequently no direct public exposure is expected (see 4.1.1.3). The indirect consumer exposure results from the formation of chloroform in chlorinated drinking water and swimming pools.

Chloroform is well absorbed, metabolized and eliminated by mammals after oral, inhalation or dermal exposure. Chloroform is hence widely distributed in the entire organism, via blood circulation and, due to its liposolubility, preferentially in fatty tissues and in the brain. Nearly all tissues of the body are capable of metabolizing chloroform, but the rate of metabolism is greatest in liver, kidney cortex, and nasal mucosa.

Chloroform can cross the placenta, transplacental transfer has been reported in mice (Danielsson et al., 1986 in WHO, 1994) and in the fetal blood in rats (Withey and Karpinski, 1985 in WHO, 1994) and it is expected to appear in human colostrum and is excreted in mature breast milk (Lechner et al., 1988; Fisher et al., 1997 in Health Council of the Netherlands, 2000; Davidson *et al.*, 1982 in US EPA, 2004).

The estimated ingestion of chloroform via breast-milk was 0.043 mg, which did not exceed the US EPA non-cancer drinking water ingestion rates for children (Fisher et al., 1997).

Human studies showed that the proportion of chloroform absorbed via inhalation ranged from 76 to 80%. The very high volatility of the substance leads to considerable low retention times of the substance on the skin, consequently dermal adsorption requires submersion or contact with chloroform in liquid form, rather than vapour. Chloroform dermal absorption increases with the temperature and the vehicle used. Human studies have showed total absorbed doses of 7.8 and 1.6% when chloroform was administered in water and ethanol respectively, furthermore the contribution to the total body burden (oral + dermal) of an immersion in bath water containing low chloroform concentrations accounted for 18% at 40°C, 17-6% at 35°C and 1-7% at 30°C. The oral administration of chloroform resulted in almost 100% of the dose absorbed from the gastrointestinal tract.

Considering the data reported, the animal inhalation, dermal and oral absorptions of chloroform are considered to be respectively 80%, 10% and 100%. Data from human studies showed that 80% of the chloroform dose is absorbed via inhalation and 10% via dermal absorption. Oral absorption of chloroform is assumed to be 100% for risk characterisation.

Acute toxicity varies depending upon the strain, sex and vehicle. In mice the oral LD_{50} values range from 36 to 1366 mg chloroform/kg body weight, whereas for rats, they range from 450

[1] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

to 2000 mg chloroform/kg body weight. Kidney damage induced in male mice are related to very sensitive strain, thus it is not considered relevant for risk characterisation.

Chloroform LC_{50} values of 6200 mg/m^3 and 9200 mg/m^3 have been reported for inhalation exposure in mice and rats respectively. Mice are more susceptible than rats to acute chloroform toxicity for both exposure routes. A systemic and local dermal LOAEL of 1.0 g/kg has been reported in rabbits for extensive necrosis of the skin and degenerative changes in the kidney tubules after chloroform exposure under occlusive conditions (Torkelson et al., 1976). An oral NOAEL of 30 mg/kg bw has been reported in rats for serum enzyme changes indicative of liver damage (Keegan *et al.*, 1998). A dose-dependent increase in the LI was present in the kidney of Osborne-Mendel rats given doses of 10 mg/kg (Templin et al., 1996b). The epithelial cells of the proximal tubules of the kidney cortex were the primary target cells for cytotoxicity and regenerative cell proliferation. The mean lethal oral dose for an adult is estimated to be about 45 g, the human inhalation LOAEC based on discomfort is ≤ 249 mg/m^3 (Verschueren, 1983 in WHO, 1994), orally a LOAEL <107 mg/kg has been determined on serious illness (WHO, 1994). However, large interindividual differences in susceptibility occur in human. NOAEL(C) and LOAEL(C) selected as starting point for risk characterisation are reported in Table 4.1.

Chloroform is an irritant substance for skin, eye and upper airways. Rabbit dermal studies showed slight to high irritation potency (LOAEL = 1000 mg/kg bw, Torkelson et al., 1976). In man, dermal contact with chloroform caused dermatitis. Severe eye irritation was observed in animals with liquid chloroform, reported effects are various but one rabbit study indicate slight but definitive corneal injury. In man, eye contact with liquid chloroform caused temporary corneal epithelium injury. Mainly repeated dose studies have been reported for irritation, chloroform induced lesion and cell proliferation in the olfactory epithelium but also bone growth. In respiratory tract of mice and rats, inhaled chloroform induced lesions and cell proliferation in the olfactory epithelium and the nasal passage, the LOAEC reported in rats for enhanced bone growth and hypercellularity in the lamina propria of the ethmoid turbinates of the nose at the early time point (4 days) is 10 ppm (50 mg/m^3, Templin et al., 1996a). A sensitisation test on chloroform was reported (Chiaki et al., 2002). This study was designed to evaluate the skin sensitizing potency of chloroform, and it was performed to further evaluate the differences between Guinea Pig Maximization Test (GPMT) and Local Lymph Node Assay (LLNA, RI Method). No positive reaction was observed in any method for sensitization.

Laboratory animal studies identify the liver kidneys and the nasal cavity as the key target organs of chloroform's toxic potential. The lowest reported oral LOAEL was 15 mg/kg/day in dog livers based on fatty cysts and elevated ALAT levels is a starting point for risk characterisation (Heywood et al., 1979 in US EPA, 2001). For mice, reported oral LOAELs were 50 mg/kg bw/day for the hepatic effects and 37 mg/kg bw for renal effects (mineralization, hyperplasia and cytomegaly) (Condie *et al.*, 1983; Munson *et al.*, 1982 in WHO, 2004). The reported inhalation NOAEC for a 90 days sub-chronic exposure was 25 mg/m^3 (5 ppm) in male mice for the renal effects (vacuolation, basophilic appearance, tubule cell necrosis and enlarged cell nuclei) and a NOAEC of 25 mg/m^3 (5 ppm) was reported in male mice for hepatic effects (vacuolated hepatocytes and necrotic foci) (Templin et al., 1998). A chronic (104 weeks) inhalation NOAEC of 25 mg/m^3 (5ppm) was reported in mice for increased renal cytoplasmic basophilia in both exposed males and females, and increased atypical tubule hyperplasia and nuclear enlargement in the kidneys in the males (Yamamoto et al., 2002). Nasal lesions have also been observed in rats and mice exposed by inhalation or via the oral route. Following a sub-chronic inhalation exposure, the lowest reported effect level

was LOAEC= 9.8 mg/m^3 (2 ppm), which caused cellular degeneration and regenerative hyperplasia in nasal passage tissues of rats. Lesions and cell proliferation in the olfactory epithelium and changes in the nasal passages were observed at LOAEL=34 mg/kg bw/d (Larson et al., 1995). In human, limited data on repeated dose toxicity suggest that the liver and kidneys are the likely target organs. Human studies were poorly reported in the reviews so animal data were selected as the starting point for risk characterisation.

Data on the mutagenicity of chloroform have recently been reviewed and evaluated by several groups: IARC, US EPA, ILSI and WHO. Most of the reviews concluded that chloroform is not a strong mutagen but a weak genotoxic effect was not excluded. Studies presented in this report were chosen based on their reliability (1 or 2) according to Klimish scoring system. Although negative in vivo results are reported, several in vivo tests published in international rewiews demonstrated that chloroform could induce micronuclei and chromosomal aberrations. Positive results are observed in the target organ (kidney) or after at least three administrations in bone marrow cells, which might be consistent with a mechanism of oxidative damage due to glutathione depletion. Besides, it should be noted that MN and CA tests performed in rats were all positive whereas mixed results were observed in mice.

Studies in animals reveal that chloroform can cause an increased incidence of kidney tumors in male rats or mice and an increased incidence of liver tumors in mice of either sex. These induced tumors responses are postulated to be secondary to sustained or repeated cytotoxicity and secondary regenerative hyperplasia, according to the dose levels tested. For the renal effects in male mice the oral NOAEL was 17 mg/kg bw (Roe et al., 1979) and the inhalation NOAEC was 5 ppm (25 mg/m^3, Yamamoto et al., 2002).

Two studies showed nasal lesion in rats or mice due to chloroform inhalation, for nasal lesions a LOAEC of 5 ppm was determined (Yamamoto et al., 2002). The weight of evidence of chloroform weak genotoxicity is consistent with the hypothesis that the liver and kidney tumors induced depend on persistent cytotoxic and regenerative cell proliferation responses. The persistent cell proliferation presumably would lead to higher probabilities of spontaneous cell mutation and subsequent cancer.

There have been no reported studies of toxicity or cancer incidence in humans chronically exposed to chloroform (alone) via drinking water. Relevant studies contain little information on specific exposure, and it is not possible to attribute any excess risk specifically to chloroform.

Regarding fertility, only one author reported increased mice abnormal sperm following exposure to an air concentration of 400 or 800 ppm chloroform (estimated inhalation LOAEC = 400 ppm, Land *et al.*, 1979-1981). Otherwise, animal findings were epididymal lesions or increased right epipidymis weight (estimated oral NOAEC is 15.9 mg/kg, Chapin et al., 1997). As well, one occupational case study reported asthenospermia in association to chloroform exposure. No other adverse reproductive effect has been evidenced in the 90 days studies.

Concerning developmental toxicity, epidemiological studies of chloroform in drinking water no association was clearly established between exposure to chloroform and reduced fetal weight, stillbirth and cleft defects. Otherwise, we need to keep in mind that many of these epidemiological studies present limitations like the use of water concentration as the measure of exposure, which can lead to exposure misclassification.

By inhalation, the effects of chloroform on the various animals tested include effects on pregnancy rate, resorption rate, litter size and live fetuses. These effects have been observed

with concentrations causing a decrease of maternal weight and food consumption. Other effects as fetal weight and CRL decrease, as well as skeletal and gross abnormalities or variations have been mentioned. An inhalation NOAEC of 10 ppm was based on decreased fetal weight & CRL (Baeder & Hoffman, 1991) and an oral LOAEL of 20 mg/kg/day was based on decreased fetal weight (Thompson et al., 1974).

Table 4.1 Summary of the selected NOAEL(C)s or LOAEL(C)s

Substance name	Inhalation (N(L)OAEC)	Dermal (N(L)OAEL)	Oral (N(L)OAEL)
Acute toxicity	LOAEC ≤ 249 mg/m^3 60 min, Man, Verschueren, 1983 in WHO, 1994	LOAEL= 1000 mg/kg bw 24h, Rabbit, Torkelson et al., 1976	LOAEL ≤ 107 mg/kg Single administration, Man, Winslow & Gerstner, 1978 in WHO, 1994 LOAEL = 10 mg/kg bw Single administration, Rat, Templin et al., 1996b
Irritation / corrositivity	LOAEC= 10 ppm - 50 mg/ m^3 Early time pojnts (4 days), 90d, Rat, Templin et al., 1996a	-	-
Repeated dose toxicity (local)	LOAEC= 2 ppm - 10 mg/ m^3 90d, Rat, Templin et al., 1996a	-	LOAEL= 34 mg/kg bw 90d, Rat, Larson et al., 1995
Repeated dose toxicity (systemic)	NOAEC= 5 ppm - 25mg/ m^3 90d, Mouse, Templin et al., 1998; 104w, Yamamoto et al., 2002	-	LOAEL= 15 mg/kg bw 7.5y, Dog, Heywood et al., 1979
Carcinogenicity (local)	LOAEC= 5 ppm - 25 mg/ m^3 104w, Mouse, Yamamoto et al., 2002	-	-
Carcinogenicity	NOAEC= 5 ppm - 25 mg/ m^3 104w, Mouse, Yamamoto et al., 2002	-	NOAEL= 17 mg/kg bw 80w, Mouse, Roe et al., 1979
Fertility impairment	LOAEC= 400 ppm – 2000 mg/m^3 5d, Mouse, Land et al. 1979, in US EPA, 2004	-	NOAEL= 16 mg/kg bw 31w, Mouse, Chapin et al., 1997, in US EPA, 2004
Developmental toxicity	NOAEC= 10 ppm - 50 mg/m^3 GD7-16 Rat, Baeder & Hoffman, 1991, in US EPA, 2004	-	LOAEL= 20 mg/kg-day GD6-18, Rabbit, Thompson *et al.*, 1974, in US EPA, 2004

4.1.3.2 Workers

Assuming that oral exposure is prevented by personal hygienic measures, the risk characterisation for workers in scenario 3.1 (Swimming instructor/lifeguard in a swimming pool) is limited to the dermal and the inhalation routes of exposure.

Table 4.2 Summary of Workers Reasonable Worst Case exposure and Total systemic dose.

Scenario	RWC Inhalation exposure	RWC Dermal exposure	RWC Ingestion exposure
3.1 Swimming instructor/lifeguard in a swimming pool	0.027 ppm 0.136 mg/m^3	0	0
3.2 Competitive swimmers	0.042 ppm 0.206 mg/m^3	0.98 mg/l	0.98 mg/l

Scenario	Systemic dose per day via inhalation (mg/kg/day)	Systemic dose per day via skin (mg/kg/day)	Systemic dose per day via ingestion (mg/kg/day)	Total systemic dose (mg/kg/day)
3..1 Swimming instructor/lifeguard in a swimming pool	0.0078	0	0	0.0078
3.2 Competitive swimmers	0.0141	0.156	0.0056	0.176

4.1.3.2.1 Acute toxicity

Inhalation

The human acute inhalation LOAEC ≤ 249 mg/m^3 based on discomfort, (Verschueren, 1983 in WHO, 1994) is compared with exposure estimations for each scenario. Calculated MOSs are reported in Table 4.4 and compared with Reference MOS reported in Table 4.3.

Table 4.3 Reference MOS for acute toxicity

Assessment factor criteria	Value
Interspecies differences	1[1]
Intraspecies differences	5 workers
Duration of study	2 [2]
Type of effect	1
Extrapolation LOAEC to NOAEC	3
Reference MOS	30

1 Human data for oral and inhalation route

2 An assessment factor was added for the differences between exposure (8h) and study (1h) duration. Based on the low severity of the effects observed (discomfort) this factor was set at 2.

For acute toxicity by inhalation, conclusion **ii** is reached for scenario 3.

Dermal

The rabbit acute dermal LOAEL of 1000 mg/kg bw, was derived from a 24h exposure study under an impermeable plastic cuff (Torkelson et al., 1976). Considering the high volatility of chloroform, the reported effects have been maximised by the occlusive conditions and thus the LOAEL is not relevant for risk assessment.

An internal dose of 3.56 mg/kg has been calculated from the human acute inhalation LOAEC ≤ 249 mg/m^3 (Verschueren, 1983 in WHO, 1994) considering a respiratory volume of 1.25 mg/m^3 (1.25 mg/m3/h * 1 hour), a worker body weight of 70 kg and an absorption factor of 80% for inhalation uptake.

249 * 1.25 * 0.8 / 70 = 3.56 mg/kg

This internal dose is divided by the systemic dose per day via skin value for each scenario (see Table 4.2) to calculate the MOS. Calculated MOSs are compared with Reference MOS in Table 4.4.

For acute toxicity by dermal route, **conclusion ii** is reached for all scenarios.

Combined exposure

For combined exposure an internal dose of 3.56 mg/kg has been calculated from the human acute inhalation LOAEC ≤ 249 mg/m^3 (Verschueren, 1983 in WHO, 1994) considering a respiratory volume of 1.25 mg/m^3 (1.25 mg/m3/h * 1 hour), a worker body weight of 70 kg and an absorption factor of 80% for inhalation uptake.

249 * 1.25 * 0.8 / 70 = 3.56 mg/kg

This value is compared with the total systemic dose reported in Table 4.2 to calculate the MOS. Calculated MOSs are compared with Reference MOS in Table 4.4.

For acute toxicity by combined exposure, conclusion **ii** is reached for scenario 3.

Table 4.4 Occupational risk assessment for acute toxicity

	Inhalation				Dermal				Combined			
	Exposure	N(L)OAEC	MOS	Conclusion	Systemic dose/day	N(L)OAEL	MOS	Conclusion	Total systemic dose	N(L)OAEL	MOS	Conclusion
	mg/m^3	mg/m^3			mg/kg	mg/kg			mg/kg /day	mg/kg		
Swimming Pool												
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0.136	249	1831	**ii**	0	3.56	-	**-**	0.0078	3.56	456	**ii**
3.2 Competitive swimmers	0.206	249	1209	**ii**	0.156	3.56	91	**ii**	0.176	3.56	20	**ii**

4.1.3.2.2 Irritation and corrosivity

Skin irritation

Given the results of the acute dermal toxicity studies, it is concluded that chloroform is irritating to the skin.

For competitive swimmers no data or occupational case on skin irritation, neither case study on animal and human for skin irritation with water containing chloroform, were reported thus it is not possible to conduct a quantitative or a qualitative risk characterisation.

No reliable repeated dose toxicity study with regard to dermal irritation of chloroform is available and thus it is not possible to make a quantitative risk assessment for local effects after repeated dermal exposure.

Eye irritation

In the available animal study, chloroform was found to be irritating to the eyes.

For competitive swimmers no data or occupational case on eye irritation, were reported thus it is not possible to conduct a quantitative risk characterisation. Competitive swimmers usually wear swimming goggles and this equipment should be recommended to prevent eye irritation.

Respiratory irritation after single exposure

Given the results of acute inhalation studies, it is concluded that chloroform is irritating to the respiratory tract. No study reported irritating effects on respiratory tract after a single exposure.

In rats, enhanced bone growth and hypercellularity in the lamina propria of the ethmoid turbinates of the nose have been reported at the early time points of the 13 weeks study at concentrations of 50 mg/m^3 (10 ppm, Templin et al., 1996a).

The LOAEC of 50 mg/m^3 is used with exposure estimations to calculate the MOS (Table 4.6) and then compared to Reference MOS reported in Table 4.5.

Table 4.5 Reference MOS for respiratory irritation

Assessment factor criteria	Value (local)
Interspecies differences	2.5 [1]
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	3
Reference MOS	37.5

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

Table 4.6 Occupational risk assessment for respiratory irritation

	Inhalation

	Exposure	N(L)OAEC	MOS	Conclusion
	mg/m³	mg/m³		
Swimming pool				
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0.136	50	368	**ii**
3.2 Competitive swimmers	0.206	50	243	**ii**

For respiratory irritation **conclusion ii** is reached for scenario 3.

4.1.3.2.3 Sensitisation

No data were available for sensitisation and no occupational case of sensitisation was reported for workers/people exposed to chloroform in human studies. A sensitisation test on chloroform was reported (Chiaki et al., 2002). This study was designed to evaluate the skin sensitizing potency of chloroform, and it was performed to further evaluate the differences between Guinea Pig Maximization Test (GPMT) and Local Lymph Node Assay (LLNA, RI Method). No positive reaction was observed in any method for sensitization.

Conclusion (**ii**) is drawn for sensitisation.

4.1.3.2.4 Repeated dose toxicity

Inhalation (local)

Effects of atrophy on the upper airways have been observed in rats and a LOAEC of 10 mg/m^3 (2 ppm) has been derived from a 13 weeks study (Templin et al., 1996a).

The LOAEC is used with exposure estimations to calculate the MOS (Table 4.9) and then compared to Reference MOS reported in Table 4.7.

Table 4.7 Reference MOS for local RDT

Assessment factor criteria	Value (local)
Interspecies differences	2.5 [1]
Intraspecies differences	5 workers
Duration of study	2
Type of effect	1
Extrapolation LOAEC to NOAEC	3
Reference MOS	75

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

For local repeated dose toxicity by inhalation, **conclusion iii** is reached for all scenarios.

Inhalation (systemic)

A NOAEC of 25 mg/m^3 (5 ppm) has been derived for induced hepatic cell proliferation in mice and renal histological changes and regenerative cell proliferation in male mice (Templin et al., 1998); renal cytoplasmic basophilia, atypical tubule hyperplasia, nuclear enlargement in the kidneys were observed in mice at the same concentration (Yamamoto et al., 2002). This NOAEC is used for calculation of MOS, the results and comparison to Reference MOS are reported in Table 4.8.

Table 4.8 Reference MOS for systemic RDT

Assessment factor criteria	Value (systemic)
Interspecies differences	2.5 [1]
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	1
Reference MOS	12.5

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

For systemic repeated dose toxicity by inhalation **conclusion ii** is reached for scenario 3.

Table 4.9 Occupational risk assessment for repeated dose toxicity by inhalation

	Inhalation (local)				Inhalation (systemic)			
	Exposure	N(L)OAEC	MOS	Conclusion	Exposure	N(L)OAEC	MOS	Conclusion
	mg/m³	mg/m³			mg/m³	mg/m³		
Swimming pool								
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0.136	10	74	iii	0.136	25	184	ii
3.2 Competitive swimmers	0.206	10	49	iii	0.206	25	121	ii

Dermal

For MOS calculation: the mouse inhalatory NOAEC of 25 mg/m^3 (Templin et al., 1998; Yamamoto et al., 2002) has been converted into dermal NOAEL (in mg/kg bw/day) by using a 6h respiratory volume of 0.41 m^3/kg bw (45 ml/min / 40g bw = 1.125 l/min/kg bw) for the mouse and a correction for differences in absorption between mouse and humans.

$$\text{Corrected Dermal N(L)OAEL} = \text{inhalatory N(L)OAEC} \times \text{sRV}_{\text{mouse}} \times \frac{\text{ABS}_{\text{inh-mouse}}}{\text{ABS}_{\text{derm-human}}}$$

sRV = standard respiratory volume

$\text{ABS}_{\text{inh – mouse}}$ = 80%

$\text{ABS}_{\text{derm – Human}}$ = 10%

25 * 0.41 * 80 / 10 = 82 mg/kg bw/day

The dermal NOAEL is converted to internal dose taking into account 10% absorption via skin and compared to the systemic dose per day via skin for each scenario (see Table 4.2) to calculate the MOS.

Table 4.10 Reference MOS for dermal RDT

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	87.5

Calculated MOSs are compared with Reference MOS in Table 4.11.

[2] TGD 2005 Appendix VIII, part 2 B4

For repeated dose toxicity by dermal route **conclusion iii** is reached for competitive swimmers.

Table 4.11 Occupational risk assessment for dermal and combined RDT

	Dermal				Combined			
	Systemic dose/day	N(L)OAEL	MOS	Conclusion	Total systemic dose	N(L)OAEL	MOS	Conclusion
	mg/kg /day	mg/kg			mg/kg /day	mg/kg		
Swimming pool								
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0	8.2	-	**-**	0.0078	8.2	1051	**ii**
3.2 Competitive swimmers	0.156	8.2	53	**iii**	0.176	8.2	47	**iii**

Combined exposure

For MOS calculation: the mouse inhalatory NOAEC of 25 mg/m^3 (Templin et al., 1998; Yamamoto et al., 2002) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$MOS = \frac{N(L)OAEC_{inh\text{-}mouse} \times sRV_{mouse} \times ABS_{inh\text{-}mouse}}{\left[Expo_{inh\text{-}human} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[Expo_{derm\text{-}human} \times ABS_{derm-human}\right] + \left[Expo_{oral\text{-}human} \times ABS_{oral-human}\right]}$$

6h sRV_{mouse} = 0.41 m^3/kg bw (45 ml/min / 40g bw = 1.125 l/min/kg bw)

$ABS_{inh\text{-}mouse}$ = 80%

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume light activity for worker (10 m^3/person)

bw = 70 kg (worker body weight)

[3] TGD 2005 Appendix VIII, Part 2 B7

Table 4.12 Reference MOS for combined RDT

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	1
Reference MOS	87.5

Calculated MOSs are compared with Reference MOS in Table 4.11.

For combined exposure **conclusion iii** is reached for scenario 3.2 (Competitive swimmers), **conclusion ii** is reached for scenario 3.1 (Swimming instructor).

4.1.3.2.5 Mutagenicity

Data on the mutagenicity of chloroform have recently been reviewed and evaluated by several groups: IARC, US EPA, ILSI and WHO. Most of the reviews concluded that chloroform is not a strong mutagen but a weak genotoxic effect was not excluded. Studies presented in this report were chosen based on their reliability (1 or 2) according to Klimish scoring system. Although negative in vivo results are reported, several in vivo tests published in international rewiews demonstrated that chloroform could induce micronuclei and chromosomal aberrations. Positive results are observed in the target organ (kidney) or after at least three administrations in bone marrow cells, which might be consistent with a mechanism of oxidative damage due to glutathione depletion. Besides, it should be noted that MN and CA tests performed in rats were all positive whereas mixed results were observed in mice.

A test protocol for micronucleus assay in Sprague Dawley rats according to OECD guideline no. 474 was proposed and circulated to Member States (MS). A discussion took place at the Technical Committee on New and Existing Chemicals I'08 (TCNES) on the further information needed for mutagenicity evaluation. Two MS expressed their support on the testing proposal. Three MS were not in favour of the protocol for further testing since they were in favour instead of a classification Category 3 for mutagenicity. One MS and the Rapporteur reminded the TCNES group that further testing was requested to confirm the database and the disputed Fujie et al., (1990) study. One MS answered that a confirmatory study should be a chromosomal aberrations test on bone marrow (BM) following Fujie's protocol instead of the MN test proposed with in addition an exploration in the targeted organs such as liver and kidney. Other MS indicated that if a test should be conducted, a Comet assay should be carried out instead. The Industry justified the choice of the MN based on the sensitivity of this test in comparison to the BM test. It was also stressed that international bodies do not consider chloroform as a non-threshold carcinogen. According to the Industry, the dataset is not sufficient for a classification on mutagenicity, the Industry would like to perform the test as proposed in the protocol and requested a recommendation of the TCNES.

ECB concluded that the majority of the expressed Member States (6) did not support the test proposal.

Conclusion open applies with regard to mutagenicity of chloroform following TCNES discussion.

4.1.3.2.6 Carcinogenicity

Inhalation (local)

A LOAEC of 25 mg/m^3 (5 ppm) was determined for nasal lesions including thickening of the bone and atrophy and respiratory metaplasia of the olfactory epithelium in rats of both sexes and female mice (Yamamoto et al., 2002). This LOAEC is used with occupational values to calculate the MOSs, which are compared to Reference MOS given in Table 4.13. Results and conclusions are presented in Table 4.14.

Table 4.13 Reference MOS for local carcinogenicity

Assessment factor criteria	Value
Interspecies differences	2.5 [1]
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	3
Reference MOS	37.5

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

Table 4.14 Occupational risk assessment for local carcinogenicity

	Inhalation (local)			
	Exposure	N(L)OAEC	MOS	Conclusion
	mg/m³	mg/m³		
Swimming pool				
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0.136	25	184	**ii**
3.2 Competitive swimmers	0.206	25	121	**ii**

For inhalation (local) **conclusion ii** is reached for scenario 3.

Inhalation (systemic)

The liver and kidney tumors induced by chloroform depend on persistent cytotoxic and regenerative cell proliferation responses. The persistent cell proliferation presumably would lead to higher probabilities of spontaneous cell mutation and subsequent cancer. The weight of the evidence indicates that a mutagenic mode of action via DNA reactivity is not a significant component of the chloroform carcinogenic process (US EPA, 2001).

The risk characterisation for carcinogenicity can be conducted on a threshold basis.

A NOAEC of 25 mg/m^3 was reported in mice for induction of renal adenomas and carcinomas (Yamamoto et al., 2002). This NOAEC is used with occupational values to calculate the MOSs, which are compared to Reference MOS given in Table 4.15. Results and conclusions are presented in Table 4.18.

For inhalation **conclusion ii** is reached for scenario 3.

Table 4.15 Reference MOS for carcinogenicity

Assessment factor criteria	Value
Interspecies differences	2.5 [1]
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	1
Reference MOS	12.5

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

Dermal

For MOS calculation: the mouse inhalatory NOAEC of 25 mg/m^3 (Yamamoto et al., 2002) has been converted into dermal NOAEL (in mg/kg bw/day) by using a 6h respiratory volume of 0.41 m^3/kg bw (45 ml/min / 40g bw = 1.125 l/min/kg bw) for the mouse and a correction for differences in absorption between mice and humans.

$$\text{corrected dermal N(L)OAEL} = \text{inhalatory N(L)OAEC} \times \text{sRV}_{\text{mouse}} \times \frac{\text{ABS}_{\text{inh-mouse}}}{\text{ABS}_{\text{derm-human}}} \quad [4]$$

sRV = standard respiratory volume

$\text{ABS}_{\text{inh – mouse}}$ = 80%

$\text{ABS}_{\text{derm – Human}}$ = 10%

25 * 0.41 * 80 / 10 = 82 mg/kg bw/day

The dermal NOAEL is converted to internal dose taking into account 10% absorption via skin and compared to the systemic dose per day via skin for each scenario (see Table 4.2) to calculate the MOS.

Table 4.16 Reference MOS for dermal carcinogenicity

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	87.5

Calculated MOSs are compared with Reference MOS in Table 4.18.

For dermal route **conclusion iii** is reached for competitive swimmers.

Combined exposure

For MOS calculation: the mouse inhalatory NOAEC of 25 mg/m^3 (Yamamoto et al., 2002) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$\text{MOS} = \frac{\text{N(L)OAEC}_{\text{inh-mouse}} \times \text{sRV}_{\text{mouse}} \times \text{ABS}_{\text{inh-mouse}}}{\left[\text{Expo}_{\text{inh-human}} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[\text{Expo}_{\text{derm-human}} \times ABS_{derm-human}\right] + \left[\text{Expo}_{\text{oral-human}} \times ABS_{oral-human}\right]}$$

6h $\text{sRV}_{\text{mouse}}$ = 0.41 m^3/kg bw (45 ml/min / 40g bw = 1.125 l/min/kg bw)

[4] TGD 2005 Appendix VIII, part 2 B4

[5] TGD 2005 Appendix VIII, Part 2 B7

$ABS_{inh\text{-}mouse}$ = 80%

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume light activity for worker (10 m^3/person)

bw = 70 kg (worker body weight)

Table 4.17 Reference MOS for combined carcinogenicity

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	1
Reference MOS	87.5

Conclusion iii is reached for scenario 3.2 (Competitive swimmers), **conclusion ii** is reached for scenario 3.1 (Swimming instructor).

Table 4.18 Occupational risk assessment for carcinogenicity

	Inhalation				Dermal				Combined			
	Exposure	N(L)OAEC	MOS	Conclusion	Systemic dose/day	N(L)OAEC	MOS	Conclusion	Total systemic dose	N(L)OAEC	MOS	Conclusion
	mg/m^3	mg/m^3			mg/kg/day	mg/kg			mg/kg/day	mg/kg		
Swimming pool												
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0.136	25	184	**ii**	-				0.0078	8.2	1051	**ii**
3.2 Competitive swimmers	0.206	25	121	**ii**	0.156	8.2	53	**iii**	0.176	8.2	47	**iii**

4.1.3.2.7 Toxicity for reproduction

Effects on fertility

Inhalation

The inhalation LOAEC of 2000 mg/m^3 (400 ppm, Land et al., 1979) was reported in mouse for fertility effects following chloroform exposition.

MOS calculated for inhalation are presented in Table 4.22 and compared to Reference MOS given in Table 4.19.

Conclusion ii is reached for all occupational scenarios.

Table 4.19 Reference MOS for inhalation effects on fertility

Assessment factor criteria	Value
Interspecies differences	2.5 [1]
Intraspecies differences	5 workers
Duration of study	2
Type of effect	1
Extrapolation LOAEC to NOAEC	3
Reference MOS	75

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

Dermal

For MOS calculation: the mouse oral NOAEL of 16 mg/kg (Chapin et al., 1997) has been converted into dermal NOAEL (in mg/kg bw/day) by using a correction for differences in absorption between mice and humans.

$$\text{corrected dermal N(L)OAEL} = \text{oral N(L)OAEL} \times \frac{\text{ABS}_{\text{oral-mouse}}}{\text{ABS}_{\text{derm-human}}}$$

$\text{ABS}_{\text{oral–mouse}} = 100\%$

$\text{ABS}_{\text{derm–Human}} = 10\%$

16 / 0.1 = 160 mg/kg bw/day

The dermal NOAEL is converted to internal dose taking into account 10% absorption via skin and compared to the systemic dose per day via skin for each scenario (see Table 4.2) to calculate the MOS.

[6] TGD 2005 Appendix VIII, Part 2 B5

Table 4.20 Reference MOS for dermal effects on fertility

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	87.5

Calculated MOSs are compared with Reference MOS in Table 4.22.

For fertility toxicity by dermal route, **conclusion ii** is reached for all scenarios.

Combined exposure

For MOS calculation: the mouse oral NOAEL of 16 mg/kg (Chapin et al., 1997) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$MOS = \frac{N(L)OAEL_{oral\text{-}mouse} \times ABS_{oral\text{-}mouse}}{\left[Expo_{inh\text{-}human} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[Expo_{derm\text{-}human} \times ABS_{derm-human}\right] + \left[Expo_{oral\text{-}human} \times ABS_{oral-human}\right]}$$

$ABS_{oral\text{-}mouse}$ = 100%

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume light activity for worker (10 m^3/person)

bw = 70 kg (worker body weight)

[7] TGD 2005 Appendix VIII, Part 2 B7

Table 4.21 Reference MOS for combined effects on fertility

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	1
Reference MOS	87.5

Conclusion ii is reached for scenario 3.

Table 4.22 Occupational risk assessment for effects on fertility

	Inhalation				Dermal				Combined			
	Exposure	N(L)OAEC	MOS	Conclusion	Systemic dose/day	N(L)OAEC	MOS	Conclusion	Total systemic dose	N(L)OAEC	MOS	Conclusion
	mg/m^3	mg/m^3			mg/kg	mg/kg			mg/kg/day	mg/kg		
Swimming pool												
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0.136	2000	14706	ii	-	16			0.0078	16	2051	ii
3.2 Competitive swimmers	0.206	2000	9709	ii	0.156	16	103	ii	0.176	16	91	ii

Developmental toxicity

Inhalation

The inhalation NOAEC of 50 mg/m^3 (10 ppm, Baeder & Hoffman, 1991) was reported in rat for developmental effects following chloroform exposition.

MOS calculated for inhalation are presented in Table 4.26 and compared to Reference MOS given in Table 4.23.

Table 4.23 Reference MOS for developmental toxicity

Assessment factor criteria	Value
Interspecies differences	2.5 [1]
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	1
Reference MOS	12.5

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

For inhalation **conclusion ii** is reached for scenario 3.

Dermal

For MOS calculation: the rat inhalatory NOAEC of 50 mg/m^3 (Baeder & Hoffman, 1991) has been converted into dermal NOAEL (in mg/kg bw/day) by using a 7h respiratory volume of 0.34 m^3/kg bw (200 ml/min / 250g bw = 0.8 l/min/kg bw) for the rat and a correction for differences in absorption between rats and humans.

$$\text{corrected dermal N(L)OAEL} = \text{inhalatory N(L)OAEC} \times \text{sRV}_{\text{rat}} \times \frac{\text{ABS}_{\text{inh-rat}}}{\text{ABS}_{\text{derm-human}}}$$

sRV = standard respiratory volume

$\text{ABS}_{\text{inh-rat}}$ = 80%

$\text{ABS}_{\text{derm-Human}}$ = 10%

50 * 0.34 * 80 / 10 = 136 mg/kg bw/day

The dermal NOAEL is converted to internal dose taking into account 10% absorption via skin and compared to the systemic dose per day via skin for each scenario (see Table 4.2) to calculate the MOS.

Table 4.24 Reference MOS for dermal developmental toxicity

Assessment factor criteria	Value
Interspecies differences	2.5 * 4 (rat data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	50

Calculated MOSs are compared with Reference MOS in Table 4.26.

For developmental toxicity by dermal route, **conclusion ii** is reached for all scenarios.

Combined exposure

For MOS calculation: the rat inhalatory NOAEC of 50 mg/m^3 (Baeder & Hoffman, 1991) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$MOS = \frac{N(L)OAEC_{inh\text{-}rat} \times sRV_{rat} \times ABS_{inh\text{-}rat}}{\left[Expo_{inh\text{-}human} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[Expo_{derm\text{-}human} \times ABS_{derm-human}\right] + \left[Expo_{oral\text{-}human} \times ABS_{oral-human}\right]}$$

7h sRV_{rat} = 0.34 m^3/kg bw (200 ml/min / 250g bw = 0.8 l/min/kg bw)

$ABS_{inh\text{-}rat}$ = 80%

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume light activity for worker (10 m^3/person)

bw = 70 kg (worker body weight)

[8] TGD 2005 Appendix VIII, Part 2 B7

Table 4.25 Reference MOS for combined developmental toxicity

Assessment factor criteria	Value
Interspecies differences	2.5 * 4 (rat data)
Intraspecies differences	5 workers
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	1
Reference MOS	50

Conclusion ii is reached for scenario 3.

Table 4.26 Occupational risk assessment for developmental toxicity

	Inhalation				Dermal				Combined			
	Exposure	N(L)OAEC	MOS	Conclusion	Systemic dose/day	N(L)OAEC	MOS	Conclusion	Total systemic dose	N(L)OAEC	MOS	Conclusion
	mg/m³	mg/m³			mg/kg	mg/kg			mg/kg/day	mg/kg		
Swimming pool												
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	0.136	50	368	**ii**	-				0.0078	13.6	1744	**ii**
3.2 Competitive swimmers	0.206	50	243	**ii**	0.156	13.6	87	**ii**	0.176	13.6	77	**ii**

4.1.3.2.8 Summary of risk characterisation for workers

		Acute toxicity			Local toxicity after single or repeated exposure			Sensitisation	Repeated dose toxicity Systemic			Mutagenicity	Carcinogenicity	Toxicity for reproduction,	
		Inhalation	Dermal	Combined	Inhalation	Dermal	Eye		Inhalation	Dermal	Combined			Fertility	Development
Scenario 3.1: Swimming instructor / lifeguard in a swimming pool	**MOS**	1831	-	3654	456	**-**			74 (local) **184 (syst)**	**-**	1051		**184** **-** **1051**	**14706** **-** **2051**	**368** **-** **1744**
	Concl.	**ii**	**-**	**ii**	**ii**	**-**		**ii**	**iii** (local) **ii (syst)**	**-**	**ii**	**i**	**ii inh local** **ii inh** **ii combi**	**ii inh** **ii combi**	**ii inh** **ii combi**
3.2 Competitive swimmers	**MOS**	1209	91	162	20				49 (local) **121 (syst)**	53	**47**		**121** **53** **47**	**9709** **103** **91**	**243** **87** **77**
	Concl.	**ii**	**ii**	**ii**	**ii**			**ii**	**iii** (local) **ii (syst)**	**iii**	**iii**	**i**	**ii inh local** **ii inh** **iii dermal** **iii combi**	**ii inh** **ii dermal** **ii combi**	**ii inh** **ii dermal** **ii combi**

4.1.3.3 Consumers

As the use of chloroform is limited to professional and industrial applications through regulation, there is no direct consumer use of chloroform and consequently no direct public exposure is expected.

A physiologically based pharmacokinetic (PBPK) model was developed for a lactating woman to estimate the amount of chemical that a nursing infant ingests for a given nursing schedule (24h) and maternal occupational exposure (10 ppm for an intermittent exposition of 6.5h on a 8h period). The estimated ingestion of chloroform via breast-milk was 0.043 mg, which did not exceed the US EPA non-cancer drinking water ingestion rates for children (Fisher et al., 1997).

During their presence in the swimming pool, child swimmers and adult swimmers remain in contact with water and air containing chloroform. The calculations of systemic doses for child swimmers and adult swimmers are done according the worst case and moderate exposure scenarios detailed in the part 4.1.1.2.3 "Scenario 3: exposure of workers to chloroform in swimming pools".

The systemic doses per day via inhalation, skin and ingestion (4.1.1.3) are presented in the following table:

Scenario	RWC Inhalation exposure	RWC Dermal exposure	RWC Ingestion exposure
Child or Adult swimmers	0.042 ppm 0.206 mg/m^3	0.980 mg/l	0.980 mg/l

Scenario	Systemic dose per day via inhalation (mg/kg/day)	Systemic dose per day via skin (mg/kg/day)	Systemic dose per day via ingestion (mg/kg/day)	Total systemic dose (mg/kg/day)
Child swimmers: Worst case	0.00059	0.0101	0.0007	0.0114
Adult swimmers: Worst case	0.00117	0.0196	0.0007	0.0215

The risk assessment for the consumer in swimming pool will be done only for the worst case.

4.1.3.3.1 Acute toxicity

Combined exposure

In a pragmatic approach, the risk characterisation for systemic effects was conducted for combined exposure only.

For combined exposure an internal dose has been calculated from the human acute inhalation LOAEC ≤ 249 mg/m^3 (Verschueren, 1983) considering a respiratory volume of 0.5 m^3/h for 1h/day, a body weight of 10 kg for child or a respiratory volume of 1 m^3/h for 1h/day, a body weight of 60 kg for an adult with an absorption factor of 80% for inhalation uptake.

249 * 0.5 * 0.8 / 10 = 9.96 mg/kg for child

249 * 1 * 0.8 / 60 = 3.32 mg/kg for adult

Calculated MOSs are reported in Table 4.28 and compared with Reference MOS reported in Table 4.27.

Table 4.27 Reference MOS for acute toxicity

Assessment factor criteria	Value
Interspecies differences	1[1]
Intraspecies differences	10
Duration of study	2
Type of effect	1
Extrapolation LOAEL to NOAEL	3
Reference MOS	60

1 Human data for oral and inhalation route

2 An assessment factor was added for the differences between exposure (8h) and study (1h) duration. Based on the low severity of the effects observed (discomfort) this factor was set at 2.

Table 4.28 Consumer risk assessment for acute toxicity

	Combined			
	Total systemic dose	N(L)OAEL	MOS	Conclusion
	mg/kg /day	mg/kg		
Swimming pool				
Child swimmers	0.0114	9.96	874	**ii**
Adult swimmers	0.0215	3.32	154	**ii**

For acute toxicity via combined exposure, **conclusion ii** is reached for all scenarios.

4.1.3.3.2 Irritation and corrosivity

As the use of chloroform is limited to professional and industrial applications through regulation, there is no direct consumer use of chloroform and consequently no direct public exposure is expected. During their presence in the swimming pool, child swimmers and adult

swimmers remain in contact with water containing chloroform at a concentration assumed to be 980 µg/litre for the worst case exposure (the highest concentration measured; Lahl et al., 1981).

Skin irritation

No data or case study was reported on animal and human for skin irritation with water containing chloroform. For consumers, the risk for skin irritation caused by water containing chloroform is considered to be low (**conclusion ii**).

Eye irritation

No data or case study was reported on animal and human for eye irritation with water containing chloroform. For consumers, the risk for eye irritation caused by water containing chloroform might be anticipated to be low due to the high dilution of chloroform in water (**conclusion ii**).

Respiratory irritation after single exposure

Given the results of acute inhalation studies, it is concluded that chloroform is irritating to the respiratory tract. No study reported irritating effects on respiratory tract after a single exposure.

In rats, enhanced bone growth and hypercellularity in the lamina propria of the ethmoid turbinates of the nose have been reported at the early time points of the 13 weeks study at concentrations of 50 mg/m^3 (10 ppm, Templin et al., 1996a).

For MOS calculation: the rat inhalatory LOAEC of 50 mg/m^3 has been compared to the inhalation reasonable worst case in swimming pools (concentration in the air is assumed to be 0.206 mg/m^3 for a swimmer 20 cm above the water surface, see 4.1.1.3).

MOS calculated are presented in Table 4.30 and compared to Reference MOS given in Table 4.29.

Table 4.29 Reference MOS for respiratory irritation

Assessment factor criteria	Value (local)
Interspecies differences	2.5 [1]
Intraspecies differences	10
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	3
Reference MOS	75

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

Table 4.30 Occupational risk assessment for respiratory irritation

	Inhalation			
	Exposure	N(L)OAEL	MOS	Conclusion
	mg/m³	mg/m³		
Swimming pool				
Child swimmers	0.206	50	243	**ii**
Adult swimmers	0.206	50	243	**ii**

For respiratory irritation **conclusion ii** is reached for adult and child swimmers.

4.1.3.3.3 Sensitisation

No data were available for sensitisation and no occupational case of sensitisation was reported for workers/people exposed to chloroform in human studies. A sensitisation test on chloroform was reported (Chiaki et al., 2002). This study was designed to evaluate the skin sensitizing potency of chloroform, and it was performed to further evaluate the differences between Guinea Pig Maximization Test (GPMT) and Local Lymph Node Assay (LLNA, RI Method). No positive reaction was observed in any method for sensitization.

Moreover, the limitation to professional and industrial applications use of chloroform lowers the concern for sensitisation.

Conclusion ii is drawn for sensitisation.

4.1.3.3.4 Repeated dose toxicity

Inhalation (local)

Effects of atrophy on the upper airways have been observed in rats and a LOAEC of 10 mg/m^3 (2 ppm) has been derived from a 13 weeks study (Templin et al., 1996a).

The LOAEC is used with exposure estimations to calculate the MOS (Table 4.31) and then compared to Reference MOS reported in Table 4.32.

Table 4.31 Reference MOS for local RDT

Assessment factor criteria	Value (local)
Interspecies differences	2.5 [1]
Intraspecies differences	10
Duration of study	2
Type of effect	1
Extrapolation LOAEC to NOAEC	3
Reference MOS	150

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

Table 4.32 Consumer risk assessment for repeated dose toxicity by inhalation

	Inhalation (local)			
	Exposure	N(L)OAEC	MOS	Conclusion
	mg/m³	mg/m³		
Swimming pool				
Child swimmers	0.206	10	49	**iii**
Adult swimmers	0.206	10	49	**iii**

For local repeated dose toxicity by inhalation, **conclusion iii** is reached for adult and child swimmers.

Combined exposure

In a pragmatic approach, the risk characterisation for systemic effects was conducted for combined exposure only.

For MOS calculation: the mouse inhalatory NOAEC of 25 mg/m^3 (Templin et al., 1998; Yamamoto et al., 2002) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$MOS = \frac{N(L)OAEC_{inh\text{-}mouse} \times sRV_{mouse} \times ABS_{inh\text{-}mouse}}{\left[Expo_{inh\text{-}human} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[Expo_{derm\text{-}human} \times ABS_{derm-human}\right] + \left[Expo_{oral\text{-}human} \times ABS_{oral-human}\right]}$$

6h sRV_{mouse} = 0.41 m^3/kg bw (45 ml/min / 40g bw = 1.125 l/min/kg bw)

9 TGD 2005 Appendix VIII, Part 2 B7

$ABS_{inh\text{-}mouse}$ = 80%

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume for child or adult

bw = child or adult body weight

Calculated MOSs are reported in Table 4.34 and compared with Reference MOS reported in Table 4.33.

Table 4.33 Reference MOS for combined RDT

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	10
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	175

Table 4.34 Consumer risk assessment for combined RDT

	Combined			
	Total systemic dose	N(L)OAEL	MOS	Conclusion
	mg/kg /day	mg/kg		
Swimming pool				
Child swimmers	0.0114	8.2	719	**ii**
Adult swimmers	0.0215	8.2	381	**ii**

For RDT via combined exposure **conclusion ii** is reached for adult and child swimmers.

4.1.3.3.5 Mutagenicity

Data on the mutagenicity of chloroform have recently been reviewed and evaluated by several groups: IARC, US EPA, ILSI and WHO. Most of the reviews concluded that chloroform is not a strong mutagen but a weak genotoxic effect was not excluded. Studies presented in this report were chosen based on their reliability (1 or 2) according to Klimish scoring system.

Although negative in vivo results are reported, several in vivo tests published in international rewiews demonstrated that chloroform could induce micronuclei and chromosomal aberrations. Positive results are observed in the target organ (kidney) or after at least three administrations in bone marrow cells, which might be consistent with a mechanism of oxidative damage due to glutathione depletion. Besides, it should be noted that MN and CA tests performed in rats were all positive whereas mixed results were observed in mice.

A test protocol for micronucleus assay in Sprague Dawley rats according to OECD guideline no. 474 was proposed and circulated to Member States (MS). A discussion took place at the Technical Committee on New and Existing Chemicals I'08 (TCNES) on the further information needed for mutagenicity evaluation. Two MS expressed their support on the testing proposal. Three MS were not in favour of the protocol for further testing since they were in favour instead of a classification Category 3 for mutagenicity. One MS and the Rapporteur reminded the TCNES group that further testing was requested to confirm the database and the disputed Fujie et al., (1990) study. One MS answered that a confirmatory study should be a chromosomal aberrations test on bone marrow (BM) following Fujie's protocol instead of the MN test proposed with in addition an exploration in the targeted organs such as liver and kidney. Other MS indicated that if a test should be conducted, a Comet assay should be carried out instead. The Industry justified the choice of the MN based on the sensitivity of this test in comparison to the BM test. It was also stressed that international bodies do not consider chloroform as a non-threshold carcinogen. According to the Industry, the dataset is not sufficient for a classification on mutagenicity, the Industry would like to perform the test as proposed in the protocol and requested a recommendation of the TCNES.

ECB concluded that the majority of the expressed Member States (6) did not support the test proposal.

Conclusion open applies with regard to mutagenicity of chloroform following TCNES discussion.

4.1.3.3.6 Carcinogenicity

Inhalation (local)

A LOAEC of 25 mg/m^3 (5 ppm) was determined for nasal lesions including thickening of the bone and atrophy and respiratory metaplasia of the olfactory epithelium in rats of both sexes and female mice (Yamamoto et al., 2002). This LOAEC is used with occupational values to calculate the MOSs, which are compared to Reference MOS given in Table 4.35. Results and conclusions are presented in Table 4.36.

Table 4.35 Reference MOS for local carcinogenicity

Assessment factor criteria	Value
Interspecies differences	2.5 [1]
Intraspecies differences	10
Duration of study	1
Type of effect	1
Extrapolation LOAEC to NOAEC	3
Reference MOS	75

1 For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements.

Table 4.36 Occupational risk assessment for local carcinogenicity

	Inhalation (local)			
	Exposure	N(L)OAEC	MOS	Conclusion
	mg/m³	mg/m³		
Swimming pool				
Child swimmers	0.206	25	121	**ii**
Adult swimmers	0.206	25	121	**ii**

For inhalation (local), **conclusion ii** is reached for adult and child swimmers.

Combined exposure

In a pragmatic approach, the risk characterisation for systemic effects was conducted for combined exposure only.

For MOS calculation: the mouse inhalatory NOAEC of 25 mg/m^3 (Yamamoto et al., 2002) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$MOS = \frac{N(L)OAEC_{inh\text{-}mouse} \times sRV_{mouse} \times ABS_{inh\text{-}mouse}}{\left[Expo_{inh\text{-}human} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[Expo_{derm\text{-}human} \times ABS_{derm-human}\right] + \left[Expo_{oral\text{-}human} \times ABS_{oral-human}\right]}$$

6h sRV_{mouse} = 0.41 m^3/kg bw (45 ml/min / 40g bw = 1.125 l/min/kg bw)

$ABS_{inh\text{-}mouse}$ = 80%

[10] TGD 2005 Appendix VIII, Part 2 B7

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume for child or adult

bw = child or adult body weight

Calculated MOSs are reported in Table 4.38 and compared with Reference MOS reported in Table 4.37.

Table 4.37 Reference MOS for combined carcinogenicity

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	10
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	175

Table 4.38 Consumer risk assessment for carcinogenicity

	Combined			
	Total systemic dose	N(L)OAEL	MOS	Conclusion
	mg/kg /day	mg/kg		
Swimming pool				
Child swimmers	0.0114	8.2	719	**ii**
Adult swimmers	0.0215	8.2	381	**ii**

For carcinogenicity via combined exposure **conclusion ii** is reached for child and adult swimmers.

4.1.3.3.7 Toxicity for reproduction

Effects on fertility

Combined exposure

In a pragmatic approach, the risk characterisation was conducted for combined exposure only.

For MOS calculation: the mouse oral NOAEL of 16 mg/kg (Chapin et al., 1997) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$MOS = \frac{N(L)OAEL_{oral\text{-}mouse} \times ABS_{oral\text{-}mouse}}{\left[Expo_{inh\text{-}human} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[Expo_{derm\text{-}human} \times ABS_{derm-human}\right] + \left[Expo_{oral\text{-}human} \times ABS_{oral-human}\right]}$$

$ABS_{oral\text{-}mouse}$ = 100%

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume for child or adult

bw = child or adult body weight

Calculated MOSs are reported in Table 4.40 and compared with Reference MOS reported in Table 4.39.

Table 4.39 Reference MOS for combined effects on fertility

Assessment factor criteria	Value
Interspecies differences	2.5 * 7 (mouse data)
Intraspecies differences	10
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	175

Table 4.40 Consumer risk assessment for effects on fertility

	Combined			
	Total systemic dose	N(L)OAEL	MOS	Conclusion
	mg/kg /day	mg/kg		
Swimming pool				
Child swimmers	0.0114	16	1404	**ii**
Adult swimmers	0.0215	16	744	**ii**

[11] TGD 2005 Appendix VIII, Part 2 B7

For effects on fertility via combined exposure **conclusion ii** is reached for child and adult swimmers.

Developmental toxicity

Combined exposure

In a pragmatic approach, the risk characterisation was conducted for combined exposure only.

For MOS calculation: the rat inhalatory NOAEC of 50 mg/m^3 (Baeder & Hoffman, 1991) has been converted in the following formula and compared to the total systemic dose via inhalation, skin and ingestion.

$$MOS = \frac{N(L)OAEC_{inh\text{-}rat} \times sRV_{rat} \times ABS_{inh\text{-}rat}}{\left[Expo_{inh\text{-}human} \times \frac{RV_{human}}{bw_{human}} \times ABS_{inh-human}\right] + \left[Expo_{derm\text{-}human} \times ABS_{derm-human}\right] + \left[Expo_{oral\text{-}human} \times ABS_{oral-human}\right]}$$

7h sRV_{rat} = 0.34 m^3/kg bw (200 ml/min / 250g bw = 0.8 l/min/kg bw)

$ABS_{inh\text{-}rat}$ = 80%

$ABS_{inh\text{-}human}$ = 80%

$ABS_{derm\text{-}human}$ = 10%

$ABS_{oral\text{-}human}$ = 100%

wRV = Respiratory volume for child or adult

bw = child or adult body weight

Calculated MOSs are reported in Table 4.42 and compared with Reference MOS reported in Table 4.41.

Table 4.41 Reference MOS for combined developmental toxicity

Assessment factor criteria	Value
Interspecies differences	2.5 * 4 (rat data)
Intraspecies differences	10
Duration of study	1
Type of effect	1
Extrapolation LOAEL to NOAEL	1
Reference MOS	100

[12] TGD 2005 Appendix VIII, Part 2 B7

Table 4.42 Consumer risk assessment for developmental toxicity

	Combined			
	Total systemic dose	N(L)OAEL	MOS	Conclusion
	mg/kg /day	mg/kg		
Swimming pool				
Child swimmers	0.0114	13.6	1193	**ii**
Adult swimmers	0.0215	13.6	633	**ii**

For effects on development via combined exposure **conclusion ii** is reached for child and adult swimmers.

4.1.3.3.8 Summary of risk characterisation for consumers

	Acute		Irritation		RDT local		RDT		Carcinogenicity local		Carcinogenicity		Effects on fertility		Developmental toxicity	
	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
Child swimmers	874	**ii**	243	**ii**	49	**iii**	719	**ii**	121	**ii**	719	**ii**	1404	**ii**	1193	**ii**
Adult swimmers	154	**ii**	243	**ii**	49	**iii**	381	**ii**	121	**ii**	381	**ii**	744	**ii**	633	**ii**

5 RESULTS [13]

5.1 INTRODUCTION

5.2 ENVIRONMENT

5.3 HUMAN HEALTH

5.3.1 Human health (toxicity)

5.3.1.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to:

- Scenario 3, Swimming pools for acute toxicity, sensitisation, irritation, RDT (inhalation systemic, combined for swimming instructors), carcinogenicity (swimming instructor, inhalation for competitive swimmers), fertility and development (dermal).

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to:

- Scenario 3, Swimming pools for RDT (inhalation local, dermal and combined for competitive swimmers), carcinogenicity (dermal and combined for competitive swimmers).

5.3.1.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to:

- Child and Adult swimmers for acute toxicity, irritation, RDT, carcinogenicity, fertility and development.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

[13] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to:

- Child and Adult swimmers for RDT (local).