European Union Risk Assessment Report

CHLOROFORM

CAS No: 67-66-3

EINECS No: 200-663-8

RISK ASSESSMENT

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CHLOROFORM

CAS No: 67-66-3

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RISK ASSESSMENT

Draft of May 2008

France

Rapporteur for the risk assessment of Chloroform is France

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Review of report by MS Technical Experts finalised: 05-2008
Final report: 2008

Foreword

This Draft Risk assessment Report is carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

This Draft Risk Assessment Report is currently under discussion in the Competent Group of Member State experts with the aim of reaching consensus. During the course of these discussions, the scientific interpretation of the underlying scientific information may change, more information may be included and even the conclusions reached in this draft may change. The Competent Group of Member State experts seek as wide a distribution of these drafts as possible, in order to assure as complete and accurate an information basis as possible. The information contained in this Draft Risk Assessment Report does not, therefore, necessarily provide a sufficient basis for decision making regarding the hazards, exposures or the risks associated with the priority substance.

This Draft Risk Assessment Report is the responsibility of the Member State rapporteur. In order to avoid possible misinterpretations or misuse of the findings in this draft, anyone wishing to cite or quote this report is advised to contact the Member State rapporteur beforehand.

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[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

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0 OVERALL RESULTS OF THE RISK ASSESSMENT[1]

CAS Number: 67-66-3
EINECS Number: 200-663-8
IUPAC Name: [click here to insert IUPAC name]

Environment

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) is applied to the use of chloroform as a solvent for all compartments.
Conclusion (iii) is also applied to production sites A, C, E and J, to all uses and to unintended releases for the sewage compartment.

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) is applied to all levels of the life cycle of chloroform (except the use as a solvent) for the following compartments: aquatic, sediment, atmosphere, terrestrial and non-compartment specific effects relevant to the food chain.

Human health

Human health (toxicity)

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 1, Manufacture of chloroform and HCFC 22 for acute toxicity (inhalation and dermal), sensitisation, RDT (dermal), carcinogenicity (dermal), fertility (inhalation and dermal) and development (dermal).
- Scenario 2, Chloroform as intermediate or solvent in the synthesis of chemicals for acute toxicity (dermal), sensitisation, RDT (dermal), carcinogenicity (dermal), fertility (inhalation and dermal) 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.

[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.

Conclusion (iii) applies to:

- Scenario 1, Manufacture of chloroform and HCFC 22 for acute toxicity (combined), irritation, RDT (inhalation and combined), carcinogenicity (inhalation and combined), fertility (combined) and development (inhalation and combined).
- Scenario 2, Chloroform as intermediate or solvent in the synthesis of chemicals for acute toxicity (inhalation and combined), irritation, RDT (inhalation and combined), carcinogenicity (inhalation and combined), fertility (combined) and development (inhalation and combined).

Consumers

Conclusion for Consumers are reported in Annex 1

Humans exposed via the environment

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:

- Human exposed via the environment for exposure via air, food and water.

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:

- Human exposed via the environment at local scale for RDT (local) via air; RDT and carcinogenicity via air, food and water.
-

Human health (physico-chemical properties)

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.

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1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: [click here to insert CAS No.]
EINECS Number: [click here to insert EINECS No.]
IUPAC Name: [click here to insert IUPAC name]
Molecular formula: [click here to insert molecular formula]
Structural formula: [click here to insert structural formula]
Molecular weight: [click here to insert molecular weight]
Synonyms: [click here to insert synonyms]

[delete or click here to insert additional text if necessary]

1.2 PURITY/IMPURITIES, ADDITIVES

[click here to insert text]

1.3 PHYSICO-CHEMICAL PROPERTIES

[delete or click here to insert additional comments on a specific property]

Table 1.1 Summary of physico-chemical properties

Property	Value	[enter comment/reference or delete column]
Physical state		
Melting point		
Boiling point		
Relative density		
Vapour pressure		
Water solubility		
Partition coefficient n-octanol/water (log value)		
Granulometry		
Conversion factors		
Flash point		
Autoflammability		
Flammability		
Explosive properties		
Oxidizing properties		
Viscosity		
Henry's constant		
Surface tension		
[enter other property or delete row]		

[click here to insert table note or Table X.X continued overleaf or delete if not appropriate]

1.4 CLASSIFICATION

[click here to insert text]

1.4.1 Current classification

Symbol: **Xn**
R phrases:
• 1 % ≤ conc. < 5 %
R 40 [Limited evidence of a carcinogenic effect]
• 5% ≤ conc. < 20 %
R 22 [Harmful if swallowed]
R 40-48/20/22 [Harmful: danger of serious damage to health by prolonged exposure through inhalation and if swallowed]
• conc. ≥ 20 % **R 22-38** [Irritating to skin] **40-48/20/22**
S-phrases:
S 2: Keep out of the reach of children
S 36/37: Wear suitable protective clothing and gloves

1.4.2 Proposed classification

- Xn; R20/22
- Xn; R48/20
- Xi ; R36/38
- [Muta cat. 3; R68]
- Carc. Cat. 3; R40
- Repr. Cat. 3; R63

- Not classified for the environment

No agreement could be reach by the TC C&L on mutagenicity and the classification for this endpoint is submitted to ECHA.

Revision of the classification of chloroform was discussed and agreed by the TC C&L in september 2007:

The TC C&L agreed not to classify chloroform with Xi; R37 as the nasal effects reported were rather covered by Xn; R48/20. Further, the TC C&L agreed that R48/22 could be deleted as effects were only seen at high doses. They also agreed on classification with Repr. Cat. 3; R63 based on the FR proposal. The narcotic effects that would be covered by Xn; R20 under the current system would trigger classification with STOT Single 3 under the CLP Regulation.

Proposed classification based on GHS criteria:

- Acute Tox. 3 – H331
- Acute Tox. 4 – H 302
- STOT Rep. 1 – H 372
- STOT Single 3 – H336
- Eye Irrit. 2 – H319
- Skin Irrit. 2 – H315
- [Muta. 2 – H341]
- Carc. 2 – H351
- Repr. 2 – H361d

- Not classified for the environment

Proposed labelling:

Xn

R:20/22-36/38-40-48/20-63-68

S: 2-36/37

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

2.1.1 Production processes

[click here to insert text]

2.1.2 Production capacity

[click here to insert text]

Table 2.1 [Production volume or appropriate text]

[Country or appropriate text]	[Volume or appropriate text]
[Total or appropriate text]	

[click here to insert table note or Table X.X continued overleaf or delete if not appropriate]

2.2 USES

2.2.1 Introduction

[click here to insert text]

Table 2.2 [click here to enter appropriate text]

Industry category	Use category	Quantity used [click here to add unit]	Percentage of total use
Total			

[click here to insert table note or Table X.X continued overleaf or delete if not appropriate]

2.2.2 Scenarios

[click here to insert text]

2.3 TRENDS

[click here to insert text]

2.4 LEGISLATIVE CONTROLS

[click here to insert text]

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

[click here to insert text]

3.1.1 General discussion

[click here to insert text]

3.1.2 Environmental releases

[click here to insert text]

3.1.2.1 Release from production

[click here to insert text]

3.1.2.2 Release from formulation

[click here to insert text]

3.1.2.3 Release from industrial/professional use

[click here to insert text]

3.1.2.4 Release from private use

[click here to insert text]

3.1.2.5 Release from disposal

[click here to insert text]

3.1.2.6 Summary of releases

[click here to insert text and table]

3.1.3 Environmental fate

[click here to insert text]

3.1.3.1 Degradation in the environment

[click here to insert text]

3.1.3.1.1 Atmospheric degradation

[click here to insert text]

3.1.3.1.2 Aquatic degradation (incl. sediment)

[click here to insert text]

3.1.3.1.3 Degradation in soil

[click here to insert text]

3.1.3.1.4 Summary of environmental degradation

[click here to insert text and table]

3.1.3.2 Distribution

[click here to insert text]

3.1.3.2.1 Adsorption

[click here to insert text]

3.1.3.2.2 Precipitation

[click here to insert text]

3.1.3.2.3 Volatilisation

[click here to insert text]

3.1.3.2.4 Distribution in wastewater treatment plants

[click here to insert text]

3.1.3.3 Accumulation and metabolism

[click here to insert text]

3.1.4 Aquatic compartment (incl. sediment)

[click here to insert text]

3.1.4.1 Calculation of predicted environmental concentrations (PEC_{local})

[click here to insert text]

3.1.4.1.1 Calculation of PEC_{local} for production

[click here to insert text or delete if subdivision is not necessary]

3.1.4.1.2 Calculation of PEC_{local} for formulation

[click here to insert text or delete if subdivision is not necessary]

3.1.4.1.3 Calculation of PEC_{local} for industrial/professional use

[click here to insert text or delete if subdivision is not necessary]

3.1.4.1.4 Calculation of PEC_{local} for private use

[click here to insert text or delete if subdivision is not necessary]

3.1.4.1.5 Calculation of PEC_{local} for disposal

[click here to insert text or delete if subdivision is not necessary]

3.1.4.2 Measured levels

[click here to insert text]

3.1.4.3 Comparison between predicted and measured levels

[click here to insert text]

3.1.5 Terrestrial compartment

[click here to insert text]

3.1.5.1 Calculation of PEC_{local}

[click here to insert text]

3.1.5.1.1 Calculation of PEC_{local} for production

[click here to insert text or delete if subdivision is not necessary]

3.1.5.1.2 Calculation of PEC_{local} for formulation

[click here to insert text or delete if subdivision is not necessary]

3.1.5.1.3 Calculation of PEC_{local} for industrial/professional use

[click here to insert text or delete if subdivision is not necessary]

3.1.5.1.4 Calculation of PEC_{local} for private use

[click here to insert text or delete if subdivision is not necessary]

3.1.5.1.5 Calculation of PEC_{local} for disposal

[click here to insert text or delete if subdivision is not necessary]

3.1.5.2 Measured levels

[click here to insert text]

3.1.5.3 Comparison between predicted and measured levels

[click here to insert text]

3.1.6 Atmosphere

[click here to insert text]

3.1.6.1 Calculation of PEC_{local}

[click here to insert text]

3.1.6.1.1 Calculation of PEC_{local} for production

[click here to insert text or delete if subdivision is not necessary]

3.1.6.1.2 Calculation of PEC_{local} for formulation

[click here to insert text or delete if subdivision is not necessary]

3.1.6.1.3 Calculation of PEC_{local} for industrial/professional use

[click here to insert text or delete if subdivision is not necessary]

3.1.6.1.4 Calculation of PEC_{local} for private use

[click here to insert text or delete if subdivision is not necessary]

3.1.6.1.5 Calculation of PEC_{local} for disposal

[click here to insert text or delete if subdivision is not necessary]

3.1.6.2 Measured levels

[click here to insert text]

3.1.6.3 Comparison between predicted and measured levels

[click here to insert text]

3.1.7 Secondary poisoning

[click here to insert text]

3.1.8 Calculation of $PEC_{regional}$ and $PEC_{continental}$

[click here to insert text and table]

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

[Please consider using overview tables to summarise the test results for the different species]

3.2.1 Aquatic compartment (incl. sediment)

[click here to insert text]

3.2.1.1 Toxicity test results

[click here to insert text]

3.2.1.1.1 Fish

[click here to insert text]

Acute toxicity

[click here to insert text]

Long-term toxicity

[click here to insert text]

3.2.1.1.2 Aquatic invertebrates

[click here to insert text]

Acute toxicity

[click here to insert text]

Long-term toxicity

[click here to insert text]

3.2.1.1.3 Algae

[click here to insert text]

Acute toxicity

[click here to insert text]

Long-term toxicity

[click here to insert text]

3.2.1.1.4 Microorganisms

[click here to insert text]

3.2.1.1.5 Amphibians

[click here to insert text]

3.2.1.2 Calculation of Predicted No Effect Concentration (PNEC)

[click here to insert text]

3.2.1.3 Toxicity test results for sediment organisms

[click here to insert text]

3.2.1.4 Calculation of Predicted No Effect Concentration (PNEC) for sediment organisms

[click here to insert text]

3.2.2 Terrestrial compartment

[click here to insert text]

3.2.2.1 Toxicity test results

[click here to insert text]

3.2.2.1.1 Plants

[click here to insert text]

Acute toxicity

[click here to insert text]

Long-term toxicity

[click here to insert text]

3.2.2.1.2 Earthworm

[click here to insert text]

Acute toxicity

[click here to insert text]

Long-term toxicity

[click here to insert text]

3.2.2.1.3 Microorganisms

[click here to insert text]

3.2.2.1.4 Other terrestrial organisms

[click here to insert text]

Acute toxicity

[click here to insert text]

Long-term toxicity

[click here to insert text]

3.2.2.2 Calculation of Predicted No Effect Concentration (PNEC)

[click here to insert text]

3.2.3 Atmosphere

[click here to insert text]

3.2.4 Secondary poisoning

[click here to insert text]

3.2.4.1 Effect data

[click here to insert text]

3.2.4.2 Calculation of $PNEC_{oral}$

[click here to insert text]

3.3 RISK CHARACTERISATION [1]

[click here to insert text; consider using overview tables with PEC and PNEC ratios]

3.3.1 Aquatic compartment (incl. sediment)

[click here to insert text]

[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.

Conclusions to the risk assessment for the aquatic compartment:

[keep only appropriate conclusion(s)]

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 () applies to [click here to insert text in accordance with conclusion(s)]

3.3.2 Terrestrial compartment

[click here to insert text]

Conclusions to the risk assessment for the terrestrial compartment:

[keep only appropriate conclusion(s)]

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 () applies to [click here to insert text in accordance with conclusion(s)]

3.3.3 Atmosphere

[click here to insert text]

Conclusions to the risk assessment for the atmosphere:

[keep only appropriate conclusion(s)]

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 () applies to [click here to insert text in accordance with conclusion(s)]

3.3.4 Secondary poisoning

[click here to insert text]

Conclusions to the risk assessment for secondary poisoning:

[keep only appropriate conclusion(s)]

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 () applies to [click here to insert text in accordance with conclusion(s)]

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

It is recalled that a short assessment study (risks, advantages/drawbacks) was carried out in 1995 on request of DG III within the framework of Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations, to answer an Austrian claim concerning several chlorinated solvents. The results of that study led to the adoption, in 1996, of Directive 96/55/EEC of the Commission (2nd adaptation of Directive 76/769) which prohibits the use of chloroform "in concentrations equal to or greater than 0,1 % by weight in substances and preparations placed on the market for sale to the general public and/or in diffusive applications such as in surface cleaning and cleaning of fabrics. The provisions entered into force on June 30th 1998. 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 the use of product.

Mainly based on this previous assessment, the French rapporteur asked during a CA's meeting to limit the work in term of the Risk Assessment. It was finally agreed to follow a fast track procedure; this is why the human health assessment is mainly based on published reviews.

Humans may be exposed to chloroform at workplace and indirectly via the environment.

Chloroform is also a chemical by-product associated with disinfection of swimming pool water; chloroform is originated by the reaction of disinfecting agents with organic substances; the chloroform exposure will be assessed for workers as swimming instructors, lifeguards, competitive swimmers (they will be considered as workers) and for consumers as child swimmers and adult swimmers.

Workers are primarily exposed via inhalation and dermal routes (and ingestion route for competitive swimmers). Consumers in swimming pools are exposed by inhalation, dermal and ingestion routes.

For workers, there are two possible exposure pathways: from industrial processes and from the formation of chloroform in chlorinated swimming pool water.

In swimming pool, people are exposed to chloroform present in the water and in the air.

For the industrial activities, exposure may occur mainly during manufacture and use as intermediate for the production of chlorodifluoromethane (HCFC 22); chloroform is also used as a chemical intermediate or solvent in the synthesis of various chemicals and pharmaceuticals.

The vast majority of chloroform (95.4 %) is consumed as feedstock, in closed continuous processes, for the production of chlorodifluoromethane (HCFC 22, also known as refrigerant

R 22). When the productions of chloroform and HCFC 22 are integrated in the same site, chloroform is supplied to the consuming units by pipeline inside the industrial site. In the other cases, transport to customer occurs by rail or truck tank or occasionally by vessel.

Chloroform is used in other applications (4.6 %) as feedstock (2.8%) or extraction solvent (1.8%), generally in batch processes, especially in the pharmaceutical industry (for example in the extraction of penicillin and other antibiotics) and in the production of dyes, pesticides and other substances. In these cases, chloroform is distributed in liquid form in tanks and drums and transported via rail or by road trucks.

General remark: The operations and tasks described hereafter are typical of standard chloroform production or handling facilities. There could be slight variations in the operating procedures but these will not affect the human exposure pathways and levels.

4.1.1.2 Occupational exposure

Definitions

In this document, unless otherwise stated, the term exposure is used to denote external personal exposure as measured or otherwise assessed without taking into account the attenuating effect of any personal protective equipment (PPE) which might have been worn. This definition permits the effects of controls, other than PPE, to be assessed and avoids the considerable uncertainty associated with attempting to precisely quantify the attenuation of exposure brought about by the proper use of PPE. Furthermore, inappropriate use of gloves may even increase dermal uptake.

The worst-case estimates generated in this exposure assessment are considered to be reasonable worst-case estimates, as they describe high-end or maximum exposures in feasible but not unrealistic situations. They are not intended to account for extreme or unusual use scenarios. The majority of exposures are expected to be well below these estimates.

Air sampling data are provided by the manufacturers and users of chloroform and have been tabulated in this section. There is little information on the sampling strategy and measurement methods.

Measured exposure data are compared with that predicted from the EASE (Estimation and Assessment of Substance Exposure) model version 2. EASE is a general-purpose predictive model for workplace exposure assessments. It is an electronic, knowledge based, expert system which is used where measured exposure data is limited or not available. The model is in widespread use across the European Union for the occupational exposure assessment of new and existing substances.

No measured dermal exposure data were provided by industry for chloroform.

All models are based upon assumptions. Their outputs are at best approximate and may be wrong. EASE is only intended to give generalised exposure data; it predicts inhalation exposure as ranges for concentrations for continuous exposure. Dermal exposure estimates are provided by EASE as the quantity of a product adhering to the skin due to a task, they do not take into account evaporation of the product.

In the present assessment all inhalation exposures are expressed in parts per million (ppm), and in mg/m3. All mg/m3 have been converted to ppm using the following approximation:

1 ppm = 4.88 mg/m3 at 25°C and 1 Atm.

Routes of exposure and relevant scenarios

The occupational routes of exposure to chloroform are inhalation and skin contact. Assuming proper hygiene measures are applied, oral exposure would normally not occur in the workplace (except for competitive swimmers).

Literature data

In HSE (Health and Safety Executive, 1994) it is reported that chloroform is manufactured on a substantial tonnage scale by one UK company by hydrochlorination of methanol to methylchloride, followed by chlorination. A large proportion is used as a raw material in the production of chlorodifluoromethane (HCFC 22) but it is also used as an industrial process solvent and in laboratory work. It is estimated that not more than 2000 UK workers are regularly exposed to chloroform, in many cases intermittently. The majority of exposure measurements have been less than 10 ppm at manufacturing and packaging operations. In a large user plant, all measured exposures were =< 5 ppm and 98% =< 1 ppm.

Production and use are described in WHO (World Health Organization, 2004) : the total production in the European Union has been estimated at 316000 tonnes. Chloroform's main use is in HCFC 22 production and this accounts for 90-95% of its use in the European Union. Although use of HCFC 22 in refrigerant application is decreasing, increasing use of HCFC 22 as the feedstock for fluoropolymers such as polytetrafluoroethylene means that demand for chloroform has remained relatively constant. Earlier use of chloroform as an anaesthetic has been largely discontinued in most countries, but it still has limited use in some dental procedures and in certain pharmaceuticals.

In NTP (National Toxicology Program, 2005) it is mentioned that approximately 96% to 98% produced in the United States is used to make HCFC 22. It is used as a refrigerant (70% of the HCFC 22 produced) and in the production of fluoropolymers (30%). However, this use is expected to diminish because of the phaseout of chlorine containing fluorocarbons. Other uses include the following: as a solvent in the extraction and purification of some antibiotics, alkaloids, vitamins and flavours.

In NPI (National Polluant Inventory, 2005), common uses as the production of refrigerants, manufacture of chemicals and solvent extraction are described; it is also reported that chloroform is steadily being replaced by less toxic solvents and may no longer be used in some of applications less common.

The use of chloroform in endodontics is described in SHUUR (2004): chloroform is used to dissolve gutta-percha from root canals. It is questioned whether the use of the solvent could affect the health of patients or of the dental team.
Endodontics treatments consist in filling root canals of the tooth with gutta percha to isolate the canal system from the oral environment ; sometimes it is necessary to eliminate the gutta percha from the canal to do the treatment again; the elimination is done with specific tools and also with chloroform as solvent to dissolve Gutta percha; these treatments are conducted by a dentist and are not so frequent, and the quantity of chloroform used is very small (a few drops of chloroform injected with a syringe).
It seems warranted to conclude that the amounts and concentrations of chloroform used in endodontic retreatment are very low and safe. No scenario should be developed for this use.

Another scenario of exposure to chloroform is reported in ERDINGER (2004): chlorination of pool water leads to the formation numerous disinfection by-products (DBPs), chloroform usually being most abundant. Bathers and pool guardians (workers walking around the pool without swimming) take up various amounts of DBPs by different pathways as inhalation, dermal absorption or orally. In this experimental study involving up to 17 participants, the body burden resulting from exposure to three different concentrations of chloroform in water and air of an indoor swimming pool was quantified during a 60 min exercising period. Chloroform concentration of the water was 0.0207, 0.0071, and 0.0248 mg/l. Corresponding air chloroform concentrations were measured and ranged to 0.085 mg/m3 to 0.235 mg/m3 or 0.017 ppm to 0.05 ppm, a value (0.05 ppm) which is about 40 times lower than the european OEL value of 2 ppm recommended for the 8-hour TWA.

An other study from WHO (2000) reviews the routes of exposure to chemicals in swimming pools and similar recreational-water environments, estimated and measured intakes of chemicals by users (workers and consumers), and the hazards with exposure to the chemicals. It is reported that the main constituent among trihalomethanes produced by reactions between disinfectants and other substances present in the swimming pool is chloroform.

In view of data from literature source and data from European producers/importers, occupational exposure assessment will be carried out through the three following main categories of scenarios:

- Scenario 1: the manufacture of chloroform and its use as an intermediate for the production of chlorodifluoromethane (both in closed continuous system);
- Scenario 2: its use as intermediate or solvent in the synthesis of various chemicals and pharmaceuticals (both in closed batch processes).
- Scenario 3: exposure of workers (swimming instructors, lifeguards, competitive swimmers) to chloroform in swimming pools

Occupational exposure limits (OELs)

OELs apply to workplace air concentrations of chemicals. They are normally intended to protect workers against short-term adverse effects (irritation, acute effects) or long-term effects (e.g. on liver, lungs, kidneys, or chronic effects) after months or years of exposure. When applicable, a "short-term exposure limit" (STEL) may be proposed or imposed for the first ones, and/or a "time-weighted average" (TWA) for the second. The first value ordinarily refers to a 15 minutes or so duration, the second to a shift (generally considered as an 8-hour shift).

Table 4.1 details the OELs recommended for chloroform in various countries. They are provided for information and are not an indication of the level of control of exposure achieved in practice in workplaces.

Table 4.1 OEL values BGIA (2005)

	8-hour TWA		STEL, 15 min	
Country	mg/m^3	ppm	mg/m^3	ppm
EU*	10	2		
Austria	10	2		
Belgium[a]	10	2		
Denmark	10	2	20	4
France	10	2	250	50
Germany (MAK)	2.5	0.5	10	2
Hungary	10		10	
Italy	10	2		
Spain	10	2	-	-
Sweden	10	2	25	5
United Kingdom[a]	10	2	-	
USA (OSHA)		-	240	50
USA (ACGIH)		10		

*Directive 2000/39/CE of 8 June 2000

a : values given by Belgium and UK in their comments on the RAR of chloroform (May 2007).

The EU Directive 2000/39 proposed an Indicative Limit Value (ILV) for chloroform. The ILV is considered indicative for the limit of daily exposure for a worker which probably gives no rise to adverse health effects. The EU value, also noted ILV-TWA (for time weight average), is 10 mg/m3 on the basis of 8 h work, 40 h/week. This corresponds to a 2 ml/m^3 (ppm) OEL value accepted in Europe.

It is to be pointed out that important variations are observed between the different recommended threshold values.

4.1.1.2.1 Scenario 1: the manufacture of chloroform and its use as an intermediate for the production of chlorodifluoromethane (HCFC 22); closed continuous system

As previously indicated under 2.2., two industrial processes are used to produce chloroform:

- the esterification of methanol with hydrogen chloride to produce methyl chloride which is subsequently chlorinated with chlorine gas in the same way as methane
- and the thermal non catalytic chlorination of methane using chlorine gas

Typical process description

These processes are all closed continuous systems.

The continuous, closed production of chloroform by chlorination is followed by purification and by distillation in rectification columns, separating chloroform in high purity and transferring it into on-site storage vessels. From there it is dispatched in bulk via pipeline on site, or rail & road tanks and ISO containers and bulk ships to external customers. All down stream operations after distillation are carried out batch-like in closed systems.

As the operating conditions for the workers are very similar (as far as occupational safety is concerned) in both the chloroform production sites and in the sites using chloroform as raw material for the production of chlorodifluoromethane (HCFC 22), the task description, the safety procedures and the exposure levels will be jointly described hereafter. The use of chloroform in the other applications will be considered separately.

This option is justified by the fact that both chloroform and HCFC 22 are produced in continuous closed processes, with very limited exposure of workers in normal operation, with similar safety procedures and similar worker tasks.

Description of workers' tasks

In a chloroform or HCFC 22 plant, workers can generally perform one of the following tasks: production work, maintenance, sampling, and packaging of the end product.

Production work consists of process control: operation of manual valves, control of process parameters, loading or unloading, preparation of maintenance activities; doing rounds including visual checks of piping, pumps, valves, etc. In many plants remote control devices are used but a site survey is made by operators.

The processes are closed and during normal work, exposure to chloroform is possible only in case of accident. All equipment has been designed to meet appropriate Engineering Standards and the integrity of the pressurised systems is ensured by compliance with Engineering Procedures which covers piping, relief streams, components, testing etc.

During standard operations the exposure of workers to chloroform is limited as there is no direct contact with liquid chloroform or admixtures (no 'open' handling except sampling) and in addition the production building is well ventilated (in and out) and the air inside the building is monitored at several places via on-line GC or the production equipment is located outside. For most of the time of a working day/shift the operating staff stays outside the production building as the plant is largely automated and operated by remote control from a room placed in a spatially separated building. The interim storage building is usually only entered for short-time operations (switching pumps, adding stabilisers and sampling). Storage tanks and dispatch filling stations are installed without surrounding building and freely ventilated by the atmosphere.

When chloroform is used as raw material it is supplied in tankers and pumped into a storage tank. Couplings are of the 'dry break' type resulting normally in no emission of chloroform.

The rest of the process operates in a closed system. The liquid is fed through an alumina drier into a header tank, then into the reactor via a central dip pipe.

Maintenance consists of control, revision, repair of all mechanical or electronic components, including replacement of fittings, valves, instruments and the cleaning of the reactors. Coupling and decoupling of pipelines can also take place for maintenance purposes. The opening of the system takes place only after its emptying, purging and isolation via blank flange, and disconnection. Maintenance and repairs of pumps, dosing systems and automatic control systems is only carried out by specialised companies or trained workers after complete degassing of the system.

Sampling generally consists of the collection of small volumes of liquid or gas phases from the reaction medium for analytical purposes and quality control. The sample is taken from the system at well identified sampling stations in plant or from the tank of road or rail tanker. Special sampling devices are used by trained persons. Manual samplings are often made to check the reliability of the automated remote control systems. Protective equipment (safety shoes, long sleeved shirt, long pants, safety goggles and respiratory protection mask) is often used. The analysis is made in the laboratory in a fume-hood or in a vented area. As, in the process, the analytical controls are made automatically, the sampling procedure is only used to check the quality and reliability of the system and consequently, analyses in the laboratory are not very frequent e.g. once a day.

Loading and unloading: Chloroform is transferred via pipelines to on-site users and is filled into the reaction vessel through closed systems, while off-gases from the reactor are treated before release to the atmosphere. Chloroform is also transported via rail or road tankers or via smaller packages. In all cases, the transfer of chloroform is done through loading stations adapted to the size of the tank or vessel. The main elements of these stations for road trucks or rail tankers are coupling for emission-free loading/unloading. Chloroform is unloaded from train containers to pressure controlled storage tanks with N2 blanketing.

All personnel who enter the area of a loading installation receive a special training and have available personal respiratory protection. Advice concerning the method of operation is permanently available as well as emergency plans and precise instructions in case of emergency; they are brought to the attention of the personnel involved by regular trainings. Self-contained breathing sets and protective clothing suitable for dealing with a chloroform leak are generally available near to the discharge point, and accessible at all times in case of emergency.

Safety procedures

General remark:
The safety procedures in the chloroform production or in HCFC 22 plants are very strict because they are imposed by the use of very toxic chlorine or hydrogen fluoride gas.

Inhalation exposure

Measured data

Measured data are available on chloroform atmospheric concentration in the workplace in different parts of the plant, conducted with fixed detectors placed in locations where the workers have to frequently pass. Moreover in some plants the workers are also wearing personal detectors (in their breathing zone but outside of any respiratory protective equipment), to measure exposure in a continuous way (integration over 8 hours).These detectors are working by adsorption and also detect other chlorinated organic substances. The amount of chloroform is analysed in the laboratory by gas chromatography.

Table 4.2 presents the workers exposure to chloroform in the atmosphere during chloroform or HCFC 22 production. The reported values summarise TWA data. Median values, 75 and 90 percentiles and range are expressed both in mg/m³ and ppm. These data cover 7 different production sites in the EU and refer to all functions in the plants. As most of the workers cover different functions in the plants over a long range period, it is not possible to split the TWA values into the various functions. They provide however a complete picture of worker's exposure in chloroform and HCFC 22 production plants.

It has to be pointed out that chloroform concentrations used to calculate TWA values have been measured also when the workers are wearing a mask or a PPE.. Generally, all releases should be avoided. In cases where release cannot be avoided, and a considerable percentage of the occupational exposure limit is reached, workers shall wear masks or other PPE. Consequently, as Table 4.2 represents the full range of raw data, the calculated 90 percentile clearly define the worst case exposure levels.

In some cases, the limit 2 ppm (10 mg/m³) was exceeded. However, as the operators were wearing their sensors outside of any PPE being worn this does not mean that they were necessarily over-exposed. It has to be stressed that most of values exceeding 2 ppm (10 mg/m³) were measured in very specific situations that normally required the compulsory wearing of PPE (either masks with filters or, for longer exposure, self-contained breathing apparatus) and to follow specific safety procedures. This is reflected by the low value of the 90 percentile, indicating that the cases where the 2 ppm limit are exceeded are infrequent and correspond to specific conditions.

Table 4.2 Workers exposure to chloroform in the atmosphere during chloroform or HCFC 22 production. Summary of TWA data (2003-2005). Average values, 75 - 90 percentiles and ranges are expressed in mg/m³ and in ppm

N of sites	Countries covered	Functions covered	Number of workers	Number of samples	Range TWA exposure	Average TWA exposure	75 percentile exposure	90 percentile exposure
7	B, D,F, SP, UK	All functions, process operations, maintenance, filling, laboratory	About 200	1576	mg/m³ <0.05 - 472	mg/m³ 2,45	mg/m³ 3.78	mg/m³ 5.6
					ppm <0.01- 97	ppm 0.50	ppm 0.78	ppm 1.15

Modelled data

The EASE model used to predict exposure during production in closed system with full containment provides an exposure estimation of 0 - 0.1 ppm. If the system is breached in some activities (like maintenance, sampling, cleaning, filling), concentrations could be in the range of 20-50 ppm (non dispersive use, moderate/high tendency to become airborne, presence of LEV).

Summary/statement of the exposure level

The comparison between model results and measured data should be made based on similarity of situations. However, the similarity is difficult to assess because the control pattern in the Table 4.2 of measured data is not presented with the results : both “closed system” and “closed system breached” are possible. Considering this, the two ranges 0-0.1 ppm and 20-50 ppm from EASE are in line with the range <0.01-97 ppm of TWA mentioned in the Table 4.2.

Using as a reasonable worst case “the 90 percentile of the distribution of exposure levels observed in all locations” the long term (8 hours) **inhalation exposure** to chloroform of workers in chloroform or HCFC 22 production plants is **1.15 ppm or 5.6 mg/m³**. Higher exposure may occur during non-routine maintenance activities or during rare incidents as mentioned in the Table 4.2 or for the case of breached system. Such incidents are presented as exceedingly rare by industry adding that workers would wear PPE in such circumstances.

This value is very conservative for the following reasons:

- the measured value takes into account the exposure coming from several production plants (chloroform and HCFC 22)
- the detectors are also measuring exposure when the operators are using PPE, including masks
- the 90 percentile is calculated on the distribution of all measured values
- <u>the 75 percentile (O.78 ppm) could be also used for the reasonable worst case</u>
- <u>in HSE(1994), 98% of measured exposures were lower than 1 ppm</u>

<u>Dermal exposure</u>

Measured data

No measured data are available.

Modelled data

The EASE model estimated a dermal exposure in the range of 0 - 0.1 mg/cm^2/day for the case “non dispersive use with direct handling and incidental contact” and in the range 0.1 – 1 mg/cm^2/day for the case “non dispersive use with direct handling and intermittent contact”. Assuming exposed skin surface area is 420 cm^2 (palms of hands for consistency with other EU occupational risk assessments), maximum external dermal exposure would be 42 - 420 mg/day. This exposure will be mitigated by the use of suitable gloves.

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems Moreover, the extent of protection by PPE (here gloves) depends on thesuitability of the recommended material with regard to the permeation properties of substance.

In the case of chloroform, the predominant effect reducing potential dermal exposure is the very high volatility of the substance (vapour pressure 20.9 kPa at T = 20°C) which leads to considerable low retention times of the substance on the skin or on the protective gloves. This exposure reducing effect cannot be considered if workers have continuous direct contact with the substance, e.g. dipping hands into the substance. For the area of production and further processing of chloroform, this situation is regarded to be rather non-probable. Furthermore, it is assumed, that non occlusive exposure is the predominant exposure situation.

For the purpose of determining the evaporation rate of chloroform, an equation was used which was derived within the framework of a research project (Weidlich and Gmehling 1986; Gmehling et al., 1989). This project was aimed at calculating airborne concentrations of substances when emitted from liquid mixtures under consideration of the evaporation and the spreading of the substance at the workplace. For calculating the evaporation times of substances, an equation was derived based on the mass transfer at the interface between the liquid and the vapour (two-film-theory). Mass transfer during evaporation occurs until the equilibrium state is achieved. The main influence on evaporation is the transfer through the interface.

For pure substances, the following equation is used:

$$t = \frac{m \times R \times T \times K}{M \times \beta \times \mathrm{p} \times \mathrm{A}}$$

t: time [s]
m: mass, EASE estimate [mg] (per cm²)
R: gas constant: 8.314 $J.K^{-1}.mol^{-1}$
T: skin temperature [K]
M: molar mass [$g.mol^{-1}$]
β: coefficient of mass transfer in the vapour phase [$m.h^{-1}$], for calculation:
β = 8.7 m/h, see below
p: vapour pressure of the pure substance [Pa]
A: area, EASE: 1 cm^2
K(conversion factor) = 3.6 10^4

The skin temperature amounts normally to 28-32°C (ambient temperature: 20-22°C). The reduction of the skin temperature and accordingly of the vapour pressure caused by the evaporation process is not considered in the equation. This might be done by choosing a lower mean temperature for the evaporation process.
The coefficient of mass transfer β is described based on empirical studies:
$\beta = (0.0111 * v^{0.96} .* D_g^{0.19}) / (\nu^{0.15} .* X^{0.04})$
D_g : coefficient of diffusion, gas phase
v: velocity of air [m/h]
ν: kinematic viscosity of air [m²/h]
X: length of the area of evaporation in the
direction of the air stream [m]

In the above given equation, the main influencing parameter is the velocity of the air (v). At workplaces v is often between 0.3 m/s and 0.6 m/s (a velocity higher than 0.5 m/s is felt as non-convenient). Since the hands from which a substance evaporates are often in motion, the air velocity might be higher. For a conservative approach, a low value (0.3 m/s) was chosen. For different organic solvents, Dg is approx. 0.05 m^2/h. so that Dg $^{0.19}$ is 0.566.

A literature value was taken for the kinematic viscosity of air ($5.4396.10^{-2}$ m^2/h).

The parameter X, representing the length of the area of evaporation in the direction of the air stream [m] is because of its low exponent (0.04) not very influencing. For the calculation, a length of 10 cm was taken.

Taking into account a rather low velocity of air (0.3 m/s), β is about 8.7 m/h.

For chloroform with the EASE estimate of 1 mg/cm^2, an evaporation time of 3 seconds (T = 25°C) is calculated. For chloroform on the gloves, an assumed temperature of 20°C leads to an evaporation time of 4 seconds. These values should be regarded to represent the order of magnitude, since it is not known in how far the interaction of the skin with the substance influences the evaporation time.

This short-retention time of chloroform on the skin leads to much lower dermal exposures than predicted by the EASE model which considers dermal exposure during the whole shift (42-420 mg/person/day). Taking into account the high volatility of the substance, daily dermal exposure during the production and further processing of the substance is assessed as low (<< 42-420 mg/person/day).

Summary/statement of the exposure level

Considerations on evaporation and skin absorption

Chloroform is a liquid with a high vapour pressure of 209 hPa at 20°C. In Section 4.1.1.2 it is reported that neat chloroform (1 mg/cm^2) would evaporate within 3-4 seconds from skin (T: 20-25°C) under usual working conditions of non-occlusive exposure. It is assumed that chloroform could be well absorbed as long as it is available for absorption, but quantitative data on skin absorption rates (e.g. flux value) is not known. As a worst-case assumption the highest flux value (human skin *in vivo*) for neat liquids (33 mg/cm^2/h; ethyl benzene) of a summary report (Leung and Paustenbach, 1994) is used for a model calculation to estimate skin absorption.

Applied dose: 1 mg/cm^2/d
Maximal flux: 33 mg/cm^2/h (= 0.0092 mg/ cm^2/sec)
Time of skin contact: 4 seconds

A maximal skin exposure of 0.04 mg/cm2/d (= 4% of the applied dose) is calculated for the above conditions. The calculation is uncertain due to its theoretical nature and the general caution as to dermal absorption studies and the applicability of flux values (DEN, 1999; de Heer, 1999), but overall it is expected, that the major part of neat chloroform will evaporate before absorption.

Moreover, a precautionary approach is always used because, in case of opening the chloroform system, workers are wearing protective clothing made of gloves, facial or respiratory protection mask and overalls if necessary (made of fluoro rubber, PVA, nitrile rubber, etc) to fully protect them from dermal exposure.

Consequently the following value of the daily dermal exposure has been adopted as the worst reasonable case exposure:

Dermal exposure = 420 * 0.04 = 16.8 mg/person/day

4.1.1.2.2 Scenario 2: chloroform as intermediate or solvent in the synthesis of various chemicals and pharmaceuticals; closed batch processes.

If the main chloroform use (95.4%) is as a raw material in the continuous synthesis of HCFC 22, (which has been reviewed under chapter 4.1.1.2.1.), it is also used as a chemical intermediate or solvent in the synthesis of various chemicals and pharmaceuticals, in batch processes (4.6 %). The details concerning sector applications are mentioned under section 2.2.

Chloroform is supplied in liquid form to the consuming industries by pipeline if they are located on the same site or by rail tanker or road truck. For the synthesis of chemicals and pharmaceuticals or the use as solvent in batch processes, the supply is made by tankers or drums. In all applications, occupational exposure to chloroform may occur during handling (filling) operations and/or production of chemicals. In most processes, chloroform is completely transformed during the reaction.

Typical process description

Chloroform is delivered in bulk by tankers and unloaded via closed system connections with vapour balance piping into a storage tank and transferred into the reactor by gravity or vaporisation. All down stream operations thereafter are carried out in closed systems. The reactors are glass lined (enamel) or stainless steel. The chloroform is generally completely consumed in the chemical reaction and consequently, during the use of chloroform as a raw material for production of a pharmaceutical active substance, nearly no emission into the work environment is possible.

Chloroform alone or in combination with other solvents is also used as a solvent for extraction of pharmaceutical active ingredients, either from natural resources or from the reaction medium. Afterwards, the product is separated, mainly by crystallisation and filtration and the chloroform is concentrated up by phase separation and/or distillation and then dried (continuously or by batch) to be recycled. The extraction and distillation are also done in closed systems. During the drying processes emission into the work environment is possible. In this area, the chloroform concentration is continuously monitored (by mass spectrometry for example). In general, all points in the manufacturing process where there is potential for personnel to be exposed to chloroform are fitted with local exhaust ventilation equipment. Off-gas is transferred then to a chilled trap in order to recover the chloroform.

In batch processes, chloroform is vaporised from storage on an “on-demand” basis and fed into the batch reactors via a closed system. Un-reacted chloroform, if any, is vented through scrubbers or chilled traps to be recovered after separation and distillation or to be destroyed by incineration.

During standard operations the exposure of workers to chloroform is limited as there is no direct contact with liquid chloroform or admixtures (no 'open' handling except sampling & analysis) and in addition the production building is well ventilated and the air inside the building is monitored. The operators are generally wearing detectors measuring air exposure to chloroform by adsorption over 8 hours. Most of the time, the operating staff stays outside the production building as the plant is automated and operated by remote control from a room in a separate building.

Descriptions of worker's tasks and safety procedures

Production work consists of process control: operation of manual valves; control of process parameters, loading or unloading, preparation of maintenance activities; doing rounds including visual checks of piping, pumps, valves, etc. The operating staff must wear standard protecting equipment, i.e. chemical resistant gloves and safety shoes or boots, working clothes, helmet, goggles and escape mask equipped with appropriate filter. In case of emergency self-contained breathing apparatus are available.
In general, the production is carried out in campaigns and limited to a few months per year.

Maintenance consists in control, revision, repair of all mechanical or electronic components. Coupling and decoupling of pipelines can take place for maintenance purposes. The opening of system takes place only after its emptying, purging, complete degassing and disconnection. Maintenance and repairs of pumps, dosing systems and automatic control systems is only carried out by specialised companies or trained workers after complete degassing of the system.
In most plants maintenance personnel have to follow written procedures dictated by the plant supervisor. In general maintenance work is carried out only if a "work permit" from the plant supervisor is issued when the status of the plant has been checked. Safety procedures and personal protective equipment to be used to prevent exposure are dictated by the plant supervisor and documented in the work permit. In case of opening of the system, PPE used is goggles, face shield, gloves, rubber overall, rubber boots, gas mask or self-contained breathing apparatus. Particular precautions should be taken for the cleaning of filters.
Maintenance operations generally take place for only a few days per year

Sampling generally consists of the collection of liquid or gas samples from the reaction medium for analytical purposes or quality control. The sample is taken from the system at well identified sampling stations on the plant. Special sampling devices are used by trained persons with sufficient knowledge. Manual sampling is often only done to check the reliability of the automated remote control systems. During sampling there is the possibility of coming into contact with liquid chloroform and operators are obliged to use personal protective equipment, in particular chemical resistant gloves and overalls as well as RPE, e.g. respiratory gas mask equipped with appropriate filter. Sampling usually takes approximately 30 minutes, and can be repeated 3 to 4 times a day.

All personnel who enter the area of a chloroform loading/unloading installation have available at least personal respiratory protection. Tanker loading uses a delivery pipe fitted with a conical ventilated collar that is seated in the man-way on top of the tanker. Tanker offloading uses dry break connections at ground level and vapour balancing (e.g. negative pressure in receiving vessel). Advice concerning the method of operation is permanently available. An emergency plan and precise instructions in case of emergency is permanently available and brought to the attention of the personnel involved. Canister facial masks and gloves are worn

during product transfer in particular when drums are emptied or filled. Self-contained breathing sets and protective clothing suitable for dealing with a leak is generally available in lockers located near to the discharge point, and accessible at all times in case of emergency. Loading/unloading operations are generally limited to a few hours per day and most often to 30 to 50 days a year.

Exposure scenario

In this type of production units, the personnel are required to be flexible and to cover all the functions. It is therefore difficult to distinguish the exposure scenarios between the normal production activities, the maintenance, the sampling and the loading-unloading operation.
Moreover, as the personal detectors worn by the workers are monitoring the exposure by collecting air samples by adsorption over an 8 hour period of time, it is technically not possible to differentiate the various functions and to have short term exposure data.
Consequently, we should consider a global, long term (8 hours) exposure scenario covering all operating tasks. In all cases, safety procedures and the use of appropriate protective equipment limit the exposure to chloroform to accidental events. Potential for exposure exists as a result of leaks. In case of a leak, workers shall wear the appropriate PPE, all personnel normally carrying a mask. Most of the plants perform TWA (8 hours) analysis.

Inhalation exposure

Measured data

The measured data provided by several chloroform users are representative of the multi-functional tasks carried out by the workers and are covering normal work, maintenance, sampling as well as loading-unloading. Even if the amount of data is not sufficient to be considered as statistically representative, it appears that two exposure scenarios should be considered depending on whether chloroform is used as a solvent or as a raw material. The exposure levels corresponding to these two scenarios are illustrated in Table 4.3 hereafter. These data are considered as good examples of the exposure levels in batch processes.

Table 4.3 Workers exposure to chloroform in the atmosphere during batch production using chloroform as a solvent or as raw material. Summary of TWA data (2003-2005). Average values, 75 - 90 percentiles and ranges are expressed in mg/m³ and in ppm

.Scenario	Functions covered	Type of measurement	Range TWA exposure	Average TWA exposure	75 percentile exposure	90 percentile exposure
Chloroform used as intermediate (closed batch process)	All functions, process operations, maintenance, filling, laboratory	Continuous mass spectrometry	mg/m³ 0.05 - 0.15 ppm 0.01 – 0.03	mg/m³ 0.10 ppm 0.02	mg/m³ 0.124 ppm 0.026	mg/m³ 0.15 ppm 0.03
Chloroform used as solvent in the synthesis of chemicals (closed batch process)	All functions, process operations, maintenance, filling, laboratory	Continuous mass spectrometry and 8 hours adsorption detectors	mg/m³ 0.1 – 37.5 ppm 0.02 – 7.5	mg/m³ 9.2 ppm 1.9	mg/m³ 11.4 ppm 2.35	mg/m³ 13.7 ppm 2.8

It has to be pointed out that chloroform concentrations presented in Table 4.3 have been measured also when the workers are wearing a mask or other PPE. Generally all releases should be avoided. In cases where release cannot be avoided, and a considerable percentage of the occupational exposure limit is reached, workers shall wear masks or other PPE
When chloroform is used as solvent, the limit 2 ppm (10 mg/m³) was from time to time exceeded. However, as the operators were wearing their sensor all the time and/or the air concentration is continuously monitored, most of values exceeding 2 ppm (10 mg/m³) were measured in very specific situations (drying, sampling and cleaning) where it is compulsory to wear respiratory personal protection (masks with filters or, for longer exposure, self-contained breathing apparatus) and to follow specific safety procedures. This is reflected by the fact that the 75 and 90 percentile values are relatively closed to the average value, indicating that the cases where the 2 ppm (10 mg/m³) limit are exceeded are infrequent and correspond to specific conditions. Moreover, these special situations are of relatively limited duration.

Modelled data

The EASE model used to predict exposure during use as intermediate or solvent in the synthesis of various chemicals and pharmaceuticals in closed system with full containment provides an exposure estimation of 0 - 0.1 ppm. If the system is breached in some activities (like maintenance, sampling, cleaning, filling), concentrations could be in the range of 20-50 ppm (non dispersive use, moderate/high tendency to become airborne, presence of LEV).

Summary/statement of the exposure level

The comparison between model results and measured data should be made based on similarity of situations. However, the similarity is difficult to assess because the control pattern in the Table 4.3 of measured data is not presented with the results : both “closed system” and “closed system breached” are possible. Considering this, the two ranges 0-0.1 ppm and 20-50 ppm from EASE are in line with the range <0.01-7.5 ppm of TWA mentioned in the Table 4.3.
Taking into account

- the available information,
- the fact that the measured values are coming from production plants where chloroform is used as raw material or as a solvent
- the fact that exposures are also measured when the operators are using PPE
- the fact that the operations where exposure is expected to be the most important are of short duration and submitted to particular safety conditions
- the fact that the 75 and 90 percentile (respectively 11.4 and 13.7 mg/m^3), calculated on the distribution of all measured values, are relatively closed to the EU value ILV TWA of 10 mg/m^3 or 2 ppm
- the fact that in HSE(1994), 98% of measured exposures were lower than 1 ppm

it is proposed to consider as reasonable worst case **long term inhalation exposure** of workers (equivalent to TWA) the EU value ILV TWA of 10 mg/m^3 or 2 ppm. This value covers all the operating functions in plants using chloroform as raw material or as solvent.

Dermal exposure

As for the scenario 1 “manufacture of chloroform and its use as an intermediate for the production of chlorodifluoromethane (HCFC 22); closed continuous system” no measured data are available.

In the case of chloroform, the predominant effect reducing potential dermal exposure is the very high volatility of the substance (vapour pressure 20.9 kPa at T = 20°C) which leads to low retention times of the substance on the skin. For chloroform with the EASE estimate of 1 mg/cm^2, an evaporation time of 4s at 20°C bas been calculated using an equation derived within the framework of a research project (Weidlich and Gmehling 1986;Gmehling et al., 1989). This project was aimed at calculating airborne concentrations of substances when emitted from liquid mixtures under consideration of the evaporation and the spreading of the substance at the workplace. The calculations leading to an evaporation time of 4s have been detailed above in the paragraph 4.1.1.2.1 Scenario 1/ Dermal exposure p. 33.

It is assumed that chloroform could be well absorbed as long as it is available for absorption, but quantitative data on skin absorption rates (e.g. flux value) is not known. As a worst-case assumption the highest flux value (human skin *in vivo*) for neat liquids (33 mg/cm2/h; ethyl benzene) of a summary report (Leung and Paustenbach, 1994) is used for a model calculation to estimate skin absorption.

Applied dose: 1 mg/cm2/d
Maximal flux: 33 mg/cm2/h (= 0.0092 mg/ cm2/sec)
Time of skin contact: 4 seconds

A maximal skin exposure of 0.04 mg/cm2/d (= 4% of the applied dose) is calculated for the above conditions. The calculation is uncertain due to its theoretical nature and the general caution as to dermal absorption studies and the applicability of flux values (DEN, 1999; de Heer, 1999), but overall it is expected, that the major part of neat chloroform will evaporate before absorption.
Consequently,as for the scenario 1, the following value of the daily dermal exposure has been adopted as the worst reasonable case exposure:

Dermal exposure = 420 * 0.04 = 16.8 mg/person/day

4.1.1.2.3 Scenario 3: exposure of workers to chloroform in swimming pools

People working as swimming instructors or life guards in the swimming halls may be exposed to chloroform originated by the reaction between disinfecting agents (chlorine/hypochlorite) with organic substances (amino-acids or proteins from urine, perspiration, oils, cosmetics and insoluble detritus).

Measured data

The following table presents concentrations of chloroform in air and water of European swimming pools in recent studies. Data show that chloroform concentration is highly variable, depending on operational practices (chlorine dose, pool occupancy, swimmers' hygiene and water and air renewal). The competition swimmers who are competitive adult swimmer in regular training spending at least four hours in the swimming pools will be considered as workers.

Table 4.4 Chloroform concentrations in swimming pools in water and air

By product	Concentration		Pool type	Reference
	Mean	Range		
Concentration in pool water (µg/l)				
Chloroform		19-94	indoor	Aggazzotti et al., 1993
	93.7	9-179	indoor	Aggazzotti et al., 1995
	33.7	25-43	indoor	Aggazzotti et al., 1998
	80.7		indoor	Purchert, 1994
	74.9		outdoor	
		3-27.8	indoor	Cammann & Hübner, 1995
		1.8-28	indoor	Jovanovic t al., 1995
	14	0.51-69	indoor	Stottmeiser, 1998,1999
	30	0.69-114	outdoor	
	83	70-95 (90 P = 92)	indoor	Universidad de Barcelona, 1996
	128	99-178 (90 P = 163)	outdoor	
	24		indoor	Baudisch et al., 1997

By product	Concentration		Pool type	Reference
	Mean	Range		
		7.1-24.8	indoor	Erdinger (2004)
	198	43-980	indoor	Lahl et al., 1981
Concentration in the air above the pool water surface (µg/m^3)				
Chloroform	214	66-650	indoor (1)	Aggazzotti et al., 1995
	140	49-280	indoor (1)	Aggazzotti et al., 1993
	169	35-195	indoor (1)	Aggazzotti et al., 1998
	65		indoor (1)	Jovanovic t al., 1995
	36		indoor (2)	
	5.6		outdoor (1)	
	2.3		outdoor (2)	
	3.3	0.33-9.7	outdoor (1)	Stottmeister, 1998, 1999
	1.2	0.36-2.2	outdoor (2)	
	39	5.6-206	indoor (1)	
	30	1.7-136	indoor (2)	
		85-235	Indoor	Erdinger (2004)

All data are presented in WHO “Guidelines for safe recreational-water environments”, 2006, and in Erdinger (2004)
1: measured 20 cm above the water surface; 2: measured 150 cm above the water surface

WHO carried out an evaluation of life guards / swimming instructors exposure to chloroform in swimming pools disinfected with chlorine, using available literature data on chloroform concentration in pools water and air (WHO (2000)). WHO also estimated the exposures for three others populations:

- sporadic child swimmer
- sporadic adult swimmer
- competitive swimmers

The case of adult swimmers and child swimmers will be assessed in the part Consumer exposure. The three main routes of exposure to chloroform in swimming pools will be considered:

- inhalation
- dermal contact
- direct ingestion of the water

In order to assess the exposure of these populations, many physiological assumptions need to be made ; they are presented in the following table:

Table 4.5 Physiological and exposure assumptions for four populations

Parameter	Child (1-year) swimmer	Adult swimmer	Competitive swimmer	Swimming instructor/ life guard
Volume of water ingested (litres/hour)	0.1[c]	0.1[c]	0.1[c]	0[ad]
Exposure duration (h/day)	1[cd]	1[cd]	4[d]	6[c] (air only)
Number of events per week (events/week)	0.5[c]	3[c]	6	5[c]
Inhalation rate (m3/h)	0.5[d]	1[d]	1.5[d]	1[d]
Body weight (kg)	10 [cd]	60 [bd]	60 [bd]	60[bd]
Body surface area (cm2)	10000[cd]	19400[c]	19400[c]	19400[c]

- a: these values assume that the swimming instructor/lifeguard does not swim. A more realistic assumption that swimming instructors/lifeguard receive exposures similar to those of occasional adult swimmers, in addition to their occupationally derived exposures; so for swimming instructors/lifeguards who also swim 1h per day, exposures would be the sum total of exposures for swimming instructors/lifeguards and adult swimmers.
- b: 60kg instead 70 kg (generally used for workers) is the value retained for the body weight of swimming instructor/ lifeguard because of the proportion of women for this work.
- c: these values are the same as in the RAR for sodium hypochlorite.
- d: these values are from Guidelines for Safe Recreational-water Environments, WHO (2000)

Calculations of systemic doses per day for swimming instructor/lifeguard and competitive swimmer will be done for the following scenario:

- a worst-case scenario, in which concentrations of chloroform are assumed to be maximum concentrations indoor swimming pools and where uptake via the ingestion route is considered to be 100% (EF= exposure factor).

Inhalation exposure

The following concentrations of chloroform corresponding to the worst case scenario will be used to estimate the systemic doses per day:

For inhalation and the worst case scenario, the concentration in the air is assumed to be 206 µg/m^3 for a swimmer (20 cm above the water surface) and 136 µg/m^3 for a swimming instructor/lifeguard (150 cm above the water surface) (the maximum measured concentrations (retained as worst case in WHO, (2006)) in a study in which concentrations were measured at various levels above the pool water surface (Stottmeister, 1998, 1999).

The systemic dose per day via inhalation (mg/kg/day) is estimated as follows:

Systemic dose per day via inhalation = C × IR × T × EF × N/7 / BW
where:
C = chloroform concentration (mg/m³),
IR = inhalation rate (m³/h),
T = exposure duration (h/day),
EF = exposure factor (unitless) = 80% (results from human studies reported in the toxicological part),
N = Number of events per week (events/week), and
BW = body weight (kg).

The systemic doses per day via inhalation are reported in the following table:

Table 4.6 Systemic doses per day via inhalation

Scenario	C = chloroform concentration (mg/m3),	IR = inhalation rate (m3/h)	T = exposure duration (h/day)	EF = exposure factor	N = events per week (events/week)	BW = body weight (kg)	Systemic dose per day via inhalation (mg/kg/day)
Lifeguard Worst case	0.136	1	6	80%	5	60	0.0078
Competitive swimmers Worst case	0.206	1.5	4	80%	6	60	0.0141

Dermal exposure and ingestion exposure

The following concentration of chloroform corresponding to the worst case scenario will be used to estimate the systemic doses per day:

For ingestion and dermal exposure, the concentration of chloroform in water is assumed to be 980 µg/litre (0.98 mg/l) for the worst case exposure (the highest concentration measured; Lahl et al., 1981).

The systemic dose per day via skin (mg/kg/day) is estimated as follows:

systemic dose per day via skin (mg/kg/day) = $A \times Kp^{eff} \times C_w \times t \times N/7 / BW / 1000$
where:
A = the body surface area (cm^2),
Kp^{eff} = the effective dermal permeability coefficient (cm/h),
C_w = the chloroform concentration in water (mg/l),
t = the duration of exposure (h) ,
N = number of events per week (events/week), and
BW = body weight (kg).

Kp^{eff} is calculated according to the equation of Bogen (1994): $\log Kp^{eff} = -0.812-0.0104MM+ 0.616\log Kow$ where MM is the molecular mass.

Table 4.7 Physicochemical properties of chloroform

Chemical	Molecular mass (MM)	Experimental log Kow [a]	Estimated log Kow [b]	Kp^{eff}
Chloroform	119.4	1.97	1.52	0.144 [c]

[a] Log K_{ow} values were determined experimentally by Sangster Research Laboratories, Hansch (1993), Sangster (1994) and Hansch & Leo (1995).
[b] Log K_{ow} values were calculated by the Syracuse Research Corporation using data from the Sangster LOGKOW Databank.
[c] Experimental log K_{ow}s were used.

The systemic doses per day via skin are reported in the following table:

Table 4.8 Systemic doses per day via skin

Scenario	Cw = chloroform concentration in water (mg/l),	A = the body surface area (cm2),	t = exposure duration (h/day)	N = events per week (events/week)	Kp^{eff} = the effective dermal permeability coefficient (cm/h)	BW = body weight (kg)	Systemic dose per day via skin (mg/kg/day)
Lifeguard Worst case	0	19400	6	5	0.144	60	0
Competitive swimmers Worst case	0.98	19400	4	6	0.144	60	0.156

For the ingestion exposure, estimations of oral exposure are based upon assumed values for swallowing pool water in the course of swimming, as well an assumption of 100% of uptake of chloroform after ingestion. A 'worst case' intake of 100 ml per 1h swimming session is assumed for each kind of swimmers (WHO (2006) and RAR for sodium hypochlorite)
The systemic dose per day via ingestion (mg/kg/day) is estimated as follows:

Systemic dose per day via ingestion (mg/kg/day) = C_w x V x t x EF x N/7 /BW
where:
C_w = the chloroform concentration in water (mg/l),
V = the volume of water ingested per hour (litres),
EF = exposure factor (unitless) = 100%,
t = the duration of exposure (h),
N = number of events per week (events/week) and
BW = body weight (kg).

The systemic doses per day from ingestion are reported in the following table:

Table 4.9 Systemic doses per day via ingestion

Scenario	C = chloroform concentration in water (mg/l),	V = Volume of water ingested (l/h)	t = exposure duration (h/day)	N = events per week (events/week)	EF = exposure factor	BW = body weight (kg)	Systemic dose per day via ingestion (mg/kg/day)
Lifeguard Worst case	0	0	6	5	100%	60	0
Competitive swimmers Worst case	0.98	0.100	4	6	100%	60	0.0056

4.1.1.2.4 Summary of occupational exposure

It is assumed that the production and further processing is performed in closed system ; dermal exposure for all scenarios is limited because of the very high vapour pressure of 20.9 kPa.

Table 4.10 Summary of exposure data of chloroform (RWC : Reasonable Worst Case) concerning inhalation exposure relevant for occupational risk assessment

Scenario	Form of exposure	Activity	Duration	Frequency	Reasonable Worst Case	Method
1. Manufacture of chloroform and HCFC 22 (closed continuous process)	vapour	All functions, process operations, maintenance, filling, laboratory	Shift length : 8 h	Daily	1.15 ppm 5.6 mg/m^3	Workplace measurement
2. Chloroform as intermediate or solvent in the synthesis of chemicals (closed batch process)	vapour	All functions, process operations, maintenance, filling, laboratory	Shift length : 8 h	Daily	2 ppm 10 mg/m^3	Workplace measurement and expert judgment
3.1 Swimming instructor/lifeguard in a swimming pool	Vapour	Activity in the hall of the swimming pool	Shift length: 6 h	Daily (5 events / week)	0.027 ppm 0.136 mg/m^3	Workplace measurement
3.2 Competitive swimmers	Vapour	Regular training	Shift length: 4h	Daily (6 events / week)	0.042 ppm 0.206 mg/m^3	Workplace measurement

Table 4.11 Summary of dermal exposure data of chloroform relevant for occupational risk assessment

Scenario	Form of exposure	Activity	Contact level (according to EASE model)	Level of exposure (mg/cm2/day)	Shift average Level of exposure (mg/kg/day)	Method
1. Manufacture of chloroform and HCFC 22 (closed continuous process)	liquid	All functions, process operations, maintenance, filling, laboratory	Intermittent	0.1-1 with shortened duration of dermal exposure (1)	42-420 with shortened duration of dermal exposure leading to 0.24 mg/kg/day (1)	EASE/ expert judgment

Scenario	Form of exposure	Activity	Contact level (according to EASE model)	Level of exposure (mg/cm2/day)	Shift average Level of exposure (mg/kg/day)	Method
2. Chloroform as intermediate or solvent in the synthesis of chemicals (closed batch process)	liquid	All functions, process operations, maintenance, filling, laboratory	Intermittent	0.1-1 with shortened duration of dermal exposure (1)	42-420 with shortened duration of dermal exposure leading to 0.24 mg/kg/day (1)	EASE/ expert judgment
3.1 Swimming instructor/lifeguard in a swimming pool 3.2 Competitive swimmers	Liquid Liquid	Activity in the hall of the swimming pool Regular training	No contact Continual	 Chloroform concentration in water = 0.98 mg/l	0 Chloroform concentration in water = 0.98 mg/l leading to 0.156 mg/kg/day	Measurement and calculations

(1) The EASE estimate is largely reduced because of the short duration time of dermal exposure. The retention time of pure chloroform is calculated to 4 seconds (order of magnitude)

Table 4.12 Summary of ingestion exposure data of chloroform relevant for occupational risk assessment

Scenario	Form of exposure	Activity	Level of exposure (mg/l)	Systemic dose per day via ingestion (mg/kg/day)	Method
1. Manufacture of chloroform and HCFC 22 (closed continuous process)	liquid	All functions, process operations, maintenance, filling, laboratory	No concern	0	
2. Chloroform as intermediate or solvent in the synthesis of chemicals (closed batch process)	liquid	All functions, process operations, maintenance, filling, laboratory	No concern	0	

Scenario	Form of exposure	Activity	Level of exposure (mg/l)	Systemic dose per day via ingestion (mg/kg/day)	Method
3.1 Swimming instructor/lifeguard in a swimming pool	Liquid	Activity in the hall of the swimming pool	No concern	0	Measurement and calculations
3.2 Competitive swimmers	Liquid	Regular training	Chloroform concentration in water = 0.98 mg/l	0.0056	

4.1.1.2.5 Summary of systemic doses per day via inhalation, via skin, via ingestion and total systemic dose

Exposure assumptions for scenarios 1 and 2:
A dermal absorption of chloroform through human skin of 10% is used to calculate the systemic dose per day via skin (mg/kg/day).
Human studies showed that the proportion of chloroform absorbed via inhalation ranged from 76 to 80% (Morgan et *al*., 1970 in WHO, 1994).

The systemic dose per day via inhalation is calculated with the following values:

- exposure duration = 8h
- inhalation rate = 1.25 m^3/h
- adult weight = 70 kg

Exposure assumptions for scenario 3:
The exposure assumptions are presented in the part 4.1.1.2.3 in the table "Physiological and exposure assumptions for four populations"

Table 4.13 Systemic doses per day via inhalation, via skin, via ingestion and total systemic dose for occupational risk assessment

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)
1. Manufacture of chloroform and HCFC 22 (closed continuous process)	1.25*8*5.6*0.8/70 = 0.64	16.8*0.1/70 = 0.024	0	0.66
2. Chloroform as intermediate or solvent in the synthesis of chemicals (closed batch process)	1.25*8*10*0.8/70 = 1.14	16.8*0.1/70 = 0.024	0	1.164

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

In scenario 3, 60kg instead 70 kg (used for workers in scenarios 1 and 2) is the value retained for the body weight of swimming instructor/ lifeguard because of the proportion of women for this work.

4.1.1.3 Consumer exposure

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.

Swimming pool

During their presence in the swimming pool, child swimmers and adult swimmers remain in contact with water and air containing chloroform. The physiological and exposure assumptions are described in the part 4.1.1.2.3 “Scenario 3: exposure of workers to chloroform in swimming pools”.

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 are presented in the following table:

Table 4.14 Systemic doses per day via inhalation, via skin, via ingestion and total systemic dose for consumer risk assessment

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 will be done only for the worst case.

4.1.1.4 Humans exposed via the environment

The estimation of the indirect exposure of humans via the environment is presented in the EUSES calculation file. The total daily intake based on the local environmental concentrations due to production and the different uses are presented in Table 4.15.

Table 4.15 Total daily intake due to local environmental exposures

Scenario	DOSE TOT (MG/KG BW/DAY)
Production : Site A :	**6.73 E^{-3} $mg.kg^{-1}.d^{-1}$**
Site B :	9.87 E^{-5} $mg.kg^{-1}.d^{-1}$
Site C :	5.55 E^{-4} $mg.kg^{-1}.d^{-1}$
Site D :	3.68 E^{-3} $mg.kg^{-1}.d^{-1}$
Site E :	2.65 E^{-3} $mg.kg^{-1}.d^{-1}$
Site F :	1.96 E^{-3} $mg.kg^{-1}.d^{-1}$
Site G :	5.75 E^{-4} $mg.kg^{-1}.d^{-1}$
Site H :	7.93 E^{-4} $mg.kg^{-1}.d^{-1}$
Site I :	2.66 E^{-4} $mg.kg^{-1}.d^{-1}$
Site J :	5.19 E^{-3} $mg.kg^{-1}.d^{-1}$
HCFC Production	5.49 E^{-3} $mg.kg^{-1}.d^{-1}$
Dyes and Pesticide Production	1.17 E^{-3} $mg.kg^{-1}.d^{-1}$
Other applications	2.24 E^{-3} $mg.kg^{-1}.d^{-1}$
Uses as a solvent	**5.48 E^{-2} $mg.kg^{-1}.d^{-1}$**
Losses as a by-product during chemical manufacturing	1.71 E^{-2} $mg.kg^{-1}.d^{-1}$

Based on the regional concentrations, the total daily intake for humans is $8.07.10^{-5}$ mg/kg bw/d.

4.1.1.4.1 Exposure via air

In Section 3.1.3.4. of this report it is said that the **air concentration** of chloroform in urban areas never exceed 5 µg/m³.

4.1.1.4.2 Exposure via food and water

As far as the exposure to chloroform via drinking water, in the EU risk assessment of sodium hypochlorite (E.C., 2002), chloroform concentration in drinking water due to water chlorination was reported to be in the range of 11.7 – 13.4 µg/l (see section 3.1.1.3.2.1. of this report).

The highest indirect exposure is estimated for the production of chloroform and its use as a solvent. The human intakes via different routes due to the use of chloroform as a solvent estimated from EUSES are presented in Table 4.16.

Table 4.16 Different routes of intake from human exposure via the environment due to local and regional exposure (EUSES)

	Local exposure due to the use of chloroform as a solvent		Regional exposure	
	Predicted concentration	Estimated daily dose (mg/kg bw/d)	Predicted concentration	Estimated daily dose (mg/kg bw/d)
Drinking water	0.239 mg/L	0.00682	5.49×10^{-4} mg/L	1.57×10^{-5}
Fish	6.2 mg/kg	0.0102	10.8×10^{-3} mg/kg	1.77×10^{-5}
Leaf crops	1.75×10^{-3} mg/kg	0.00003	1.93×10^{-6} mg/kg	3.38×10^{-8}
Root crops	4.25×10^{-3} mg/kg	0.00002	1.09×10^{-3} mg/kg	6×10^{-6}
Meat	6.88×10^{-5} mg/kg	< 0.00001	1.14×10^{-7} mg/kg	4.92×10^{-10}
Milk	2.33×10^{-4} mg/kg	< 0.00001	3.88×10^{-7} mg/kg	3.11×10^{-9}
Air	0.132 mg/m^3	0.0377	0.145 µg/m^3	4.13×10^{-5}
Total daily dose (mg/kg bw/d)		0.0548		8.07×10^{-5}

The highest exposures are to be expected through intake of drinking water, intake of fish and through intake of air.

4.1.1.5 Combined exposure

4.1.2 Effects assessment: Hazard identification and dose (concentration)-response (effect) assessment

The hazard identification section of this report is mainly based on data previously assessed by International Expert Groups (ATSDR, 1997; IARC, 1999; WHO, 1999; US EPA, 2001 & 2004; WHO, 2004). When available, methodology or guideline information has been added from original publications, however parts of the citations are reported as mentioned in the Expert Group reviews.

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

In vivo studies

Inhalation

Measured radioactivity in the exhaled air, urine, feces, carcass and skin, in the 48 h following a 6-day inhalation exposure of rats and mice at various chloroform concentrations (49, 440, and 1790 mg/m^3 for mice; 460, 1740, and 5100 mg/m^3 for rats). At the low concentration, metabolism was extensive in both species. Partial saturation of metabolism was indicated at about 1800 mg/m^3(Corley *et al.*, 1990 in WHO, 1994). Following a 10-minutes inhalation exposure of mice to radiolabelled chloroform (280 mg/kg bw), autoradiography carried out after exposure showed high concentrations in the fat, blood, lungs, liver, kidneys, spinal cord and nerves, meninges and cerebellar cortex (Bergman, 1984 in WHO, 1994). The concentration in arterial blood is directly proportional to inhaled concentration. Transplacental transfer has been demonstrated with accumulation of non-volatile metabolites found in the fetal respiratory tract in mice and guinea-pigs (Danielsson *et al.*, 1986 in WHO, 1994) and in the fetal blood in rats (Withey and Karpinski, 1985 in WHO, 1994).

Metabolism of chloroform is much faster in mice than in humans: the mean peak rate of metabolism at an inhalation exposure of 49 mg/m^3 has been predicted to be approximately 78 times lower in human than in mice (Delic *et al.*, 2000 in WHO, 1994).

Dermal

A dermal absorption rate of 329 nmol/minute/cm^2 (±60 nmol/minute/cm^2) was calculated for the shaved abdominal skin of mice (Tsuruta, 1975 in ATSDR, 1997).

Islam et al. (1995 in ATSDR, 1997) investigated the fate of topically applied chloroform in male hairless rats. For exposures under 4 minutes, chloroform-laden water was applied to shaved back skin; for exposures of 4-30 minutes, rats were submerged in baths containing chloroform-laden water. Selected skin areas were tape-stripped a various number of times after various delay periods. It appeared that there was an incremental build-up of chloroform in the skin over the first four minutes. When compared to uptake measured by bath concentration differences, approximately 88% of lost chloroform was not accounted for in the stratum corneum and was assumed to be systemically absorbed.

Oral

Withey et al. (1983 in US EPA, 2001) compared the rate and extent of gastrointestinal absorption of chloroform following gavage administration in either aqueous or corn oil vehicles. Twelve male Wistar rats were administered single oral doses of 75 mg chloroform/kg via gavage. The time-to-peak blood concentration of chloroform was similar for both vehicles; however, the concentration of chloroform in the blood was lower at all time points for the animals administered chloroform in the oil vehicle compared with animals administered the water vehicle. The authors interpreted this to indicate that the rate of chloroform absorption was higher from water than from oil, although differences in the rate of first-pass metabolism in the liver might contribute to the observed difference.

In mice and rats, 45%–88% of an oral dose of chloroform was excreted from the lungs either as chloroform or carbon dioxide, with 1%–5% excreted in the urine (US EPA, 2001).

When rats, mice and monkeys were given radiolabelled chloroform at 60 mg/kg bw by the oral route, species differences can be seen in the excretion. While mice excreted about 85% of the dose as exhaled carbon dioxide and 5% as unchanged chloroform, monkeys exhaled only 18% as carbon dioxide and 79% as chloroform. The rat was intermediate, with 67% exhaled as carbon dioxide and 20% as chloroform. Excretion in the urine/faeces combined accounted for only about 2–3% of the dose in mice and monkeys and about 8% in rats (Brown *et al.*, 1974 in WHO, 1994).

In vitro studies

Chloroform is metabolized in humans and animals by cytochrome P450-dependent pathways (CYP2E1). 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 (ILSI, 1997). These tissues are also the principal sites of chloroform toxicity, indicating the importance of metabolism in the mode of action of chloroform toxicity.

In the presence of oxygen (oxidative metabolism), the chief product is trichloromethanol ($HOCCl_3$), which rapidly dehydrochlorinates to form phosgene (CCl_2O). The predominant reaction with phosgene is hydrolysis by water, yielding carbon dioxide and hydrochloric acid. However phosgene is electrophilic and reacts with cellular macromolecules (such as enzymes, proteins or the polar head of phospholipids) to form molecular adducts which in turn may lead to loss of cellular function and cell death.

In the absence of oxygen (reductive metabolism), the chief metabolite is dichloromethyl free radical ($CHCl_2$) which is also extremely reactive, forming covalent adducts with microsomal enzymes and the fatty acid tails of phospholipids, probably quite close to the site of free radical formation (cytochrome P450 in microsomal membranes). This results in a general loss of microsomal enzyme activity, and can also result in lipid peroxidation (US EPA, 2001).

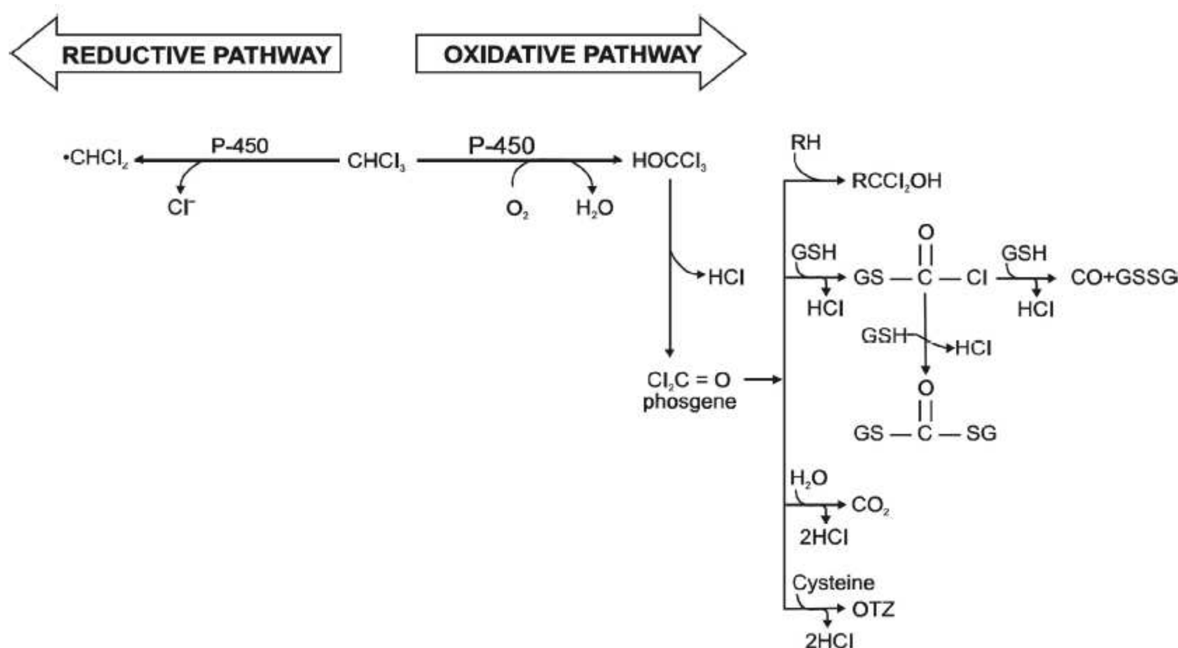


Figure 4.1 Metabolic pathways of chloroform biotransformation (US EPA, 2001)

In vitro studies using liver and kidney microsomes from mice indicate that, even under relatively low (2.6%) oxygen partial pressure (approximately average for the liver), more than 75% of the phospholipid binding was to the fatty acid heads. This pattern of adduct formation on phospholipids is consistent with phosgene, not free radicals, as the main reactive species, indicating metabolism was chiefly by the oxidative pathway (ILSI, 1997; US EPA, 2001).

4.1.2.1.2 Studies in humans

<u>*In vivo* studies</u>

Inhalation

Following a single inhalation exposure to approximately 5 mg of ^{38}Cl-Chloroform, volunteers absorbed about 80% (Morgan *et al.*, 1970 in WHO, 1994).

The half-life of chloroform in humans has been calculated to be 7.9 hours following inhalation exposure (Gordon et al. 1988 in ATSDR 1997).

Levesque et al. (1994 in ATSDR, 1997), attempted to quantitate the body burden of chloroform following exposure in an indoor pool. Scuba divers were exposed to chloroform-laden water and air on each of seven days. On each exposure day, the subjects exercised for a 55-minute period. From the first to the sixth exercise period, chloroform mean concentration in water was increased from 159 µg/l to 553 µg/l. Corresponding mean air chloroform level ranged from 597 ppb to 1630 ppb. Alveolar air samples were collected before exercise and

after 35 or 55 minutes of exercise. The authors concluded from this study that the average proportion of body burden due to inhalation after 35 and 55 minutes exercise was 76 and 78%, respectively.

Chloroform has been detected in the milk of lactating women living in industrial areas. However, the lack of appropriate data limits the assessment of chloroform effects during lactation (Lechner et al., 1988).

Fisher et al. (1997 in Health Council of the Netherlands, 2000), studied the human blood/air and milk/air partition coefficient in blood and milk samples donated by lactating women (n=9). The objective of this study was to evaluate the potential chemical exposure of a nursing infant by ingestion of contaminated milk from a mother who was occupationally exposed to vapours. To estimate infants' exposure, a generic human pharmacokinetic (PB-PK) lactation model was developed. The model was based on a 8-hour exposure of the mother to a constant vapour concentration equal to the threshold limit value for chloroform (10 ppm) in drinking water. The experimentally determined blood/air and milk/air partition coefficient values were used in the PB-PK lactation model. The predicted amount of chloroform ingested by a nursing infant over a 24-hour period was 0.043 mg. However, this model has not been validated yet and the relevance of this exposure level to the development of the human infant is unknown.

Corley et al. (1990 in ATSDR, 1997) developed a PBPK model for chloroform. In brief, the model consists of a series of differential equations that describe the rate of chloroform entry into and exiting from each of a series of body compartments, including: gastrointestinal tract, lungs, arterial blood, venous blood, liver, kidney, other rapidly perfused tissues, slowly perfused tissues, and fat.

In general, the rate of input to each compartment is described by the product of:

(a) the rate of blood flow to the compartment,
(b) the concentration of chloroform in arterial blood,
(c) the partition coefficient between blood and tissue.

Absorption of chloroform into the blood from the lungs or stomach is modeled by assuming first-order absorption kinetics. Material absorbed from the stomach is assumed to flow via the portal system directly to the liver (the "first-pass effect"), while material absorbed from the lungs enters the arterial blood. Each tissue compartment is assumed to be well mixed, with venous blood leaving the tissue being in equilibrium with the tissue. Metabolism of chloroform is assumed to occur in both the liver and the kidney. The rate of metabolism is assumed to be saturable and is described by Michaelis-Menten type equations. Chloroform metabolism is assumed to lead to binding of a fraction of the total metabolites to cellular macromolecules, and the amount bound is one indicator of the delivered dose. Binding of reactive metabolites to cell macromolecules is also assumed to cause a loss of some of the metabolic capacity of the cell. This metabolic capacity (enzyme level) is then resynthesized at a rate proportional to the amount of decrease from the normal level. Based on a review of published physiological and biochemical data, as well as several studies specifically designed to obtain model parameter estimates, Corley et al. (1990) provided recommended values for each of the model inputs for three organisms (mouse, rat, and human). On the basis of these inputs, the model predicted that the amount of chloroform metabolized per unit dose per kg of tissue (liver or kidney) would be highest in the mouse, intermediate in the rat, and lowest in the human. This difference between species is due to the lower rates of metabolism, ventilation, and cardiac output in larger species compared to smaller species. If equal amounts of metabolite binding to cellular molecules were assumed to be equitoxic to tissues, then the relative potency of chloroform would be mice > rats > humans.

Dermal

Information on occlusive conditions in dermal studies was added to the document when available.

Dick et al. (1995 in ATSDR, 1997) examined the absorption of chloroform through human skin *in vivo* using volunteers and *in vitro* using fresh, excised abdominal skin. In the *in vivo* study, fifty microlitre doses of either 1000 µg/ml chloroform in distilled water (16.1 µg/cm^2), or 5000 µg/ml of chloroform in ethanol (80.6 µg/cm^2) were applied to the forearm of volunteers with exhaled air and urine being collected for analysis. The solution remained on the skin for eight hours. When administered in water, the total absorbed dose was 7.8 +/- 1.4%. In contrast, the total absorbed dose was only 1.6 +/- 0.3% when chloroform was administered in ethanol. Of the dose absorbed *in vivo*, more than 95% was excreted via the lungs (over 88% of which was CO2), and the maximum pulmonary excretion occurred between 15 min and 2 h after dosing.

Absorption through the skin requires submersion or contact with chloroform in liquid form, rather than vapour (Davidson *et al.*, 1982 in US EPA, 2004). Dermal absorption has been studied in humans bathing in chlorinated water while breathing pure air through a facemask (Gordon *et al.*, 1998 in US EPA, 2004). Subjects bathing in 40°C water reached a near steady-state value after 6 to 9 minutes and exhaled about 30 times more chloroform than the same subjects bathing in 30 °C water. The authors concluded the difference probably results from a decline in blood flow to the skin at the lower temperatures as the body seeks to conserve heat forcing the chloroform to diffuse over a much greater path length before encountering the blood.

Levesque et al. (1994 in ATSDR, 1997), attempted to quantitate the body burden of chloroform following dermal and inhalation exposure in an indoor swimming pool. Male scuba divers were exposed to chloroform-laden water and air on each of seven days. On each exposure day the subjects exercised for a 55-minute period. On day 6 of the experiment, subjects wore scuba gear so as to determine the percentage body burden due to dermal exposure. On day 6, when scuba gear was worn, alveolar air concentrations after 35 and 55 minutes of exercise were 196 and 209 ppb, respectively. From this data it would appear that the average proportion of body burden due to dermal exposure after 35 and 55 minutes exercise was 24 and 22%, respectively.

Corley et al. (2000 in ATSDR, 1997) studied human dermal absorption of chloroform. The kinetics of chloroform in the exhaled breath of human volunteers exposed skin-only via bath water (concentrations < 100 ppb) were analyzed using a physiologically based pharmacokinetic (PBPK) model. Significant increases in exhaled chloroform (and thus bioavailability) were observed as exposure temperatures were increased from 30 to 40°C. The blood flows to the skin and effective skin permeability coefficients (Kp) were both varied to reflect the temperature-dependent changes in physiology and exhalation kinetics. At 40°C, no differences were observed between males and females. Therefore, Kps were determined (;0.06 cm/hr) at a skin blood flow rate of 18% of the cardiac output. At 30 and 35°C, males exhaled more chloroform than females, resulting in lower effective Kps calculated for females. At these lower temperatures, the blood flow to the skin was also reduced. Total amounts of chloroform absorbed averaged 41.9 and 43.6 mg for males and 11.5 and 39.9 mg for females exposed at 35 and 40°C, respectively. At 30°C, only 2/5 males and 1/5 females had detectable concentrations of chloroform in their exhaled breath. For perspective, the total intake of chloroform would have ranged from 79–194 mg if the volunteers had consumed 2 liters of water orally at the concentrations used in this study. Thus, the relative contribution of

dermal uptake of chloroform to the total body burdens associated with bathing for 30 min at 40°C and drinking 2 liters of water was predicted to be approximately 18%, on average. At 35°C, dermal absorption would contribute; 17% of the total body burdens for males and 6% for females. At the lowest temperature, 30°C, dermal absorption accounts for only 1–7% of the total body burdens.

Oral

Gastrointestinal absorption seems to be rapid and extensive: more than 90% of an oral dose was recovered from expired air (either as unchanged chloroform or carbon dioxide) within eight hours. In human given a single oral dose of 0.5 g chloroform (dissolved in olive oil in gelatine capsule), about 50-52% of the dose was absorbed and metabolised to carbon dioxide and, over a period of eight hours, pulmonary excretion of unchanged chloroform ranged from 17,8 - 66,6%. Blood levels peaked after 1.5 h and then declined in line with a two-compartment model with half-lives of 13 and 90 min, respectively for initial and second phase (Fry *et al.*, 1972 in US EPA, 2001).

Chloroform metabolism displays saturation kinetics (US EPA, 2001): the greater the dose of chloroform, the smaller proportion metabolized.

Uptake and storage of chloroform in adipose tissue can be substantial, with daily exposures potentially leading to accumulation, particularly in obese persons. There is evidence that chloroform crosses the placenta and can be expected to appear in human colostrum and mature breast milk (Davidson et al., 1982 in US EPA, 2004). Quantitative data on populations were not available from this review.

In vitro studies

The metabolism of ^{14}C[chloroform] in liver and kidney microsomes prepared from male F344, Osborne-Mendel rats, B6C3F1 mice, Syrian golden hamsters and humans was measured by trapping formed $^{14}CO_2$. The order of the rate of ^{14}C[chloroform] metabolism in liver microsomes was hamster > mouse > rat > human. Microsomes prepared from kidneys of the various species were less active than liver microsomes. The metabolism of ^{14}C[chloroform] in kidney microsomes was greatest in mice followed by hamster > rat > human, no activity being detected in human kidney microsomes (Corley et al., 1990). Amet et al. (1997) detected CYP 2E1 in human liver but not in kidney (IARC, 1999).

Dick et al. (1995 in ATSDR, 1997) examined the absorption of chloroform through human skin *in vivo* using volunteers and *in vitro* using fresh, excised abdominal skin. *In vitro*, single doses of either 0.4 µg/ml chloroform in distilled water (low dose, 0.62 µg/cm^2, 1.0 ml dosed) or 900 µg/ml chloroform in distilled water (high dose, 70.3 µg/cm^2, 50 µl dosed) were applied to discs of the excised abdominal skin placed in flow-through diffusion cells and perfused with Hepes buffered Hank's balanced salt solution, with a wash at 4 h. The percentage of dose absorbed *in vitro* (skin+perfusate) was 5.6 +/- 2.7% (low dose) and 7.1 +/- 1.4% (high dose).

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

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.

The half-life of chloroform in humans has been calculated to be 7.9 hours following inhalation exposure (Gordon et al. 1988 in ATSDR 1997). Furthermore, an oral-exposure study found most of the chloroform dose being eliminated within 8 hours postexposure (Fry et al. 1972 in ATSDR 1997).

Chloroform is mainly metabolised in liver and both oxidative and reductive pathways of chloroform have been identified, although data *in vivo* are limited. The major metabolite is carbon dioxide, generated by oxidative pathway *in vivo*; this main pathway generates also reactive metabolites, including phosgene. The reductive pathway generates the dichloromethylcarbene free radical. Both pathways proceed through a cytochrome P450-dependent enzymatic activation step ant their balance depends on species, tissue, dose and oxygen tension. Phosgene is produced by oxidative dechlorination of chloroform to trichloromethanol, which spontaneously dehydrochlorinates (WHO, 2004).

The electrophilic metabolic phosgene binds covalently to nucleophilic components of tissue proteins and also interacts with other cellular nucleophiles and, to some extent, to the polar heads of phospholipids. Phosgene can also react with water to release carbon dioxide and hydrochloric acid.Available literature data show that chloroform toxicity is due to its metabolites: phosgene is supposed to be responsible for irreversible bindings to liver components (WHO, 2004).

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).

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%.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

In vivo studies

Inhalation

Bonnet (1980) has reported an inhalation LC_{50} value, for 6-hour exposure, of 9.2 g/m^3 in rats. Depression of the central nervous system is the main symptom of acute inhalation in rats; subnarcotic effects occur at 2.1 g/m^3 for 4h (Frantik *et al.*, 1998). In female mice, an inhalation LC_{50} value of 6.2 g/m^3 for 6-hour exposure was reported (Gradiski *et al.*, 1978). (cited as in WHO, 1994)

F344 rats and BDF1 mice were exposed to chloroform vapours (500, 1000, 2000, 4000, 8000 ppm - or 2.44, 4.88, 9.760, 19520 or 39040 mg/m^3) 6h/day 5d/week during 2 weeks. Male

mice were more susceptible than females to acute toxicity, for both species 100% mortality occurred within 48h at 2000 ppm and over (see Table 4.17, Kasai *et al.*, 2002).

Table 4.17 Mortality rates for rats and mice of both sexes exposed to chloroform for 2 wk by inhalation (Kasai *et al.*, 2002)

Exposed concentration	Mice		Rats	
	Male	Female	Male	Female
0 ppm	0	0	0	0
500 ppm	9 (9/2nd)	0	0	0
1000 ppm	9 (9/2nd)	9 (4/4th) (4/5th) (1/6th)	0	0
2000 ppm	10 (10/2nd)	10 (6/2nd) (2/4th) (2/5th)	10 (9/1st) (1/2nd)	10 (8/1st) (2/2nd)
4000 ppm	10 (1/1st) (9/2nd)	10 (10/2nd)	10 (9/1st) (1/2nd)	10 (9/1st) (1/2nd)
8000 ppm	10 (10/1st)	10 (10/1st)	10 (10/1st)	10 (10/1st)

The fraction within parenthesis indicates the number of dead animals as the numerator/the day of repeated exposure at death as the denominator.

Dermal

Single application of 1.0, 2.0, or 3.98 g/kg for 24h under an impermeable plastic cuff held tightly around the clipped bellies of each of two rabbits did not result in any deaths. However, extensive necrosis of the skin and considerable weight loss occurred at all levels. Animals were sacrificed for study 2 weeks after exposure. All treated rabbits exhibited degenerative changes in the kidney tubules graded in intensity with dosage levels. The livers were not grossly affected; the dermal and systemic LOAEL is 1.0 g/kg (Torkelson et al., 1976).

Oral

In rats, acute oral LD_{50} range from 450 to 2000 mg/kg bw (Kimura *et al.*, 1971; Chu *et al.*, 1980 in WHO, 2004).. Administration of 0, 67, 135, or 338 mg/kg body weight by gavage in olive oil to male Wistar rats increased, in a dose-dependent manner, the number of necrotic hepatocytes in the centrilobular region and elevated plasma alanine aminotransferase (ALAT) levels significantly (Nakajima et al., 1995 in WHO, 2004)

Chloroform given by gavage in corn oil at 180 mg/kg per day induced kidney tumors in male Osborne-Mendel rats (NCI, 1976 in IARC, 1999) . Chloroform-induced cytotoxicity and regenerative cell proliferation have been observed in the kidneys of male F-344 rats (Templin et al; 1996b). In order to compare the acute sensitivity of male Osborne-Mendel with F-344 rats, animals from both strains were administered a single gavage dose of 0, 10, 24, 90, 180, or 477 mg/kg chloroform and necropsied 48 h later. Known target tissues were examined for histological changes. Regenerative cell proliferation was assessed as a labeling index (LI, percent of cells in S phase) as determined by nuclear incorporation of bromodeoxyuridine. The epithelial cells of the proximal tubules of the kidney cortex were the primary target cells for cytotoxicity and regenerative cell proliferation. A dose-dependent increase in the LI was present in the kidney of Osborne-Mendel rats given doses of 10 mg/kg chloroform and above and in F-344 rats given 90 mg/kg and above. The maximal increase in the LI was 4.5- or 3.7-fold over control in Osborne-Mendel or F-344 given 477 mg/kg, respectively. The only increase in the hepatocyte LI was in the F-344 rats given 477 mg/kg. Edema and periosteal hypercellularity were observed in the nasal passages of both strains at doses of 90 mg/kg and above. These data indicate that Osborne-Mendel and F-344 rats are about equally susceptible to chloroform-induced nephrotoxicity. These results provide a basis for linking the extensive data base on mechanisms of action of chloroform toxicity in F-344 rats to the Osborne-

Mendel rat and support the hypothesis that events secondary to chloroform-induced cytolethality and regenerative cell proliferation played a role in the induction of renal tumors in the Osborne-Mendel rat.

Ninety-day-old male Fischer 344 rats were gavaged with 14.9, 22.4, 29.8, 59.7, 89.5, 119.4 or 179.1 mg/kg body weight CHCl3 in 10% Alkamuls EL-620 (5 ml/kg body weight). At 24 h postgavage, serum was collected for analysis of clinical chemistry indicators of liver damage. CHCl3 induced dose-dependent hepatotoxicity; serum alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase were elevated significantly over control at 179.1, 119.4, and 59.7 mg/kg. At 29.8, 22.4, and 14.9 mg/kg, significant increases over control were not detected for any measured endpoint. A NOAEL of 30 mg/kg bw has been established for serum enzyme changes indicative of liver damage (Keegan et al., 1998).

In mice, a wide range of LD_{50} has been reported too, from 36 to 1366 mg/kg bw. Chloroform-induced death is usually due to liver damage, with the exception of male mice of very sensitive strains, whose death is caused by kidney damage. The higher susceptibility to chloroform acute toxicity in these strains of mice (such as DBA, C3H, C3Hf, CBA, Balb/c, C3H/He), with respect to other strains, is genetically controlled. Likely, cellular proliferation and lesions of liver and kidneys were observed in mice (Gemma *et al.*, 1996; Reitz *et al.*, 1982; Moore *et al.*, 1982 in WHO, 1994).

In vitro studies

No study reported.

4.1.2.2.2 Studies in humans

In vivo studies

Inhalation

Most data on the controlled exposure of man to chloroform have resulted from its clinical use as an anaesthetic. This use of chloroform was described as early as 1847 (Simpson, 1847). Induction of anaesthesia may result from inhalation of chloroform vapours at a concentration of 24 to 73 g/m^3 air. For maintenance of anaesthesia, concentrations in the range of 12 to 48 g/m^3 are required. As with animals, chloroform anaesthesia may result in death in humans due to respiratory and cardiac arrhythmias and failure. Because of the relatively high frequency of "late chloroform poisoning" (liver toxicity), its use as anaesthetic has been abandoned.

It has been reported that chloroform can cause severe toxic effects in humans exposed to 9960 mg/m^3 (2000 ppm) for 60 min, symptoms of illness at 2490 mg/m^3 (500 ppm) and can cause discomfort at levels below 249 mg/m^3 (50 ppm) (Verschueren, 1983 in WHO, 1994). The human estimated LOAEC is ≤ 249 mg/m^3. (**Considered as key study for risk characterisation).**

Dermal

No study reported.

Oral

Cases of severe intoxication after suicidal attempts, with the same pattern of symptoms as after anaesthetical use, have been reported by Schröder (1965). There are considerable inter-individual differences in susceptibility. Some persons presented serious illness after an oral dose of 7.5 g of chloroform, whereas others survived a dose of 270 g chloroform. The mean lethal dose for an adult is estimated to be about 45 g (Winslow & Gerstner, 1978 in WHO, 1994). A LOAEL of 107 mg/kg is estimated from the oral dose of 7.5g assuming a body weight of 70 kg. **Considered as key study for risk characterisation.**

A 16-year-old female who ingested an unknown amount of chloroform and arrived at a hospital semiconscious and with repeated vomiting was reported by Hakim et al. (1992). The person was treated with gastric lavage, antacids, intravenous glucose, and antiemetics. The woman had apparently recovered and was released. Seven days later, the woman presented with hepatomegaly, slightly depressed hemoglobin, and an abnormal liver sonogram, suggesting toxic hepatic disease due to chloroform toxicosis (ATSDR 1997).

A 33-year-old female had injected herself intravenously with 0.5 ml of chloroform and then became unconscious. The woman awoke approximately 12 hours later and drank another 120 ml of chloroform. The person was treated with hyperbaric oxygen, cimetidine (to inhibit cytochrome P-450 and formation of phosgene), and N-acetylcystine (to replenish GSH stores). Liver serum enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and LDH were elevated in a pattern that suggested liver cell necrosis. Generally, these enzymes were noted to peak by day 4 and decrease by day 11. Total bilirubin and direct bilirubin did not change appreciably. GGT (gamma glutamyltransferase, also known as gamma glutamyl transpeptidase), alpha-feto protein and retinol binding protein showed increases between 6 and 8 days after ingestion, but still within normal ranges for humans (Rao et al. 1993 in ATSDR, 1997).

The kidney is also a major target of chloroform-induced toxicity in humans. Oliguria was observed 1 day after the ingestion of 3,755 or 2,410 mg/kg chloroform (Piersol et al. 1933; Schroeder 1965). Increased blood urea nitrogen (BUN) and creatinine levels also indicated renal injury. Albuminuria and casts were detected in the urine. Histopathological examination at autopsy revealed epithelial swelling and hyaline and fatty degeneration in the convoluted tubules of kidneys in one fatal case of oral exposure to chloroform (Piersol et al. 1933 in ATSDR, 1997).

In vitro studies

No study reported.

4.1.2.2.3 Summary of acute toxicity

Chloroform acute toxicity data are available for inhalation and oral route in rats and mice and for the dermal route in rabbits. Some studies on clinical use and on accidental human exposure have also been reported.

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 to 2000 mg chloroform/kg body weight. Chloroform LC_{50} values of 6.2 g/m^3 and 9.2 g/m^3 have been reported for 6 h inhalation exposure in mice and rats respectively (WHO, 1994). Mice are more susceptible than rats to acute chloroform toxicity for both exposure routes. A

systemic and local LOAEL of 1.0 g/kg has been reported in rabbits by dermal route 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.

In general, chloroform elicits the same symptoms of toxicity in humans as in animals. The mean lethal oral dose for an adult is estimated to be about 45 g, but large interindividual differences in susceptibility occur. The human estimated inhalation LOAEC is ≤ 249 mg/m^3 (Verschueren, 1983 in WHO, 1994) and the oral LOAEL is <107 mg/kg (Winslow & Gerstner, 1978 in WHO, 1994). **Considered as key studies for risk characterisation**

Based on acute toxicity data, the proposed classification for chloroform is Harmful with the risk phrases R22: harmful if swallowed and R20: harmful by inhalation.

4.1.2.3 Irritation

4.1.2.3.1 Skin

<u>Studies in animals</u>

Few studies were realised to evaluate the irritating effects of chloroform to skin but results are widespread. In the first, chloroform is highly irritant; in the second, application of 1000 mg/kg for 24-hours caused a moderate skin necrosis (Duprat *et al.*, 1976 in WHO, 1994). This study is poorly reported and more details were not available.

Torkelson et al., (1976) found that chloroform, when applied to the skin of rabbits, produced slight to moderate irritation and delayed healing of abraded skin. When applied to the uncovered ear of rabbits, slight hyperemia and exfoliation occurred after one to four treatments. No greater injury was noted after 10 applications. One to two 24h applications, on a cotton pad bandaged on the shaven belly of the same rabbits, produced a slight hyperemia with moderate necrosis and a resulting eschar formation. Healing appeared to be delayed on the site as well as on abraded areas that were also covered for 24h with a cotton pad soaked in chloroform. Single application of either 1.0, 2.0 or 3.98, g/kg for 24 hours, under an impermeable plastic cuff held tightly around the clipped bellies of each of two rabbits, produced extensive necrosis of the skin at all levels.

Chloroform showed irritant responses in a sensitisation test reported in a study in Japanese (Chiaki et al., 2002), the abstract only was available in English. 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). GPMT was conducted in accordance with Magnusson and Kligman Method. On the other hand LLNA was conducted in accordance with Kimber Method. In the results, no positive reaction was observed in any method.

Studies in humans

Dermal contact with chloroform causes chemical dermatitis (symptoms: irritation, reddening, blistering and burns) (WHO, 1994).

4.1.2.3.2 Eye

Studies in animals

Duprat et al. (1976) applied undiluted chloroform into the eyes of six New Zealand white rabbits. It produced severe eye irritation, with mydriasis and keratitis in all rabbits. Translucent zones in the cornea were observed in four animals and a purulent haemorrhagic discharge was also reported (number of rabbits unknown). The effects had disappeared 2-3 weeks after application, except for one rabbit that still showed corneal opacity after 3 weeks.

Liquid chloroform, when dropped into the eyes of 3 rabbits, caused slight irritation of the conjunctiva that was barely detectable 1 week after treatment. In addition, slight but definite corneal injury occurred, as evidenced by staining with fluorescein. A purulent exudate occurred after 2 days of treatment. Washing of one eye of each rabbit with a stream of running water, 30 seconds after instilling the chloroform, did not significantly alter the response compared to the unwashed eye (Torkelson et al., 1976).

Studies in humans

Burn sensation, lacrimation and inflammation of conjunctiva are reported in human cases in contact with liquid chloroform. Reversible effects of the cornea are often observed: otherwise, its regeneration is fast (less than 3 weeks) (Grant and Schuman, 1993).

According to Oettel (1936) and Winslow & Gerstner (1978), exposure to concentrated chloroform vapours causes a stinging sensation in the eye. Splashing of the liquid into the eye evokes burning, pain and redness of the conjunctival tissue. Occasional injury of the corneal epithelium will recover fully within a few days (cited as in WHO, 1994).

4.1.2.3.3 Respiratory tract

Studies in animals

In rats and mice, lesions and cell proliferation in the olfactory epithelium and changes in the nasal passages were observed following chloroform exposure (Kasai *et al.*, 2002). In mice exposed to chloroform vapours (500, 1000, 2000, 4000, 8000 ppm - 6h/day, 5d/week) for 2 weeks, atrophy and respiratory metaplasia of olfactory epithelium was observed in males; as well as degeneration, necrosis and disarrangement of olfactory and respiratory epithelia in females. In rats exposed in the same conditions (500, 1000, 2000, 4000, 8000 ppm - 6h/d, 5d/w, 2 weeks), desquamation, atrophy and disarrangement of the olfactory epithelium but also edema of the lamina propria of the nasal cavity have been observed at all doses. The LOAEC for mice and rats is 500 ppm (2.5 g/m^3) for the two weeks study.

The authors (Kasai *et al.*, 2002) conducted a second experiment with lower doses (12, 25, 50, 100, 200 ppm for mice and 25, 50, 100, 200, 400 ppm for rats - 6h/day, 5d/week) during 13

weeks. Significant increases of the following nasal lesions were reported. Degeneration of the olfactory epithelium was observed in male mice exposed to 25 ppm and above. In females, 12 ppm and above caused thickening of the bone in nasal septum and eosinophilic changes of olfactory and respiratory epithelia. In rats of both sexes, mineralization and atrophy of the olfactory epithelium were observed at 25 ppm, for concentrations of 200 and above necrosis was observed in males. For nasal effects, a LOAEC of 12 ppm (60 mg/m^3) can be derived in female mice; a NOAEC of 12 ppm (60 mg/m^3) can be derived in male mice and a LOAEC of 25 ppm (125 mg/m^3) for rats of both sexes.

Larson et al. (1996 in ATSDR, 1997) investigated the ability of acute exposure to chloroform vapors to produce toxicity and regenerative cell proliferation in the liver, kidneys, and nasal passage of female B6C3F1 mice. Groups of 5 animals were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via inhalation for 6 hours a day for 4 consecutive days. This study found no overt clinical signs of toxicity in female mice exposed to chloroform for 4 days; however, some mild and transient changes occurred in the posterior ventral areas of nasal tissue in female mice exposed to the 10, 30, and 90 ppm concentrations of chloroform. The lesions were characterized by mild proliferative responses in the periosteum consisting of a thickening of the bone. The adjacent lamina also exhibited loss of acini of Bowman's glands and vascular congestion. US EPA (2001) determined, from this study, a NOAEC of 90 ppm (450 mg/m^3) for nasal lesions. No more detail was given on the choice of this NOAEC.

Male and female F-344 rats were exposed to airborne concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform 6 hr/day, 7 days/week for 4 days or 3, 6, or 13 weeks. Additional treatment groups were exposed 5 days/week for 13 weeks or were exposed for 6 weeks and held until week 13. The severity and type of chloroform-induced nasal lesions were dependent on both concentration and duration of exposure. The lesions were primarily confined to the ethmoid portion of the nasal passages lined by olfactory epithelium. At the early time points, enhanced bone growth and hypercellularity in the lamina propria of the ethmoid turbinates of the nose occurred at concentrations of 10 ppm and above. With continued exposure, lesions were present throughout the entire ethmoid portion of the nose. (**Considered as key study for risk characterisation, see** Table 4.21**).** At 90 days there was a generalized atrophy of the ethmoid turbinates at concentrations of 2 ppm and above. LOAEC = 2 ppm (Templin et al., 1996a).

Acute exposure to chloroform clearly can induce site-specific as well as biochemical changes in the nasal region of female B6C3F1, mice and male Fischer 344 rats (Mery et al. 1994 in ATSDR, 1997). To demonstrate the biochemical alterations, mice were exposed to 1.2, 3, 10, 29.5, 101, and 288 ppm chloroform and rats were exposed to 1.5, 3.1, 10.4, 29.3, 100, and 271 ppm for 6 hours a day for 7 days to determine the nasal cavity site-specific lesions and the occurrence of cell induction/proliferation associated with these varying concentrations of chloroform. In male rats, the respiratory epithelium of the nasopharyngeal meatus exhibited an increase in the size of goblet cells at 100 and 271 ppm chloroform, in addition to an increase in both neutral and acidic mucopolysaccharides. Affected epithelium was up to twice its normal thickness. New bone formation within the nasal region was prominently seen at 10.4 ppm and above, and followed a concentration response curve. At 29.3 and 100 ppm, new osseous spicules were present at the beginning of the first endoturbinate, while at 271 ppm, the width of the new bone was almost doubled compared to controls. The Bowman's glands were markedly reduced in size. Cytochrome P-450-2El staining was most prominent in the cytoplasm of olfactory epithelial sustentacular cells and in the acinar cells of Bowman's glands in control animals. In general, increasing the chloroform concentration tended to decrease the amount of P-450 staining in exposed animals. Exposure to chloroform resulted in a dramatic increase in the number of S-phase nuclei, with the proliferative response confined

to activated periosteal cells, including both osteogenic (round) and preosteogenic (spindle) cells. The proximal and central regions of the first endoturbinate had the highest increase of cell proliferation. Interestingly, the only detectable treatment-related histologic change observed in female mice was a slight indication of new bone growth in the proximal part of the first endoturbinate in one mouse exposed to 288 ppm chloroform. The S-phase response was observed at chloroform concentrations of 10.4 ppm and higher. The authors concluded that if similar nasal cavity changes occur in humans, the sense of smell could potentially be altered. US EPA (2001), determined a NOAEC of 3 ppm based on histological and induced cell proliferation.

Studies in humans

No Data available

4.1.2.3.4 Summary of irritation

Chloroform is an irritant substance for skin, eye and upper airways. Rabbit dermal studies showed slight to high irritation potency. 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 indicates slight but definite 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). **Considered as key study for risk characterisation**

Table 4.18 Study summary for irritation

Animal species & strain	Number of animals	Doses	Result	Reference
Rabbit Dermal	Not reported	Liquid chloroform 24h, occlusive 10 applications for ears 2 applications for bellies	ear: hyperemia and exfoliation after 1 to 4 applications belly: slight hyperemia with moderate necrosis and eschar formation delayed healing of the skin	Torkelson et al., 1976 in WHO 2004
Rabbit, NZW Ocular	6	Undiluted chloroform, doses not specified	6/6 severe eye irritation, with mydriasis and keratitis 4/6 translucent zones in the cornea	Duprat et al., 1976
Rabbit Ocular	3	Undiluted chloroform, doses not specified 1 eye rinsed after 30s	Slight irritation of the conjunctiva slight but definite corneal injury	Torkelson et al., 1976

Animal species & strain	Number of animals	Doses	Result	Reference
Rat, F344 Inhalation	10/sex/dose	vapour, 6h/d, 5d/week, 13 weeks 25, 50, 100, 200, 400 ppm	25 ppm (125 mg/m^3): mineralization and atrophy of the olfactory epithelium 200 ppm (1000 mg/m^3): necrosis of olfactory epithelium in males	Kasai et al., 2002
Rat, F344 Inhalation	10/sex/dose	vapour, 6h/d, 5d/week, 2 weeks 500, 1000, 2000, 4000, 8000 ppm	All doses desquamation, atrophy and disarrangement of the olfactory epithelium, edema of the lamina propria of the nasal cavity	Kasai et al., 2002
Rat, F344 Inhalation	Not reported	1.2, 3, 10, 29.5, 101, and 288 ppm 6 hr/day for 7 days	NOAEC= 3 ppm (15 mg/m^3) atrophy of Bowman's glands, new bone formation, and increased labeling index in S phase periosteal cells	Mery et al., 1994
Rat, F-344 rats Inhalation		0, 2, 10, 30, 90, or 300 ppm 6 h/day, 7 d/week or 5d/week, 13 weeks	Early time points (4 days) LOAEC= 10 ppm Enhanced bone growth, hypercellularity in the lamina propria 13 weeks LOAEC= 2 ppm Enhanced bone growth hypercellularity in the lamina propria of the ethmoid turbinates	Templin et al., 1996a
Mouse, BDF1 Inhalation	10/sex/dose	vapour, 6h/d, 5d/week, 13 weeks 12, 25, 50, 100, 200 ppm	25 ppm (125 mg/m^3): degeneration of the olfactory epithelium in males 12 ppm (60 mg/m^3): thickening of the bone in nasal septum, eosinophilic changes of olfactory and respiratory epithelia in females	Kasai et al., 2002
Mouse, B6C3F1 Inhalation	10/sex/dose	vapour, 6h/d, 5d/week, 2 weeks 500, 1000, 2000, 4000, 8000 ppm	All doses atrophy and respiratory metaplasia of olfactory epithelium in males degeneration, necrosis and disarrangement of olfactory and respiratory epithelia in females	Kasai et al., 2002
Mouse, B6C3F1 Inhalation	Female	0.3, 2, 10, 30, and 90 ppm 6 h/d, 4 days	NOAEC = 90 ppm (441 mg/m^3) nasal lesions	Larson et al., 1996
Mouse, B6C3F1 Inhalation	Not reported	1.2, 3, 10, 29.5, 101, and 288 ppm 6 hr/day for 7 days	NOAEC= 3 ppm (15 mg/m^3) increased labeling index in S phase periosteal cells	Mery et al., 1994

The classification proposed according to the data available is Irritant with the risk phrases R38: irritating to skin, R36 irritating to eyes and R37 irritating to respiratory system.

4.1.2.4 Corrosivity

No data available

4.1.2.5 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 in a study in Japanese (Chiaki et al., 2002) the abstract only was available in English. 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). GPMT was conducted in accordance with Magnusson and Kligman Method. Chloroform and the immunopotentiator Freund's complete adjuvant were administered intradermally to 5 guinea pigs as primary sensitization (Day 1). One day after open application of 10% sodium lauryl sulfate (SLS) to enhance sensitization (as secondary sensitization), chloroform was applied as an occlusive patch for 48 hours (Day 9, patch sensitization). For challenge, another 3 guinea pigs in the control group were used as a control group, and chloroform was applied to 5 guinea pigs in the sensitization group as an occlusive patch for 24 hours in the same manner (Day 22). Evaluation was according to the Draize criteria 48 and 72 hours after the start of challenge. Significant suppression of body weight gain ($P<0.01$) compared to the control group was seen at secondary sensitization (Day 9) after intradermal chloroform administration (Day 1). Extensive necrosis at the chloroform administration site was observed from the day after administration, and piloerection and decreased spontaneous movement were observed for 1 week following intradermal administration. In the evaluation at 48 and 72 hours after the start of challenge, erythema (score 1 or 2, slight to mild) was observed in all 8 animals including the control group. This reaction at the challenge site was observed until 8 days after the start of challenge, with a tendency for the erythema to become stronger over time in all 8 animals including the control group, confirming that chloroform, which is an organochlorine solvent, is a strongly irritant substance. Sensitization could not be definitely evaluated due to this strong irritation reaction, but since skin reactions were comparable in the chloroform sensitization group and the control group, chloroform sensitization was judged to be negative in GPMT.

On the other hand LLNA was conducted in accordance with Kimber Method. Hexyl cinnamic aldehyde (HCA) was used as the positive control substance in LLNA, and HCA was dissolved in chloroform or in acetone/olive oil solvent (AOO; acetone : olive oil = 4 : 1) to reach a concentration of 10%. Using 4 groups with 5 animals per group, chloroform, AOO, 10% HCA/chloroform or 10% HCA/AOO (25µL/ear) was applied to both auricles of the mice in each group for 3 consecutive days, and 3 days later the mice were euthanized by cervical dislocation 5 hours after ^{3}H-methyl thymidine was administered intravenously (250 µL, 2.96 MBq/mL) and the auricular lymph nodes were removed, in order to compare reactions to HCA with chloroform as vehicle and with AOO as vehicle. Then cells were isolated from the lymph nodes, cell suspensions prepared, and radioactivity was measured with a beta scintillation counter. Evaluation of LLNA was done by calculation of the Stimulation Index (SI). SI was obtained by dividing the mean measured value in each test substance

administration groups by the mean measured value in the vehicle administration groups, the AOO and chloroform administration groups. SI for chloroform alone was obtained using the value for AOO as the vehicle administration group. Sensitization was judged to be positive if SI was 3 or more and there was statistically significant difference from the vehicle control group. In LLNA, chloroform showed higher levels of radioactivity than AOO. The lymphoproliferative activity is used as an index of sensitization in LLNA, but since primary irritation also activates lymph cell proliferation through inflammatory cytokine effects, the reactions are said to be difficult to differentiate. It is very likely that the reactions to chloroform seen in the present study were due to primary irritation rather than sensitization.

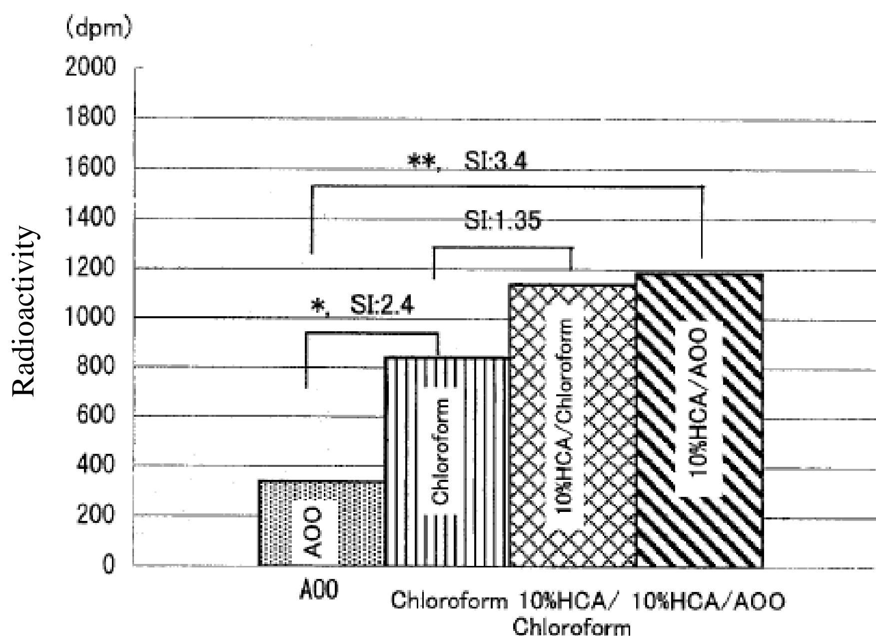


Figure 4.2 Comparison of LLNA radioactivity by difference in vehicle (*: p<0.05, **: p<0.01)

No classification is proposed for sensitisation.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

<u>*In vivo* studies</u>

Inhalation

The toxicity of 1-week exposures to inhaled chloroform was investigated in male F-344 rats exposed to chloroform vapors at concentrations of 1, 3, 10, 30, 100 or 300 ppm for 6 h/day during 7 consecutive days and necropsied on Day 8 (Larson et al., 1994). For liver lesions, a NOAEC was 30 ppm (150 mg/m^3) based on swelling and mild vacuolation of centrilobular hepatocytes. For renal effects, a NOAEC of 100 ppm (500 mg/m^3) was derived from proximal tubules lined by regenerating epithelium. And a NOAEC of 3 ppm (15 mg/m^3) was reported for histological changes in the nasal cavity of rats.

The toxicity of 1-week exposures to inhaled chloroform was investigated in female B6C3F1 mice exposed to chloroform vapors at concentrations of 1, 3, 10, 30, 100 or 300 ppm for 6 h/day during 7 consecutive days and necropsied on Day 8 (Larson et al., 1994). It was reported a NOAEC of 10 ppm (50 mg/m^3) based on liver effects (hepatocellular necrosis and vacuolar changes in the hepatocytes) and a NOAEC of 100 ppm (500 mg/m^3) based on renal lesions (proximal tubules lined by regenerating epithelium). No nasal lesions were observed in mice.

When F344 rats were exposed to chloroform vapours (500, 1000, 2000, 4000, 8000 ppm) 6h/day 5d/week during 2 weeks, 100% mortality occurred within 48h over 1000 ppm for males and females. Dead rats showed lung congestion and inflammation, probably as a result of cardiovascular toxicity. In surviving animals, a LOAEC of 500 ppm (2.5 mg/l) is based on vacuolic changes in proximal tubules of the kidneys and in the central area of the liver (Kasai *et al.*, 2002).

When BDF1 mice were exposed to chloroform vapours (500, 1000, 2000, 4000, 8000 ppm) 6h/day 5d/week during 2 weeks, male mice were more susceptible than females to toxicity. Chloroform induced necrosis and cytoplasmic basophilia of the kidney proximal tubules in males and centrilobular necrosis of the liver in females. Mortality rates for males and females were 100% within 2 days at 2000 ppm and over, deaths were histologically attributed to necrosis of proximal tubules in males and centrilobular necrosis of the liver in females. In surviving animals, a LOAEC of 500 ppm (2.5 g/m^3) can be determined for histopathological changes in male kidneys and female liver (Kasai *et al.*, 2002).

Five groups of 10 male and 10 female rats and mice were exposed 6h/day, 5 days a week, for 13 weeks to chloroform vapours by inhalation: 12, 25, 50, 100 or 200 ppm for mice and 25, 50, 100, 200 or 400 ppm for rats (Kasai *et al.*, 2002). No mortality occurred in rats and female mice but almost all the exposed male mice died after the first day of exposure. The chloroform-induced deaths of mice were histopathologically attributed to necrosis of proximal tubules in males and centrilobular necrosis of the liver in females. In surviving mice, necrosis and cytoplasmic basophilia of proximal tubules and degeneration of the olfactory epithelium were observed in males as well as liver necrosis and nasal lesions in females. In rats, renal lesions (vacuolic changes in proximal tubules), liver collapse (loss of hepatocytes and deposit of ceroid) and nasal lesions have been observed in both sexes. For the hepatic effects in rats and mice, NOAECs were 50 ppm in females and 100 ppm in males (248 mg/m^3 and 496 mg/m^3 respectively). For the renal effects, LOAEC was 12 ppm (60 mg/m^3) in male mice, in female rats the NOAEC for vacuolic changes in the kidney was 100 ppm (500 mg/m^3). For nasal lesions, LOAEC was 12 ppm (60 mg/m^3) and 25 ppm (124 mg/m^3) in the mice and the rats of both sexes, respectively.

Male and female F-344 rats were exposed to airborne concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform (Templin et al., 1996a). Rats were divided into groups exposed for periods of 4 days or 3, 6, or 13 weeks for male rats and 3 or 13 weeks for female rats. Daily exposures were conducted for 6 hr, 7 days/week. To compare the effects of a 7-days/week exposure to the conventional 5 days/week schedule, groups of rats were exposed to 30, 90, or 300 ppm chloroform for 6 hr/day, 5 days/week for 13 weeks. To investigate the reversibility of chloroform-induced alterations, additional groups of rats were exposed to 90 or 300 ppm chloroform for 6 hr/day, 7 days/week for the first 6 weeks, after which rats were housed in the control chamber for the remaining 7 weeks (6 weeks exposure, stop, 7 weeks holding). Designated subsets of rats were administered BrdU to label cells in S-phase (labeled groups) while others did not receive BrdU (unlabeled groups).

Table 4.19 Kidney Lesion Scores and Incidence in Male or Female F-344 Rats Exposed to Chloroform Vapors (Templin et al., 1996a)

Concentration (ppm)	4 days	3 weeks 7 days/week	6 weeks 7 days/week	13 weeks 7 days/week	13 weeks 5 days/week	13 weeks 6-week stop
Male rats						
0	0.0 (0.5) [a]	0.3 (4/13)	0.1 (1/12)	0.6 (8/14)	0.6 (8/14) [b]	0.6 (8/14) [b]
2	0.0 (0/5)	0.4 (5/13)	0.3 (4/13)	0.8 (10/15)	c	c
10	0.0 (0/5)	0.5(6/13)	0.6(8/13)	0.5 (7/15)	c	c
30	0.2 (1/5)	0.9 (12/13)	1.0 (11/13)	0.6 (9/14)	0.1 (2/15)	c
90	0.4 (2/5)	1.0(10/10)	0.5 (5/10)	1.2 (14/15)	0.6 (6/13)	1.1 (8/8)
300	1.0(5/5)	1.9(10/10)	2.0 (10/10)	1.4 (14/14)	2.8 (13/13)	1.4 (8/8)
Female rats						
0	—	0.0 (0/8) [a]	—	0.4 (6/14)	0.4 (6/14) [b]	0.4 (6/14) [b]
2	—	0.5 (4/8)	—	0.7 (10/15)	c	c
10	—	1.0 (8/8)	—	0 7(10/15)	c	c
30	—	1.4 (8/8)	—	0.8 (12/15)	1.8 (13/13)	c
90	—	1.4 (5/5)	—	0.7 (10/15)	0.4 (5/13)	0.9 (7/8)
300	—	1.2(5/5)	—	1.1 (14/14)	1.4 (13/13)	0.8 (6/8)

a: Chloroform-induced kidney histopathological changes were scored qualitatively for severity as follows: 0 = within normal limits, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, where 1 through 4 indicate increasing severity of the lesions ranging from vacuolation of proximal cell tubule (PCT) epithelium, enlarged PCT nuclei, pyknotic PCT nuclei, to individual tubule cell necrosis. Detailed descriptions of the lesions are given under Results. The first number in each box is the mean lesion score for the entire group of animals. The ratio in parentheses is that of the number of animals presenting with a lesion score of I or greater, relative to the total number of animals evaluated in that group.

b: Control animals are the same for all the 13-week studies.

c: Animals were not exposed at these time points.

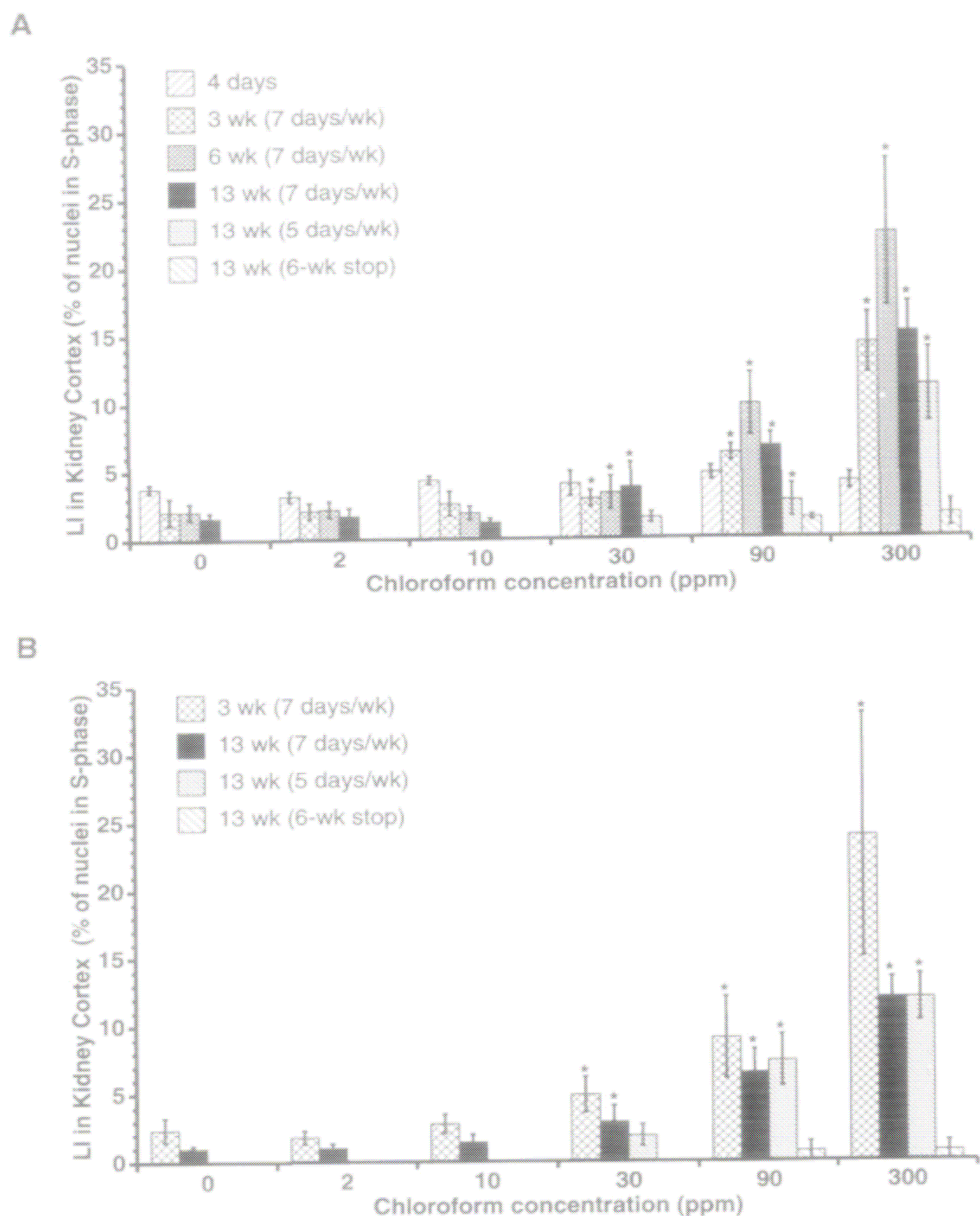


Figure 4.3 Labeling index (LI) in the kidney cortex of (A) male or (B) female F-344 rats exposed to chloroform vapors for 4 days or 3, 6, or 13 weeks (males) or 3 or 13 weeks (females).

Bars represent the mean LI ± SD (n = 5-10 rats per group). The LI is the percentage of nuclei in S-phase identified in histological sections stained immunohistochemically for BrdU. Rats were exposed 6 hr/day for 7 or 5 days/week. Additional rats were exposed for 6 hr/day, 7 days/week for 6 weeks and then housed in the control chambers for the remaining 7 weeks (6-week stop). Asterisks (*) denote groups that were statistically different from exposure- and duration-matched control groups (Williams test, $p < 0.05$).

A clear concentration response in the number of affected rats, severity of histological alterations, and increased labeling index (LI) was present in the kidneys of both male and female rats exposed to chloroform vapors. Increased cell proliferation was not found in either sex of rats exposed for 6 weeks and then held until Week 13, indicating that the proliferative response is dependent on the presence of chloroform and represents regenerative growth as a result of repetitive cytolethality. A concentration of 10 ppm in the male and the female rat was determined to be the experimental NOAEC within the proximal tubules of the cortex. No microscopic alterations were found in either sex of rats exposed 7 days/week to 10 ppm, nor was the LI within the proximal tubule epithelium elevated.

Table 4.20 Hepatic Lesion Scores and Incidence in Male or Female F-344 Rats Exposed to Chloroform Vapors (Templin et al., 1996a)

Concentration (ppm)	4 days	3 weeks 7days/week	6 weeks 7days/week	13 weeks 7 days/week	13 weeks 5 days/week	13 weeks 6-week stop
Male rats						
0	0.0 (0/5) [a]	0.0 (0/13)	0.2 (2/12)	0.1 (1/15)	0.1 (1/15) [b]	0.1 (1/15) [b]
2	0.0 (0/5)	0.0 (0/13)	0.1 (4/13)	0.2 (3/15)	c	c
10	0.4 (2/5)	0.1 (1/13)	0.2 (3/13)	0.0 (0/15)	c	c
30	0.4 (2/5)	0.0 (0/13)	0.0 (0/13)	0.1 (2/15)	0.0(0/13)	c
90	0.3 (1/4)	0.2 (2/10)	0.3 (3/10)	1.0 (14/15)	0.3 (4/13)	0.0 (0/8)
300	0.0 (0/5)	1.8 (10/10)	2.0 (10/10)	3.9 (15/15)	2.4 (13/13)	0.0 (0/8)
Female rats						
0	—	0.0 (0/8) [a]	—	0.1 (1/15)	0.1 (1/15) [b]	0.1 (1/15) [b]
2	—	0.0 (0/8)	—	0/1 (1/15)	c	c
10	—	0.0 (0/8)	—	0.0 (0/14)	c	c
30	—	0.4 (3/8)	—	0.0 (0/15)	0.0 (0/13)	c
90	—	0.8 (4/5)	—	0.8 (12/15)	0.3 (4/13)	0.1 (1/8)
300	—	2.0 (5/5)	—	3.0(15/15)	2.0(13/13)	0.0 (0/8)

a: **Chloroform-induced liver histopathological changes were scored qualitatively for severity as follows: 0 = within normal limits, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, where 1 through 4 indicate increasing severity of the lesions ranging from hepatocyte vacuolation, degenerative changes in hepatocytes, to hepatocyte necrosis. Detailed descriptions of the lesions are given under Results. The first number in each box is the mean lesion score for the entire group of animals. The ratio in parentheses is that of the number of animals presenting with a lesion score of 1 or greater, relative to the total number of animals evaluated in that group.**

b: Control animals are the same for all the 13-week studies.

c: Animals were not exposed at these time points.

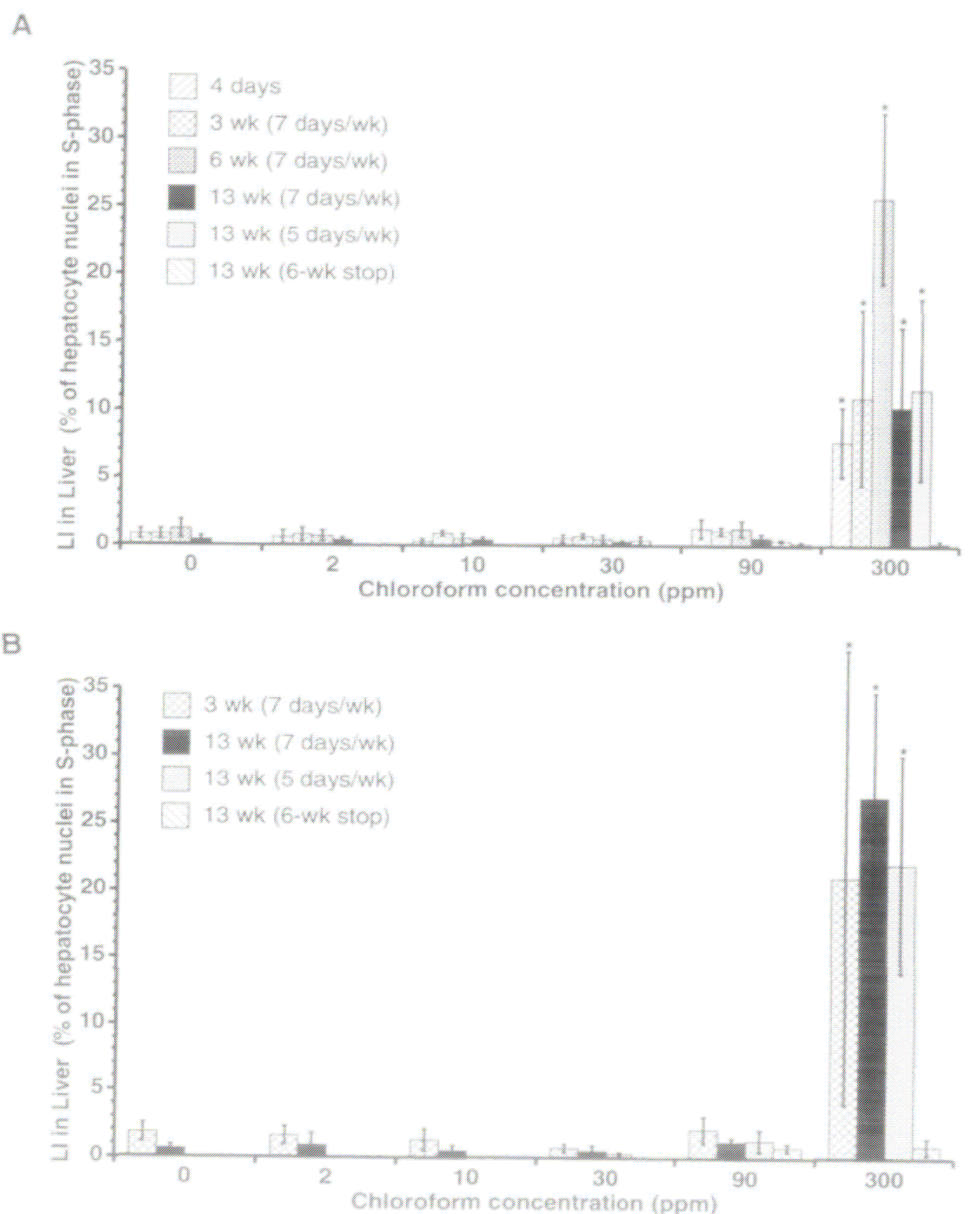


Figure 4.4 Hepatocyte labeling index (LI) in the livers of (A) male or (B) female F-344 rats exposed to chloroform vapors for 4 days or 3. 6. or 13. weeks (males) for 3 or 13 weeks (females).

Bars represent the mean LI ± SD (n = 5-10 rats per group). The LI is the percentage of nuclei in S-phase identified in histological sections stained immunohistochemically for BrdU. Rats were exposed 6 hr/day for 7 or 5 days/week. Additional rats were exposed for 6 hr/day, 7 days/week for 6 weeks and then housed in the control chambers for the remaining 7 weeks (6-week stop). Asterisks (*) denote groups that were statistically different from exposure- and duration-matched control groups (Williams test. /; < 0.05).

In males, hepatocyte alterations were primarily confined to the 300 ppm exposed rats at all time points and in the 90 ppm exposed rats at the later time points. Microscopic findings in the rats exposed 7 days/week to 300 ppm included scattered individual hepatocyte degeneration, mitotic figures, and midzonal vacuolation.

The lesions characterized by intestinal crypt-like ducts with periductular fibrosis were dramatically increased in the livers of female rats exposed to 300 ppm chloroform. Microscopically, the lesions were characterized as glandular structures lined by columnar epithelium and goblet cells and surrounded by connective tissue. The prevalence and severity of the lesions was greatest in the right and caudate lobes. The severity of alterations in livers of the female rats was greater than that of the males.

The nasal lesions were primarily confined to the ethmoid portion of the nasal passages lined by olfactory epithelium. At the early time points, alterations involved the ventral and lateral regions of the ethmoid turbinates, while the central aspects of the turbinates and nasal septum were unaffected. With continued exposure, lesions were present throughout the entire ethmoid portion of the nose. Relatively few alterations were present in the anterior portions of the nasal cavity or the posterior regions lined by respiratory epithelium. The type, severity, and distribution of the lesions were consistent and usually present in all rats within a specific concentration and duration-exposed group (see Table 4.21). The proliferative and atrophic alterations induced in the nasal passages of female rats exposed to chloroform vapor for 3 or 13 weeks were similar to those found in the male rat following 3 or 13 weeks of exposure. (LOAEC = 2 ppm)

Table 4.21 Severity of Nasal Lesions in Male F-344 Rats Exposed to Chloroform Vapors (Templin et al., 1996a)

Concentration (ppm)	4 days	3 weeks 7 days/week	6 weeks 7 days/week	13 weeks 7 days/week	13 weeks 5 days/week	13 week 6-week stop
0	1.0 (5/5) [a]	1.3 (6/8)	0.0 (0/7)	0.0 (0/10)	0.0 (0/10) [b]	0.0(0/10) [b]
2	1.0 (5/5)	1.4(5/8)	1.0 (7/8)	1.1 (10/10)	c	c
10	1.4 (5/5)	2.4 (8/8)	1 9 (8/8)	2.0(10/10)	c	c
30	2.0 (5/5)	2.4 (8/8)	2 1 (8/8)	2.0(10/10)	1.8 (8/8)	c
90	3.0 (5/5)	2.8 (5/5)	3.0 (5/5)	2.5 (10/10)	2.0 (8/8)	2.1 (5/8)
300	3.8 (5/5)	3.0 (5/5)	3.0 (5/5)	2.9 (10/10)	3.0 (8/8)	2.9 (8/8)

a: Chloroform-induced histopathological changes in the ethmoid region of the nasal passage were scored qualitatively for severity as follows: 0 = within normal limits. 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, where 1 through 4 indicate increasing severity of the lesions. Nasal sections from rats exposed for 4 days or 3 weeks were assigned severity scores for lesions in the lamina propria ranging from edema and loss of Bowman's gland, penosteal hypercellulanty. to mineralization of the basal lamina In rats exposed for 6 or 13 weeks, severity scores were assigned for lesions ranging from edema and loss of Bowman's glands, olfactory metaplasia, basal lamina mineralization, to generalized atrophy of the ethmoid turbinates. The first number in each box is the mean lesion score for the entire group of animals. The ratio in parentheses is that of the number of animals presenting with a lesion score of 1 or greater, relative to the total number of animals evaluated in that group.

b: Control animals are the same for all the 13-week studies.

c: Animals were not examined at these time points

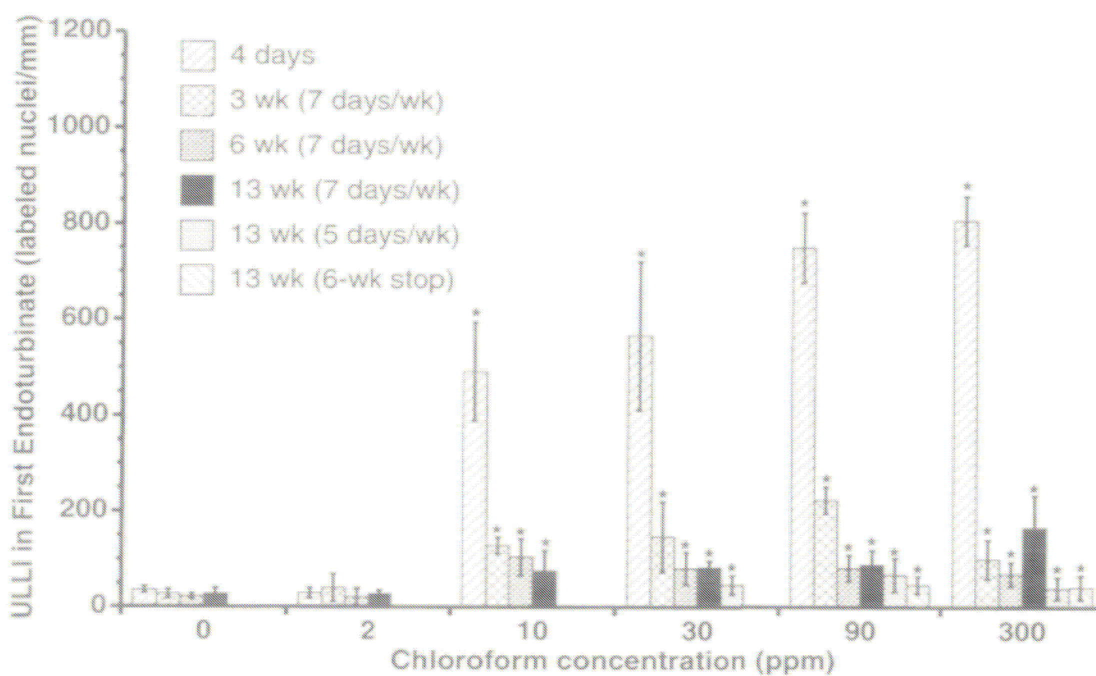


Figure 4.5 Unit length labeling index (ULLI) in the proximal portion of the dorsal scroll of the first endoturbinate of male F-344 rats exposed to chloroform vapors for 4 days or 3, 6, or 13 weeks.

Bars represent the mean ULLI ± SD (n = 5 —10 rats per group). The ULLI is the number of nuclei in S-phase in the lamina propria and adjacent periosteum. The underlying turbinate bone was used for determination of length. Rats were exposed 6 hr/day for 7 or 5 days/week. Additional rats were exposed for 6 hr/day, 7 days/week for 6 weeks and then housed in the control chambers for the remaining 7 weeks (6-week stop). Asterisks (*) denote groups that were statistically different from exposure- and duration-matched control groups (Williams test, $p < 0.05$).

Larson et al., (1996) exposed different groups of female and male B6C3Fi mice to atmospheric concentrations of 0, 0.3, 2, 10, 30, and 90 ppm chloroform 6 hr/day, 7 days/week for exposure periods of 4 days or 3, 6, or 13 consecutive weeks. Some additional exposure groups were exposed for 5 days/week for 13 weeks or were exposed for 6 weeks and then examined at 13 weeks. Bromodeoxyuridine was administered via osmotic pumps implanted 3.5 days prior to necropsy, and the labeling index (LI, percentage of nuclei in S-phase) was evaluated immunohistochemically from histological sections. Complete necropsy and microscopic evaluation revealed treatment-induced dose- and time-dependent lesions only in the livers and nasal passages of the female and male mice and in the kidneys of the male mice. Large, sustained increases in the liver LI were seen in the 90-ppm groups at all time points. The female mice were most sensitive, with a NOAEC for induced hepatic cell proliferation of 10 ppm. The hepatic LI in the 5 days/week groups were about half of those seen in the 7 days/week groups and had returned to the normal baseline in the 6-week recovery groups. Induced renal histologic changes and regenerative cell proliferation were seen in the male mice at 30 and 90 ppm with 7 days/week exposures and also at 10 ppm with the 5 days/week

regimen. Nasal lesions were transient and confined to mice exposed to 10, 30, or 90 ppm for 4 days. Assuming that chloroform-induced female mouse liver cancer is secondary to events associated with necrosis and regenerative cell proliferation, then no increases in liver cancer in female mice would be predicted at the NOAEC of 10 ppm or below based on the results reported here.

Chloroform was administered to BDF1 mice (8 per group) by inhalation 6 h/day, 5 days/week for 13 weeks (Templin et al., 1998). Because 30 and 90 ppm chloroform atmospheres are nephrotoxic and lethal to male BDF1 mice, a gradual step-up and adaptation procedure was used in the bioassay and in the studies reported here. Male mice in the 1 and 5 ppm groups were exposed to chloroform vapors for 3, 7 or 13 weeks. Male mice in the 30 ppm group were exposed to 5 ppm for 2 weeks, then to 10 ppm for 2 weeks, then to 30 ppm for the remainder of the 7 or 13-weeks. Male mice in the 90 ppm group were exposed to 5 ppm for 2 weeks, to 10 ppm for 2 weeks, to 30 ppm for 2 weeks, and then to 90 ppm for the remainder of the 7 or 13 weeks. Female BDF1 mice were exposed to 5, 30 or 90 ppm for 6 h/day, 5 days/ week for 3 or 13 weeks without step-up procedure. Chloroform induced pathology and regenerative cell proliferation, measured as the labeling index (LI, percentage of cells in S-phase), were assessed microscopically and immunohistochemically. The predominant alteration was a replacement of some or most of the proximal tubule epithelium by regenerating cells characterized by basophilic cytoplasm and variably sized heterochromatic nuclei. There were rare proximal tubules that contained necrotic cellular debris. Kidneys from female mice treated with chloroform were not different from controls.

Table 4.22 Histopathological changes and scores in the kidneys of male BDF1 mice exposed to chloroform (Templin et al., 1998)

Chloroform concentration (ppm)	Histopathological scores [a]		
	3 weeks	7 weeks	13 weeks
0	0	0.2	0
1	0.25	0.2	0.25
5	0	0.2	0.25
30		3	2.75
90		3.4	2.75

a: Chloroform-induced kidney histologic changes were scored qualitatively for severity as follows: 0 = within normal limits; 1 = minimal changes, 1–10% of cortex affected with regenerating tubules; 2 = mild changes, ~25% of cortex affected with regenerating tubules; 3 = moderate changes, ~50% of cortex affected with regenerating tubules; and 4 = severe changes, over 75% of cortex affected with regenerating tubules.

Significant, dose-related increases in LI were observed in the kidneys of male mice exposed to 30 or 90 ppm at the 7- and 13-week time points (see Figure 4.6). At 3 weeks, these dose groups were still in the step-up phase of the protocol. By the 13-week time point, the LI was elevated ~16- or 31-fold over the control in the kidneys of male mice exposed to 30 or 90 ppm respectively. No increase in the LI was observed in male mice exposed to 1 or 5 ppm at any of the time points. Thus, 5 ppm is a NOAEC for both renal toxicity and tumors, the most sensitive toxic end points. (**Considered as key study for risk characterisation**). No increase

in the LI was observed in the kidneys of the female mice at any time point or exposure concentration.

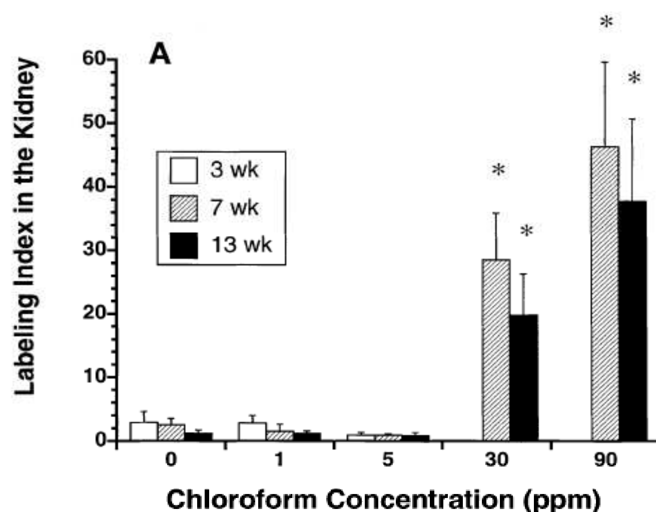


Figure 4.6 Labeling index (LI) in the kidney cortex and outer stripe of the outer medulla of male BDF1 mice exposed to chloroform vapors for 3, 7 or 13 weeks. Bars represent the mean LI ± SD (animal-to-animal variation). The LI is the percentage of nuclei in S-phase identified in histological sections stained immunohistochemically for BrdU. Asterisks (*) denote groups that were statistically different from exposure- and duration-matched control groups (Williams test, P < 0.05). (Templin et al., 1998)

In the male mice, histopathological changes were not observed at 1 or 5 ppm at any time point. Centrilobular swelling was observed at 30 ppm in 40% of male mice exposed for 7 weeks and in 88% of the male mice exposed for 13 weeks. Centrilobular to midzonal vacuolation and degeneration was observed in all male mice exposed to 90 ppm at both 7 and 13 weeks.

Yamamoto et al. (2002) performed a study on chronic toxicity of chloroform in mice exposed by inhalation to chloroform vapours for 6 h/day, 5 days a week, for 104 weeks. Groups of 50 BDF1 mice of both sexes were exposed at the concentration of 5, 30 or 90 ppm. There was no difference in the 2-year survival rate between the exposed groups and the control group. An increased incidence of renal cytoplasmic basophilia was observed in both exposed males and females, and the incidences of atypical tubule hyperplasia and nuclear enlargement in the kidneys increased in the exposed male mice only (see table below). Fatty change was observed in the liver of both exposed male and female mice whereas the incidences of total altered cell foci increased in the exposed females only. Moreover, thickening of bone, atrophy and respiratory metaplasia of the olfactory epithelium were observed in the nasal cavity of mice of both sexes. For the renal effect, the NOAEC was 5 ppm (25 mg/m^3). (**Considered as key study for risk characterisation).** For the hepatic effect, the NOAEC was 30 ppm (150 mg/m^3). For nasal lesions, the LOAEC was 5 ppm (25 mg/m^3) in mice.

Table 4.23 Incidences of selected non-neoplastic lesions in the liver and kidneys of mice exposed to chloroform vapor for 104 weeks (Yamamoto et al., 2002)

(A) Mice

Group	Male				Female			
	Control	5 ppm	30 ppm	90 ppm	Control	5 ppm	30 ppm	90 ppm
Number of animals examined	50	50	50	48	50	49	50	48
Liver								
Necrosis: central	0	0	0	3	1	0	1	2
Necrosis: focal	1	2	6	2	0	0	2	3
Fatty change	4	2	6	24**	0	0	0	6*
Total altered cell foci	10	1**	1**	5	0	1	2	6*
Clear cell foci	6	0*	0*	3	0	1	0	3
Basophilic cell foci	3	1	1	1	0	0	1	2
Mixed cell foci	1	0	0	1	0	0	1	1
Kidneys								
Nuclear enlargement : proximal tubules	0	3	43**	42**	0	0	0	4
Cytoplasmic basophilia[a)] +	33	40	8**	9**	0	4	3	5*
2+	7	1	36	34	0	0	0	2
3+	0	0	2	0	0	0	0	0
Atypical tubule hyperplasia	0	0	11**	14**	0	0	0	0
Tubular necrosis: proximal tubules	0	0	1	2	1	0	0	0

Significant difference at $P \leq 0.05$ (*) and $P \leq 0.01$ (**) by Chi square test. a) The severity of cytoplasmic basophilia was qualitatively scored as follows: +, a few lesions involving a single tubule in the whole histological section; 2+, more than 4 lesions involving two or more tubules in the whole histological section; 3+, numerous lesions throughout whole section. b) The severity of chronic progressive nephropathy was classified into four different grades according to the criteria described by Kawai[21)].

Yamamoto et al. (2002) also performed the same chronic study in rats exposed by inhalation to chloroform vapours for 6 h/day, 5 days a week, for 104 weeks. Groups of 50 F344 rats of both sexes were exposed at the concentration of 10, 30 or 90 ppm. There was no difference in the 2-year survival rate between the exposed groups and the control group. Increased incidences of nuclear enlargement and dilatation of tubular lumen were found in the kidneys of exposed males and females (see table below). An increased incidence of the vacuolated cell foci was observed in the liver of female rats. Moreover, thickening of bone, atrophy and respiratory metaplasia of the olfactory epithelium were observed in the nasal cavity of male and female rats. For the renal effect, the NOAEC was 10 ppm (50 mg/m^3) and for the hepatic effect, the NOAEC was 30 ppm (150 mg/m^3) in rats. For nasal lesions, the LOAEC was 10 ppm (50 mg/m^3).

Table 4.24 Incidences of selected non-neoplastic lesions in the liver and kidneys of rats exposed to chloroform vapor for 104 weeks (Yamamoto et al., 2002)

(B) Rats

Group	Male				Female			
	Control	10 ppm	30 ppm	90 ppm	Control	10 ppm	30 ppm	90 ppm
Number of animals examined	50	50	50	50	50	50	50	49
Liver								
Total altered cell foci	11	16	16	18	15	9	20	26
Clear cell foci	4	4	5	6	4	1	2	7
Acidophilic cell foci	2	5	2	3	0	1	0	1
Basophilic cell foci	4	6	8	8	7	5	10	4
Mixed cell foci	1	1	1	1	4	2	6	9
Vacuolated cell foci	0	0	0	0	0	0	2	5 *
Kidneys								
Nuclear enlargement : proximal tubules	0	0	5 *	32 **	0	0	6 *	34 **
Dilatation : tubular lumen	0	0	9 *	27 **	0	0	5 *	38 **
Chronic progressive nephropathy[b)] +	3	11 *	10 **	17 **	8	19 **	27 **	15 **
2+	6	10	24	14	15	7	5	3
3+	19	15	8	2	14	3	3	1
4+	19	8	2	1	4	2	0	2

Significant difference at P ≤ 0.05 (*) and P ≤ 0.01 (**) by Chi square test. a) The severity of cytoplasmic basophilia was qualitatively scored as follows: +, a few lesions involving a single tubule in the whole histological section; 2+, more than 4 lesions involving two or more tubules in the whole histological section; 3+, numerous lesions throughout whole section. b) The severity of chronic progressive nephropathy was classified into four different grades according to the criteria described by Kawai[21)].

Dermal

No data available on dermal repeated dose toxicity.

Oral

Female F-344 Rats were administered chloroform dissolved in corn oil at doses of 0, 34, 100, 200 or 400 mg/kg/day for 4 consecutive days or for 5 days/wk for 3 wk (Larson et al., 1995). Bromodeoxyuridine (BrdU) was administered through an implanted osmotic pump 3.5 days prior to autopsy to label cells in S-phase. Cells in S-phase were visualized immunohistochemically in tissue sections and the labelling index (LI) calculated as the percentage of cells in S-phase. Mild degenerative centrilobular changes and dose-dependent increases in the hepatocyte LI were observed after administration of 100 mg or more chloroform/kg/day. Rats given 200 or 400 mg/kg/day for 4 days or 3 wk had degeneration and necrosis of the proximal tubules of the renal cortex. Regenerating epithelium lining proximal tubules was seen histologically and as an increase in LI. Dose-dependent increases in LI were observed in the kidneys at doses of 100 mg or more cholorform/kg/day at both 4 days and 3 wk. Two distinct treatment-induced responses were observed in specific regions of the olfactory mucosa lining the ethmoid region of the nose. A peripheral lesion was seen at all doses used and included new bone formation, periosteal hypercellularity and increased cell replication. A central lesion was seen at doses of 100 mg or more chloroform/kg/day and was characterized by degeneration of the olfactory epithelium and superfic!al Bowman's glands. These observations define the dose-response relationships for the liver, kidneys and nasal passages as target organs for chloroform administered by gavage in the female F-344 rat. Lesions and cell proliferation in the olfactory epithelium and changes in the nasal passages were observed at LOAEL=34 mg/kg bw/d; after 3 weeks of administration, these effects were observed at 100 but not at 34 mg/kg bw/d. (**Considered as key study for risk characterisation).**

Table 4.25 Chloroform-induced cell proliferation in the nasal turbinates of female F-344 rats given chloroform by garage (Larson et al., 1995)

Dose (mg/kg/day)	ULLI [a]	
	4 Days	3 wk
0	15 ± 4	16 ± 3
34	145 ± 97*	24 ± 9
100	306 ± 48*	61 ± 10*
200	321 ± 19*	63 ± 5*
400	377 ± 121*	63 ± 17*

a: Unit length labelling index of cells in the lamina propria of the proximal portion of the dorsal scroll of the first endoturbinate expressed as labelled nuclei per 0.25 mm bone. Values are means ± SD. Asterisks indicate significant differences from the control (*P < 0.05; Williams' test).

In mice given 37 mg/kg bw/d by gavage for 14 days (Condie *et al.*, 1983 in WHO, 2004), lesions in the kidneys (mineralization, hyperplasia and cytomegaly) and liver inflammation were observed.

Chloroform was fed to mice (10/sex/dose) by gavage in corn oil or in 2% Emulphor, at concentrations of 60, 130 and 270 mg/kg bw/d for 90 days (Bull *et al.*, 1986). Both sexes showed increased liver weights and vacuolation and lipid accumulation in the liver, from the lowest dose level. When Emulphor was used as vehicle, the only effect observed at 60 mg/kg bw/d was increased liver weight in females. The authors concluded that hepatotoxic effects were enhanced by the administration of chloroform via corn oil versus chloroform administered in an aqueous suspension.

US EPA (1980) performed a 90-day subchronic toxicity study, in which male Osborne-Mendel rats (30/groups) were exposed to chloroform in drinking water at concentrations 0, 200, 400, 600, 900 or 1800 ppm. From 900 ppm, body weights of male rats were significantly reduced ($p<0.05$) only during the first week of treatment. Rats exposed to 1800 ppm showed significant reduced body weight during all the treatment. In addition, during the first week of treatment, drinking water consumption was reduced with increasing concentrations of chloroform. Consumed doses of chloroform were calculated on the basis of average body weight and drinking water: 0, 20, 38, 57, 81 and 160 mg/kg-day. No effect was reported on kidneys, testes, prostate and seminal vesicles except one case of testicular hyperplasia and one interstitial cell hyperplasia for animals exposed to 900 ppm, after 30 days of treatment.

In the same time, a 90-day subchronic toxicity study (US EPA, 1980) was performed on B6C3F1 mice (30/group), exposed to concentrations of 0, 200, 400, 600, 900, 1800 or 2700 ppm in drinking water. Seven mice died during the first three weeks of the treatment, after significant body weight reductions, probably due to refusal to drink the chloroform-treated water. Consumed doses of chloroform were 0, 20, 40, 60, 90, 180 and 270 mg/kg-day. Mice receiving 600-900-1800 or 2700 ppm showed decreased body weights during the first three

weeks, before weight stabilization at levels similar to controls. Some fatty liver changes were observed at 180 and 270 mg/kg-day. No effect was observed on ovaries and uteri.

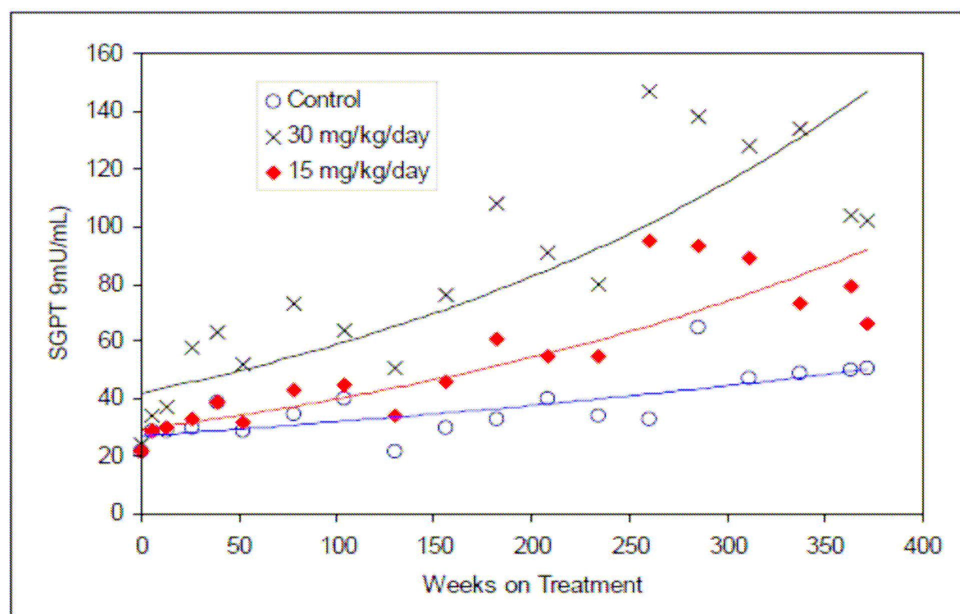
Chloroform was fed to four groups of 7-12 male and female CD1 mice, by stomach tube, at concentrations of 0-50-125 and 250 mg/kg bw/d for 90 days (Munson *et al.*, 1982). At all doses, increased liver weight and increased hepatic microsomal activity were observed in females and, in both sexes, microscopic tissue changes in the liver (hepatocyte degeneration and focal lymphocyte collection) and the kidneys (intertubular collection of inflammatory cells) were seen. The estimated LOAEL is 50 mg/kg bw (WHO, 2004).

Seven groups of 6-week-old female B6C3F1 mice (30 mice/group) were given water containing either 0, 200, 400, 600, 900, 1,800, or 2,700 ppm chloroform for 30–90 days (Jorgenson et al., 1980 in US EPA, 2001). Calculated dose levels were 0, 32, 64, 97, 145, 290, or 436 mg/kg/day based on reported water intakes. At week 1, a significant decrease in body weight was observed in the 900, 1,800, and 2,700 ppm chloroform treatment groups; however, all body weights of the treated animals were comparable to controls after week 1. On days 30, 60, and 90, ten animals from each treatment group were sacrificed for gross and microscopic pathologic examination, as well as for measurement of organ fat:organ weight ratios. A 160%–250% increase in liver fat was observed in the high-dose group. Histological examination of the liver revealed mild centrilobular fatty changes in the 1,800 and 2,700 ppm groups. On day 30, reversible fatty changes in the liver were observed at doses as low as 400 ppm chloroform. Treatment-related atrophy of the spleen was observed at the high dose. Based on the observation of mild effects of chloroform exposure via the drinking water on liver and other tissues, the LOAEL in this study was 290 mg/kg/day, while the NOAEL was 145 mg/kg/day.

Jorgenson et al. (1985, in US EPA, 2001) exposed male Osborne-Mendel rats and female B6C3F1 mice to chloroform in drinking water (0, 200, 400, 900, or 1,800 mg/L) for 104 weeks. Time-weighted average doses, based on measured water intake and body weights, were 0, 19, 38, 81, or 160 mg/kg/day for rats and 0, 34, 65, 130, or 263 mg/kg/day for mice. An additional group of animals that served as controls was limited to the same water intake as the high-dose groups. The number of animals in the dose groups (from low to high) was 330, 150, 50, and 50 for rats and 430, 150, 50, and 50 for mice. Histological slides of rat kidney from this study have been re-examined to assess whether evidence of renal cytotoxicity could be detected (ILSI, 1997; Hard and Wolf, 1999; Hard et al., 2000 in US EPA, 2001). Based on this reexamination, it was found that animals exposed to average doses of 81 or 160 mg/kg/day of chloroform displayed low-grade renal tubular injury with regeneration, mainly in the mid to deep cortex. The changes included faint basophilia, cytoplasmic vacuolation, and simple hyperplasia in proximal convoluted tubules. In some animals, single-cell necrosis, mitotic figures, and karyomegaly were also observed. Hyperplasia was visualized as an increased number of nuclei crowded together in tubule cross-sections. These changes were observable in the 160 mg/kg/day dose group at 12, 18, and 24 months, and in the 81 mg/kg/day dose group at 18 and 24 months. Cytotoxic changes were not seen in either of the lower dose groups (19 or 38 mg/kg/day). Based on histological evidence of renal cytotoxicity in rats, this study identifies a LOAEL of 81 mg/kg/day (US EPA, 2001). No mouse data on repeated dose toxicity were reported in the reviews for this study, however information on carcinogenicity was available and reported in the corresponding section.

Heywood et al. (1979, in US EPA 2001) exposed groups of eight male and eight female beagle dogs to doses of 15 or 30 mg chloroform/kg/day. The chemical was given orally in a toothpaste base in gelatin capsules, 6 days/week for 7.5 years. This was followed by a 20- to 24-week recovery period. A group of 16 male and 16 female dogs received toothpaste base

without chloroform and served as the vehicle control group. Eight dogs of each sex served as an untreated group and a final group of 16 dogs (8/sex) received an alternative nonchloroform toothpaste. Four male dogs (one each from the low- and high-dose chloroform groups, the vehicle control group, and the untreated control group) and seven female dogs (four from the vehicle control group and three from the untreated control group) died during the study. Results for alanine aminotransferase (ALAT, previously known as serum glutamate pyruvate transaminase or SGPT) levels are shown in Figure 4.7.



Data are from Heywood et al., 1979. SGPT = serum glutamate pyruvate transaminase.

Figure 4.7 ALAT (SGPT) levels in dogs exposed to chloroform for 7 years

Although there is substantial variability in individual measurements, ALAT levels tended to be about 30%–50% higher in the low-dose group (15 mg/kg/day) than in control animals. These increases were statistically significant for weeks 130-364. For the high-dose group (30 mg/kg/day), the typical increase in ALAT was about twofold, and the differences were statistically significant for the entire exposure duration (weeks 6–372). At the end of treatment, the most obvious deviation found in biochemical analyses was a dose-related elevation in ALAT values. After 14 weeks of recovery, ALAT levels remained significantly increased in the high-dose group but not in the low-dose group, when compared with the controls.

After 19 weeks of recovery, ALAT levels were not significantly increased in either treated group when compared with the controls. The authors concluded that the increases in ALAT levels were likely the result of minimal liver damage. Serum alkaline phosphatase (SAP) and serum glutamic oxaloacetic transaminase SGOT levels were also moderately increased (not statistically significant) in the treated dogs at the end of the treatment period when compared with the controls. Microscopic examinations were conducted on the major organs. The most prominent microscopic effect observed in the liver was the presence of “fatty cysts,” which were described as aggregations of vacuolated histiocytes. The fatty cysts were observed in the control and treated dogs, but were larger and more numerous (i.e., higher incidence of cysts rated as “moderate or marked,” as opposed to “occasional or minimal”) in the treated dogs at both doses than in the control dogs. The prevalence of moderated or marked fatty cysts was 1/27 in control animals, 9/15 in low-dose animals, and 13/15 in high-dose animals. Nodules of

altered hepatocytes were observed in both treated and control animals, and therefore were not considered related to treatment. No other treatment-related nonneoplastic or neoplastic lesions were reported for the liver, gall bladder, cardiovascular system, reproductive system, or urinary system. A NOAEL was not identified in this study. However, a LOAEL of 15 mg/kg/day was identified, based on elevated ALAT levels and increased incidence and severity of fatty cysts (US EPA, 2001). (**Considered as key study for risk characterisation).**

Combined exposure

A group of 50 male rats was exposed by inhalation to 0 (clean air), 25, 50, or 100 ppm (v/v) of chloroform vapor-containing air for 6 h/d and 5 d/wk during a 104 w period, and each inhalation group was given chloroform-formulated drinking water (1000 ppm w/w) or vehicle water for 104 wk, ad libitum. There was no difference in the 104-wk survival rate between the untreated control group and the three inhalation-alone groups, the oral-alone group, or the three combined-exposure groups. Incidences of non-neoplastic lesions of the kidney (cytoplasmic basophilia and dilatation of the lumen in the proximal tubule) were significantly increased in the inhalation-alone groups, the oral-alone group, and the three combined-exposure groups (see Table 4.26 below). The incidences of cytoplasmic basophilia were significantly greater in the combined-exposure groups than in the oral-alone group or the inhalation-alone groups with matched concentration. Incidence of nuclear enlargement in the proximal tubular cells was increased in both the inhalation-alone groups and the combined-exposure groups, whereas nuclear enlargement did not occur in the oral-alone group. The incidences of nuclear enlargement in the combined-exposure groups were significantly greater than those in the inhalation-alone groups with matching concentrations.

Table 4.26 Incidences of Selected Pre- and Nonneoplastic Lesions of the Kidney (Nagano et al., 2006)

	Drinking water (ppm)							
	0				1000			
Inhalation (ppm)	0	25	50	100	0	25	50	100
Estimated amount of chloroform uptake (mg/kg/d)	0	20	39	78	45	73	93	135
Number of animals examined	50	50	50	50	49	50	50	50
Kidney								
Atypical tubule hyperplasia	1	0	0	0	2	4	7[c]	15[a b c]
Cytoplasmic basophilia	0	3	7[a]	8[a]	9[a]	26[a b c]	35[a b c]	36[a b c]
Dilatation: tubular lumen	0	3	11[a]	27[a]	28[a]	46[a b c]	48[a b c]	49[a b c]
Nuclear enlargement: proximal tubule	0	0	6[a]	33[a]	0	34[a b c]	47[a b c]	50[a b c]
Chronic progressive nephropathy, +	7	21[a]	21[a]	30[a]	21[a]	2[a b c]	13[a b c]	17[a b c]
Chronic progressive nephropathy, 2+	16	15	16	10	11	1	2	1
Chronic progressive nephropathy, 3+	26	5	3	2	2	0	0	1

a : significantly different from the untreated control group (Inh-0 + Orl-0)
b: significantly different from the oral-alone group (Inh-0 + Orl-1000)
c: significantly different from each inhalation-alone group with matching concentrations (Inh-25 + Orl-0, Inh-50 + Orl-0, Inh-100 + Orl-0)
at $p \leq 0.05$ by chi-square test.

High incidence of positive urinary glucose (>80%) occurred only in the three combined-exposure groups, compared with a low incidence (<15%) in the oral-alone group or the three inhalation-alone groups. There was no untreated control rat with positive urinary glucose. Severity of positive urinary glucose was also increased in the three combined-exposure groups. On the other hand, concentrations of serum glucose and urinary protein significantly decreased in the three inhalation-alone groups, the oral-alone group, and the three combined-exposure groups, compared with that in the untreated control group. For renal effect via inhalation, the LOAEC of 25 ppm (125 mg/m3) was determined for chronic progressive nephropathy (Nagano et al., 2006).

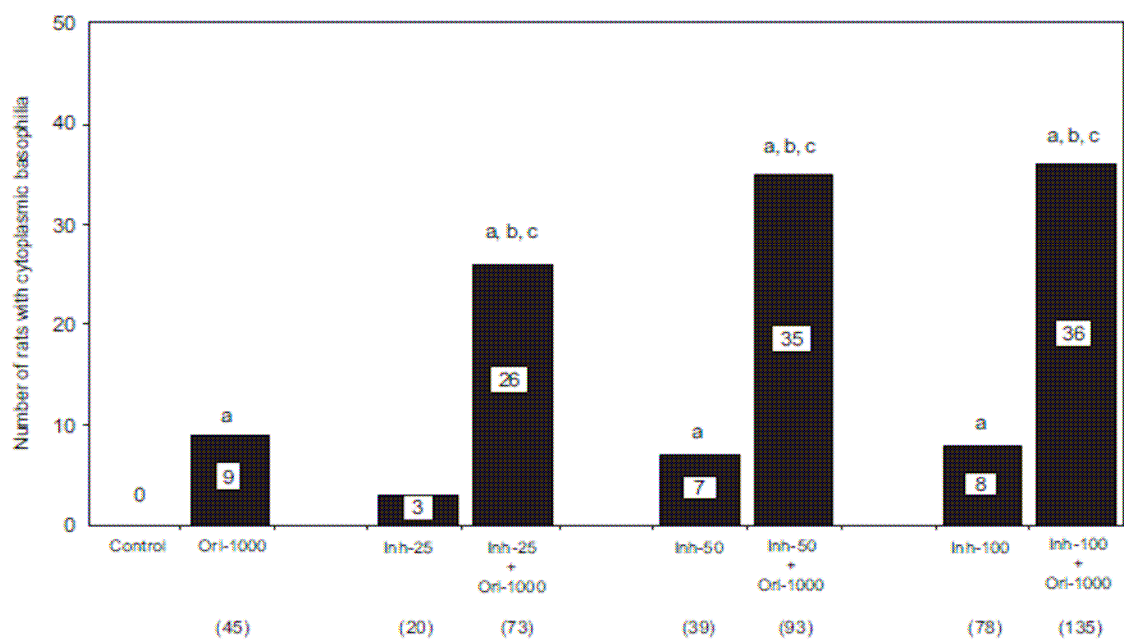


Figure 4.8 Incidences of cytoplasmic basophilia of the proximal tubule in the kidney. Parentheses indicate the estimated amount of chloroform uptake (mg/kg/d). a, b, c: significantly different from the untreated control group, from oral-alone group or from each inhalation-alone group with matching concentrations at p ≤0.05 by chi-square test (Nagano et al., 2006)

In vitro studies

No data available.

4.1.2.6.2 Studies in humans

In vivo studies

Inhalation

Gastrointestinal symptoms (nausea, dry mouth, and fullness of the stomach) were reported in female workers occupationally exposed to 22-71 ppm chloroform for lo-24 months and 77-

237 ppm chloroform for 3-10 years (Challen et al. 1958 in ATSDR, 1997). However, No clinical evidence of liver injury was observed in this study.

Toxic hepatitis (with hepatomegaly, enhanced serum glutamic pyruvic transaminase [SGPT] and serum glutamic oxaloacetic transaminase [SGOT] activities, and hypergamma-globulinemia) was observed in workers exposed to 2-205 ppm chloroform (Bomski et al. 1967 in ATSDR, 1997).

Workers exposed to 14-400 ppm chloroform for l-6 months developed toxic hepatitis and other effects including jaundice, nausea, and vomiting, without fever (Phoon et al. 1983 in ATSDR, 1997).

Li et al., (1993) carried out a series of studies in order to get necessary data for recommendation of maximum allowable concentration of chloroform in workplace. The exposure level ranged 4.27-147.91 mg/m^3 in 119 air samples collected from 3 representative worksites, with 45.4% air samples below 20 mg/m^3. The workers exposed to chloroform at 29.51 mg/m^3 had slight liver damage indicated by the higher rates of abnormal serum prealbumin and transferrin levels than those of control workers. The neurobehavioral functions of these workers were also obviously affected, manifested as increases in scores of passive mood states and dose-related negative changes in neurobehavioral testing. Mainly based on these results a Maximum Allowable Concentration of 20 mg/m^3 has been recommended in the workplace. A limitation of this study raised in ATSDR, 1997 was that the workers were probably exposed, to compounds other than chloroform (i.e., other solvents, drugs, pesticides, etc.). So the effects could not be attributed to chloroform only.

Dermal

Oral

Increased sulfobromophthalein retention was observed in an individual, who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years, indicating an impaired liver function. The changes reversed to normal after exposure was discontinued. Numerous hyaline and granular casts and the presence of albumin were observed in the urine of the subject. The urinalysis results reversed to normal after discontinuation of chloroform exposure (Wallace 1950 in ATSDR, 1997).

Biochemical tests indicate that liver function in male and female humans was not affected by the use of mouthwash providing 0.96 mg/kg/day chloroform for ≤5 years. No indications of renal effects were observed with estimated doses of 0.34 - 0.96 mg/kg/day chloroform for the same duration (De Salva et al. 1975 in ATSDR, 1997).

In vitro studies

4.1.2.6.3 Summary of repeated dose toxicity

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). **Considered as key study for risk characterisation.**

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). **Considered as key study for risk characterisation.**

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 (Templin et al., 1996a). 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). **Considered as key studies for risk characterisation.** In human, limited data on repeated dose toxicity suggest that the liver and kidneys are the likely target organs.

Based on the data available for repeated dose toxicity, the classification proposed for chloroform is R48/20/22: danger of serious damage to health by prolonged exposure.

4.1.2.7 Mutagenicity

A large number of studies have been performed to evaluate the mutagenicity of chloroform and these studies have recently been reviewed and evaluated by several groups. A more detailed presentation of available data is given in the documents from Environment Canada (1999), US EPA (2001) and WHO (2004). References are cited from IUCLID (2007). In reviewing and evaluating these studies, it is important to recognize the following potential concerns regarding study design:

- because chloroform is relatively volatile, test systems not designed to prevent chloroform escape to the air may yield unreliable results;
- because it is the metabolites of chloroform (e.g., phosgene, dichloromethyl free radical) rather than the parent compound that are most likely to react with DNA, studies in which appropriate P450-based metabolic activation systems are absent are likely to provide an incomplete result.

4.1.2.7.1 Studies *in vitro*

Studies in bacterial test systems

In tests performed using experimental conditions designed to exposed the bacteria directly to $CHCl_3$ vapour, or using appropriate precautions to prevent the evaporation of $CHCl_3$, or

exhibiting a toxic response at the higher concentrations of $CHCl_3$ - indicating that the bacteria were adequately exposed - the results of the gene mutation assays in *Salmonella typhimurium* and *Escherichia coli* are predominately negative with or without activation with microsomes from liver and/or kidney of rats and/or mice, indicating that $CHCl_3$ is not a mutagen in bacteria (Araki et al., 2004; Nestmann et al., 1980; Daniel et al., 1980: Van Abbe et al., 1982; Richold and Jones, 1981; Le Curieux et al., 1995; Roldan-Arjona et al., 1991; Kirkland et al., 1981; DeMarini et al., 1991; Gatehouse, 1981). (see Table 4.27)

A weak positive response (two-fold increases in revertants) was observed on *Salmonella typhimurium* strain TA 1535 transfected with rat theta-class glutathione S-transferase T1-1 exposed for 24 hr in a plate-incorporation assay to the vapour of $CHCl_3$ at concentrations of 19,200 and 25,600 ppm (Pegram et al., 1997). However, these vapour concentrations produce $CHCl_3$ doses of 226 and 320 mg/plate, respectively. These huge doses are well in excess of the limit dose of 5 mg/plate recommended by the international guidelines and this weak positive result seems of doubtful significance.

Gene mutation assays on fungi and yeast

Numerous investigations were carried out on *Saccharomyces cerevisiae*. Most of these investigations revealed negative results (Zimmermann and Scheel, 1981; Sharp and Parry, 1981; Kassinova et al., 1981; and Mehta and von Borstel, 1981).

One investigation carried out on *Saccharomyces cerevisiae* D7 with an increase of the gene conversion at the trp5- and ilv1-locus and a mitotic recombination at the ade2-locus gave positive results for concentrations of 21 - 54 mM which already showed a cytotoxic effect (Callen et al. 1980). It should be noted that this strain of yeast contains an endogenous cytochrome P450-dependent monooxygenase system.

Chloroform was found to be also positive in another test for deletions by intrachromosomal recombination in Saccharomyces cerevisiae (Brennan and Schiestl 1998).

Chromosome malsegregation was reported in *Aspergillus nidulans* (Crebelli et al., 1988, 1992, 1995), but only at concentrations above 0.16% (v/v), which caused also cell death, indicating that exposures were directly toxic to the test cells. When exposed to $CHCl_3$ vapour no mitotic Chromosome malsegregation was observed (Crebelli et al., 1984).

Gene mutation assays on mammalian cells

Three tests performed to detect the induction of gene mutations on mammalian cells in culture gave inconclusive or weakly positive results in a cytotoxic dose range.

A HPRT test in V79 cells (Muller, 1987) was found to be inconclusive with S9-mix in the dose range of 1000 up to 1500 µg/ml. A slight increases in mutant rates was observed in 2/3 experiments with generally very pronounced variations of the gene mutation rates (maximum mutation rate 56.2 x 10-6 , negative control 31.9 x 10-6).

In two experiments, a L5178Y TK +/- (mouse lymphoma) test was found to be weakly positive in the cytotoxic range after a metabolic activation from concentrations of 0.025 µl/ml (equivalent to approx. 1 mM) (Mitchell et al., 1988). This test was also weakly positive in the cytotoxic range in three experiments with concentrations from 0.012 µl/ml (equivalent to

approx. 0.5 mM) (Myhr and Caspary 1988). So far as the test was carried out without any metabolic activation, its result was found to be negative (Caspary et al. 1988, Mitchell et al. 1988).

Chromosomal aberration assays

Of the three available studies on the clastogenic effects of $CHCl_3$, the only reliable study was performed using meristematic cells of *Allium cepa* (Cortés et al., 1985). An increase of the frequency of the abnormal ana-telophase was observed at cytotoxic concentrations (> 1500 µg/ml). The significance of this study for human risk assessment is doubtful.

A shortly reported chromosomal aberration assay on human lymphocytes indicates a clastogenic activity without metabolic activation. This assay was not reported because reliability was not assignable (ICI, 1992).

Aneuploidy assays

The data reported by Onfelt (1987) indicate that $CHCl_3$ may affects spindle microtubules in V79 cells and suggest that $CHCl_3$ may cause aneuploidy.

Inconsistent results for mitotic aneuploidy with *Saccharomyces cerevisiae* D6 were reported by Parry and Sharp (1981). They were probably due to inadequate test conditions (exposure in plastic rather than glass containers) and therefore it can be considered that chloroform was non-mutagenic in this test.

DNA repair assays

Positive (Ono et al., 1991) or negative (Nakamura et al., 1987) results were reported in two tests on DNA repair (umu-test) with *Salmonella typhimurium.*

Two SOS-chromotests were reported negative on *Escherichia coli* (Quillardet et al., 1985; Le Curieux et al., 1995).

The ability of chloroform to induce unscheduled DNA synthesis (UDS) was examined in the *in vitro* hepatocyte DNA repair assays for the most sensitive site for tumour formation, the female mouse liver. In the *in vitro* assay, primary hepatocyte cultures from female B6C3F1 mice were incubated with concentrations from 0.01 to 10 mM chloroform in the presence of 3H-thymidine. UDS was assessed by quantitative autoradiography. No induction of DNA repair was observed at any concentration (Larson et al., 1994).

In human lymphocytes and hepatocytes from male rats, chloroform did not induce UDS (Peroccio and Prodi 1981; Althaus et al., 1982).

The ability of chloroform to induce DNA repair was examined in freshly prepared primary cultures of human hepatocytes from discarded surgical material. No activity was seen in cultures from four different individuals at concentrations as high as 1 mM chloroform (Butterworth et al., 1989).

Primary DNA damage assays

Studies showed that $CHCl_3$ induced sister-chromatid exchange (SCE) in a permanent leukaemia cell line (Fujie et al., 1993) and in meristematic cells of *Allium cepa* (Cortés et al., 1985).

In human lymphocytes, Morimoto and Koizumi (1983) found that $CHCl_3$ induced SCEs. The lowest $CHCl_3$ concentration causing a significant increase in SCE was 10 mM but it was also the concentration that induced a delay in the cell cycles. In contrast, Lindahl-Kiessling et al. (1989) did not detect the induction of SCE by $CHCl_3$ in an *in vitro* assay system using intact rat hepatocytes and human peripheral lymphocytes.

The exposure of Syrian hamster embryo cells *in vitro* to $CHCl_3$ vapours significantly enhanced the transformation of the cells by SA7 adenovirus (Hatch et al., 1983). However, the significance of this result is doubtful because the lowest positive concentration (0.25 ml/chamber) was clearly cytotoxic.

No DNA single-strand breaks were induced in the alkaline elution/rat hepatocyte assay using concentrations up to 3 mM (Sina et al., 1983). However, Ammann and Kedderis (1997) reported in an abstract that chloroform-induced DNA double-strand breaks in a time and dose-dependent fashion in freshly isolated B6C3F1 mouse and F-344 rat hepatocytes but no cytolethality was observed up to 5 mM. However, in a further publication, the same authors (Ammann et al., 1998) found that chloroform induced concentration-dependent cytotoxicity in male B6C3F1 mouse and F-344 rat hepatocyte cultures at concentrations higher than 1 mM.

Table 4.27 Summary of *in vitro* studies

Test system	Method	Metabolic activation	Dose levels	Cytotoxic dose	Result	Reference	Reliability
Gene mutation assay on bacteria - Studies reliable with or without restriction							
Salmonella typhimurium Strains: TA 98, TA 100, TA 1535, and TA 1537	Gas-phase exposure	With and without rat liver S9	0.01, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0%	5%	Negative	Araki et al., 2004	2
Salmonella typhimurium Strain: TA 1535 and TA 1535 transfected with rat theta-class glutathione S-transferase T1-1	Gas-phase exposure	Without	200-25600 ppm	No data	Weak positive ≥ 19200 ppm on GST T1-1 transfected strain	Pegram et al., 1997	2
Salmonella typhimurium Strains: TA 98, TA 100, TA 1535, TA 1537, TA 1538	Direct plate incorporation	With and without rat liver S9	No data	≥ 15 mg/plate	Negative	Nestmann et al., 1980	2
Salmonella typhimurium Strains: TA98, TA100, TA1535, TA1537 and TA1538	Direct plate incorporation	With and without - rat and mice liver S9 - rat and mice kidney S9	10, 100, 1000, 10000 µg/plate	10000 µg/plate	Negative	Daniel et al., 1980: Van Abbe et al., 1982	2
Salmonella typhimurium *Strains : TA1535, TA1537, TA1538*	Direct plate incorporation	With and without rat liver S9	0, 10, 100, 1000, 10000 µg/plate	> 10000 µg/plate	Negative	Richold & Jones, 1981	3
Salmonella typhimurium Strain: TA100	Fluctuation test	With and without rat liver S9	30 - 10000 µg/ml	10000 µg/ml	Negative	Le Curieux et al., 1995	2
Salmonella typhimurium Strains: BA 13 and BAL13	L-arabinose resistance test	With and without rat liver S9	0, 0.8, 2.7, 4.0, 6.0, 9.6, 14.4, 23.0 µmol	≥ 14.4 µmol	Negative	Roldan-Arjona et al., 1991	2
Escherichia coli Strains: WP2p, WP2uvrA-p	Preincubation assay	With and without rat liver S9	0.1, 1, 10, 100, 1000, 10000 µg/plate	≥ 100 µg/plate	Negative	Kirkland et al., 1981	2

Test system	Method	Metabolic activation	Dose levels	Cytotoxic dose	Result	Reference	Reliability
Escherichia coli WP2s (lamda)	Microscreen Prophage-Induction Assay	With and without rat liver S9	0, 0.31, 0.62, 1.25, 2.5, 5.0% v/v	5.0%	Negative	DeMarini et al., 1991	2
Escherichia coli *58-161 envA, lysogenic to bacteriophage lambda and* E. coli *C600, sensitive to lambda and resistant to streptomycin*	lambda induction assay	With rat liver S9	0.05 and 5 µl/ml	5 µl/ml	Negative	Thomson, 1981	2
Escherichia coli *Strain WP2 uvrA,* Salmonella typhimurium *Strains : TA98, TA 1535 and TA1537*	Fluctuation test	With and without rat liver S9	S. typhi: 1, 5, 10 µg/ml; E. coli: 10, 100, 1000 µg/ml	S. typhi: 10 µg/ml; E. coli: 1000 µg/ml	Negative	Gatehouse, 1981	2
Bacillus subtilis Strains: H17 and M45	Liquid Rec-assay	With and without rat liver S9	No data	No data	Positive with S9	Matsui et al., 1989	2
Gene mutation assays on fungi and yeast - Studies reliable with or without restriction							
Saccharomyces cerevisiae Strain: D7	Gene conversion and mitotic recombination	Without	0, 21, 41, 54 mM	≥ 41 mM	Positive	Callen et al., 1980	2
Saccharomyces cerevisiae Strain: D7	Gene conversion and mitotic recombination	With and without rat liver S9	2 µl/ml	> 2µl/ml	Negative	Zimmermann and Scheel, 1981	2
Saccharomyces cerevisiae Strain: JD1	Mitotic gene conversion	With and without rat liver S9	No data	No data	Negative	Sharp and Parry, 1981	2

Test system	Method	Metabolic activation	Dose levels	Cytotoxic dose	Result	Reference	Reliability
Saccharomyces cerevisiae Strains: T1 and T2	Mitotic gene conversion	With and without rat liver S9	Without S9: T1: 1000 µg/ml, T2: 100 µg/ml With S9: 1000 µg/ml for both strains	Without S9: T1: > 1000 µg/ml, T2 : 100 µg/ml With S9: 1000 µg/ml for both strains	Negative	Kassinova et al., 1981	2
Saccharomyces cerevisiae Strain XV185-14C	Reverse mutation assay	With and without rat liver S9	1.11 and 0.11 µl/ml	No data	Negative	Mehta & von Borstel, 1981	2
Saccharomyces cerevisiae *Strain RS112*	Intrachromosomal recombination assay	Without	0, 0.75, 1.49, 2.98, 4.47, 5.59 mg/ml	≥ 4.47 mg/ml	Positive	Brennan & Schiestl, 1998	2
Aspergillus nidulans	Mitotic chromosome malsegregation	Without	0.04, 0.08, 0.12, 0.16, 0.20 % v/v	0.20% v/v	Positive 0.20%	Crebelli et al., 1988, 1992, 1995	2
Aspergillus nidulans	Mitotic chromosome malsegregation	Without	5.0 and 7.5 ml/20-L desiccator	≥ 5.0 ml/20-L desiccator	Negative	Crebelli et al., 1984	2
Aspergillus nidulans haploid strain 35 and diploid strain P1	Gene mutations and somatic segregation	Without	0.5% v/v	0.5% v/v	Negative	Gualandi, 1984	2
Mammalian gene mutation assay - Studies reliable with or without restriction							
V79 Chinese hamster lung cells	HGPRT assay OECD TG 476	With and without rat liver S9	100-1500 µg/ml.	> 1500 µg/ml	Inconclusive with S9 Negative without S9	Muller, 1987	1
L5178Y mouse lymphoma cells	TK+/- assay	With and without rat liver S9	Without S9: 0.39 to 1.5 µl/ml With S9: 0.007 to 0.06 µl/ml	≥ 1.2 µl/ml without S9 ≥ 0.04 µg/ml with S9	Weak positive with S9 Negative without S9	Mitchell et al., 1988	2

Test system	Method	Metabolic activation	Dose levels	Cytotoxic dose	Result	Reference	Reliability
L5178Y mouse lymphoma cells	Mouse lymphoma assay TK+/- assay	With and without rat liver S9	Without S9: 15.6-1000 nl/ml With S9: 0.78-25.0 nl/ml	Without S9: >= 500 nl/ml With S9: > 6.25 nl/ml	Weak positive with S9 Negative without S9	Myhr and Caspary, 1988	2
Chromosomal aberration assays - Studies reliable with or without restriction							
Meristematic cells of *Allium cepa*	Cytogenetic analysis	Without	0, 250, 500, 1000, 1500, 2500 and 5000 µg/ml	≥ 1500 µg/ml	Positive ≥ 1500 µg/ml	Cortés et al., 1985	2
Assays for aneuploidy - Studies reliable with or without restriction							
V79 Chinese hamster lung cells	Cytogenetic analysis	Without	$6\ 10^{-3}$, 10^{-2} and $1.2\ 10^{-2}$ M	>$1.2\ 10^{-2}$ M	Positive	Onfelt, 1987	2
Saccharomyces cerevisiae Strain D6	Mitotic aneuploidy	With and without rat liver S9	up to 600 µg/ml	variable according to the procedure used	Negative	Parry and Sharp, 1981	2
DNA repair assays - Studies reliable with or without restriction							
Salmonella typhimuriumn TA1535/pSK1002	umu test	With and without rat liver S9	up to 620 µg/ml	No data	Negative	Nakamura et al., 1987	2
Salmonella typhimuriumn TA1535/pSK1002	umu test	With and without rat liver S9	1000 µg/ml	No data	Positive	Ono et al., 1991	2
Escherichia coli Strain: PQ37	SOS-chromotest	With and without rat liver S9	No data	No data	Negative	Quillardet et al., 1985	2

Test system	Method	Metabolic activation	Dose levels	Cytotoxic dose	Result	Reference	Reliability
Escherichia coli Strain: PQ37	SOS-chromotest	With and without rat liver S9	10 - 10000 µg/ml	≥ 3000 µg/ml	Negative	Le Curieux et al., 1995	2
Male albino rat hepatocytes	Unscheduled DNA synthesis	Without	$8.4\ 10^{-7}$ - $8.4\ 10^{-2}$ M	No data	Negative	Althaus et al., 1982	2
Female B6C3F1 Mice hepatocytes	Unscheduled DNA synthesis	Without	0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0 mM	10 mM	Negative	Larson et al., 1994	2
Human lymphocytes	Unscheduled DNA synthesis	With and without rat liver S9	0, 2.5, 5 and 10 µl/ml	> 10 µl/ml	Negative	Perocco and Prodi, 1981	2
Human hepatocytes	Unscheduled DNA synthesis	Without	0, 0.01, 0.1 and 1.0 mM	No data	Negative	Butterworth et al., 1989	2
Primary DNA damage - Studies reliable with or without restriction							
Permanent leukemia cell line K3D	Sister chromatid exchange assay	With and without rat liver S9	0, 2.10^{-3}, 2.10^{-4} and 2.10^{-5} M	No data	Positive with S9	Fujie et al., 1993	2
Human lymphocytes	Sister chromatid exchange assay	With and without co-cultured with intact rat liver cells	10^{-4}, 10^{-5}, or 10^{-6} M	No data	Negative	Lindahl-Kiessling et al., 1989	2
Human lymphocytes	Sister chromatid exchange assay	Without	$1.6\ 10^{-5}$, $8\ 10^{-5}$, $4\ 10^{-4}$, $2\ 10^{-3}$, $1\ 10^{-2}$, $5\ 10^{-2}$ M	Concentrations >= $1\ 10^{-2}$ M induce a delay in the cell cycles	Positive ≥ $1\ 10^{-2}$ M	Morimoto and Koizumi, 1983	2
Rat hepatocytes	Alkaline elution assay	Without	0.03, 0.3, 3 mM	> 3 mM	Negative	Sina et al., 1983	2

Test system	Method	Metabolic activation	Dose levels	Cytotoxic dose	Result	Reference	Reliability
Syrian hamster embryo cells	Enhancement of DNA viral transformation assay	Without	2.0, 1.0, 0.5, 0.25, 0.12 ml/chamber (equivalent to 640, 320, 160, 80, 40 mg/l air)	>= 0.25 ml/chamber (160 mg/l air)	Positive ≥ 0.25 ml/chamber	Hatch et al., 1983	2
Meristematic cells of *Allium cepa*	Sister chromatid exchange assay	Without	0, 250, 500, 1000, and 1500 µg/ml	>= 1500 µg/ml	Positive	Cortés et al., 1985	2

4.1.2.7.2 Studies *in vivo*

Gene mutation assays in transgenic animals

Butterworth *et al.*, 1998:

- Gene mutation in hepatocytes of B6C3F1 lacI mice.

Female B6C3F1 lacI mice were exposed daily for 6 hr/day 7 days/week up to 180 days to 0, 10, 30 or 90 ppm (equivalent to 0, 50, 166 and 500 mg/kg bw/ day) chloroform by inhalation. Results are presented in Table 4.28.

Table 4.28 LacI mutant frequencies in Chloroform-treated Mice.

Chloroform exposure (ppm)	Timepoint (days)[a]	Mutant frequency ($\times 10^{-5}$)[b]
0	10	10.1 ± 5.1
10	10	11.7 ± 2.4
90	10	12.7 ± 4.4
0	30	9.5 ± 2.3
90	30	10.4 ± 3.5
0	90	13.0 ± 3.1
90	90	14.7 ± 6.1
0	180	12.3 ± 0.8
90	180	13.7 ± 3.6

[a]Duration of exposure to chloroform. Exposures were 6 hr/day 7 days/week. Animals were held for 10 days after completion of exposures to allow for fixation of mutations and for complete clearance of test chemical.

[b]Mutant frequency is calculated as the number of mutant plaques isolated per total plaques screened. Values are the mean ± SD (animal-to-animal variation) from five animals per dose group for each timepoint. At least 200,000 plaques were screened per animal. As chloroform clearly did not induce an increase in mutant frequency, the remaining five animals in the group were not analyzed because of cost limitations.

The results presented here show that chloroform administered by inhalation does not increase mutant frequency in the lacI assay.

Cytogenetic assays

Shelby & Witt 1995:

- Chromosomal aberration test in bone marrow by i.p route.

Tests for the induction of chromosomal aberrations (CA) in bone marrow cells of mice have been conducted on 65 chemicals including chloroform.

Chloroform was tested for induction of chromosomal aberrations in the mouse bone marrow cells using two different sacrifice times (17 h or 36 h). Male B6C3F1 mice (8 per dose group)

received a single i.p. injection with chloroform dissolved in corn oil at doses: 200, 400, 800, 1000 mg/kg pending harvest time. The total dosing volume per mouse was 0.4 ml (chloroform or solvent control). A concurrent positive control group of mice was included for each test (data not presented). Fifty well-spread first-division metaphase cells from each animal per treatment group were scored for presence of chromosomal aberrations (see Table 4.29). This study was conducted according to OECD guideline 473, no major deviation was noted.

Table 4.29

	Harvest time (hr)	Trend P value	Dose (mg/kg)	% Cells with ABS	Survival
Chromosome aberrations (CO)	17	0.004*	0	0.25 ± 0.25	8/8
			200	1.75 ± 0.70	8/8
			400	2.50 ± 0.98*	8/8
			800	1.75 ± 0.45	8/8
	17	0.500	0	1.50 ± 0.73	8/8
			800	0.50 ± 0.33	8/8
			1,000	1.25 ± 0.37	8/8
	36	0.781	0	1.00 ± 0.53	8/8
			200	2.00 ± 1.00	8/8
			400	1.75 ± 0.70	8/8
			800	1.25 ± 0.53	8/8

[a]Tests performed at BNL.
*Significant positive effect.

One CA trial with a 17 h sample time gave a statistically significant effect at 400 mg/kg only but the concurrent solvent control value was very low, 0.25% aberrant cells (historical control value is 3.26%). This effect was not confirmed in a second trial with higher doses. Results of a trial with a 36 h sample time were also negative, so the final result was concluded to be negative.

Fujie *et al*., 1990:

- Chromosomal aberration test in bone marrow by intraperitoneal administration (i.p.):

Chloroform has been studied for its ability to induce chromosome aberrations (CA) in vivo in rats.

Chloroform was administered by intraperitoneal injection in water to male and female Long-Evans rats at doses of 1.2, 11.9 or 119.4 mg/kg body weight (10-2, 10-1 or 1 mmole/kg). Non-diluted benzene (234.3 mg/kg or 3 mmole/kg) was administered i.p. as a positive control. Dose-response relationship was studied in cells sampled 12 h after i.p. administration. A significant increase in the incidence of aberrant cells was noted for chloroform at doses of 1.2 mg/kg bw and greater with a significant dose-response trend (see Table 4.30). This study was conducted according to OCDE guideline 473, no major deviation was noted.

Table 4.30 Relationship between dose and THM-induced CA 12h after intraperitoneal injection

Chemical	Dose [a] (mmole/kg)	Sex [b]	Number of cells examined	Number of cells with		Number of aberrations/cell (mean ± SD) [c]	Incidence of aberrant cells (mean ± SD) [c]	χ^2-test	Trend test (*P* value) [d]
				gaps	breaks				
$CHCl_3$	10^{-2}	Male (3)	300	5	13	0.043 ± 0.005	4.3 ± 0.5 (%)	*	M 0.001
		Female (3)	300	3	10	0.033 ± 0.004	3.3 ± 0.5	*	F 0.001
		Total (6)	600	8	23	0.038 ± 0.007	3.8 ± 0.7	**	T 0.001
	10^{-1}	Male (3)	300	9	23	0.077 ± 0.012	7.7 ± 1.2	**	
		Female (3)	300	9	19	0.063 ± 0.004	6.3 ± 0.5	**	
		Total (6)	600	18	42	0.070 ± 0.011	7.0 ± 1.2	**	
	1	Male (3)	300	9	22	0.073 ± 0.005	7.3 ± 0.5	**	
		Female (3)	300	7	19	0.063 ± 0.013	6.3 ± 1.2	**	
		Total (6)	600	16	41	0.068 ± 0.011	6.8 ± 1.1	**	
Positive control (benzene)	3	Male (3)	525	14	70	0.133 ± 0.019	13.3 ± 1.9	**	
		Female (3)	525	10	38	0.072 ± 0.014	7.2 ± 1.4	**	
		Total (6)	1050	24	108	0.103 ± 0.035	10.3 ± 3.5	**	
Vehicle control (physiological saline)		Male (3)	300	4	3	0.010 ± 0.000	1.0 ± 0.0		
		Female (3)	300	1	2	0.007 ± 0.005	0.7 ± 0.5		
		Total (6)	600	5	5	0.008 ± 0.003	0.8 ± 0.4		

[a] Doses of 10^{-2}–1 mmole/kg body weight for each chemical are as follows: $CHCl_3$, 1.2–119.4 mg/kg; $CHCl_2Br$, 1.6–163.8 mg/kg; $CHClBr_2$, 2.1–208.3 mg/kg; $CHBr_3$, 2.5–253 mg/kg.
[b] Figures in parentheses indicate the number of animals examined.
[c] Not including the cells with gaps. Values indicate the mean and standard deviation of the results from 3 or 6 rats.
[d] Trend test indicates the significance of the dose response for each chemical at each *P* value. M indicates the value for males, F for females, and T for the total of male and female rats.
* Significantly different from untreated control at $P < 0.05$.
** Significantly different from untreated control at $P < 0.01$.

In a second experiment, the percentage of aberrant metaphase cells was determined for 6, 12, 18 and 24 h after i.p. injection of 11.9 mg/kg bw (see Table 4.31). Compared to the values for the untreated control, statistically significant increases were noted at 6, 12 and 18 h after chloroform i.p. injection. The incidence of aberrant cells reached the maximum level at 12 h, and decreased to the control level within 24 h.

Table 4.31 Variation over time of THM-induced CA in rat bone marrow cells after intraperitoneal injection

Chemical	Dose [a] (mmole/kg)	Time (h)	Sex [b]	Number of cells examined	Number of cells with		Number of aberrations/cell (mean ± SD) [c]	Incidence of aberrant cells (mean ± SD) [c]	χ^2-test
					gaps	breaks			
$CHCl_3$	10^{-1}	6	Male (3)	300	4	14	0.047 ± 0.005	4.7 ± 0.5 (%)	**
			Female (3)	300	4	9	0.030 ± 0.008	3.0 ± 0.8	*
			Total (6)	600	8	23	0.038 ± 0.011	3.8 ± 1.1	**
		12	Male (3)	300	9	23	0.077 ± 0.012	7.7 ± 1.2	**
			Female (3)	300	9	19	0.063 ± 0.004	6.3 ± 0.5	**
			Total (6)	600	18	42	0.070 ± 0.011	7.0 ± 1.2	**
		18	Male (3)	300	5	12	0.040 ± 0.008	4.0 ± 0.8	*
			Female (3)	300	4	11	0.037 ± 0.005	3.7 ± 0.5	*
			Total (6)	600	9	23	0.038 ± 0.007	3.8 ± 0.7	**
		24	Male (3)	300	4	3	0.010 ± 0.000	1.0 ± 0.0	
			Female (3)	300	4	4	0.013 ± 0.005	1.3 ± 0.5	
			Total (6)	600	8	7	0.012 ± 0.004	1.2 ± 0.4	
Vehicle control (physiological saline)		12	Male (3)	300	4	3	0.010 ± 0.000	1.0 ± 0.0	
			Female (3)	300	1	2	0.007 ± 0.005	0.7 ± 0.5	
			Total (6)	600	5	5	0.008 ± 0.003	0.8 ± 0.4	

[a] Doses of 10^{-1} mmole/kg body weight for each chemical are as follows: $CHCl_3$, 12.0 mg/kg; $CHCl_2Br$, 16.3 mg/kg; $CHClBr_2$, 20.8 mg/kg; $CHBr_3$, 25.3 mg/kg.
[b] Figures in parentheses indicate the number of animals examined.
[c] Not including the cells with gaps. Values indicate the mean and standard deviation of the results from 3 or 6 rats.
* Significantly different from untreated control at $P < 0.05$.
** Significantly different from untreated control at $P < 0.01$.

In conclusion, positive results were obtained for chloroform in dose-dependent manner after intraperitoneal injection in rat bone marrow cells

- Chromosomal aberration test in bone marrow by oral administration:

Chloroform was administered by gastric intubation to male Long-Evans rats at doses of 1.2, 11.9 or 119.4 mg/kg bw/day with 24-h interval for 5 days. Potassium bromate (250.5 mg/kg or 1.5 mmole/kg) was administered orally as a positive control. Dose-response relationships were studied in cells sampled 18 h after the last day of treatment. For oral treatment, male rats were used because they showed a slightly higher sensitivity to the chemicals than female rats with i.p. treatment. A statistically and dose-related significant increase in the incidence of aberrant cells and of the number of aberration / cells was noted with 119.4 mg/kg chloroform (6%) compared to the untreated control (1%) (see Table 4.32). This study was conducted according to OCDE guideline 473, no major deviation was noted.

Table 4.32 Relationships between dose and THM-induced CA after oral treatment

Chemical	Dose [b] (mmole/ kg)	Time (h)	Sex [c]	Number of cells examined	Number of cells with		Number of aberrations/cell (mean ± SD) [d]	Incidence of aberrant cells (mean ± SD) [d]	χ^2-test	Trend test [e]
					gaps	breaks				
$CHCl_3$	10^{-2}	24 h×5+18 h	Male (3)	300	5	6	0.020±0.008	2.0±0.8 (%)		$P < 0.001$
	10^{-1}		Male (3)	300	6	10	0.033±0.004	3.3±0.5		
	1		Male (3)	300	7	18	0.060±0.008	6.0±0.8	**	
Positive control ($KBrO_3$)	1.5	24 h×5+18 h	Male (3)	525	16	41	0.078±0.018	7.8±1.8	**	
Vehicle control (physiological saline)		24 h×5+18 h	Male (3)	300	2	3	0.010±0.000	1.0±0.0		

The percentage of aberrant metaphase cells over time was determined 6, 12, 18 and 24 h after the last day of oral treatment with 119.4 mg/kg chloroform (see Table 4.33). A slight but statistically significant increase in the incidence of CA were observed at 12h and clearly confirmed at 18h.

Table 4.33 Variation of THM-induced CA at various times after oral treatment

Chemical	Dose [b] (mmole/ kg)	Time (h)	Sex [c]	Number of cells examined	Number of cells with		Number of aberrations/cell (mean ± SD) [d]	Incidence of aberrant cells (mean ± SD) [d]	χ^2-test
					gaps	breaks			
$CHCl_3$	1	24 h×5+ 6 h	Male (3)	300	14	10	0.033±0.004	3.3±0.5 (%)	
		24 h×5+12 h	Male (3)	300	9	11	0.037±0.005	3.7±0.5	*
		24 h×5+18 h	Male (3)	300	7	18	0.060±0.008	6.0±0.8	**
		24 h×5+24 h	Male (3)	300	6	3	0.010±0.000	1.0±0.0	
Vehicle control (physiological saline)		24 h×5+18 h	Male (3)	300	2	3	0.010±0.000	1.0±0.0	

[a] 1 mmole/kg body weight of each THM was administered orally (gastric intubation) 5 times at 24-h intervals. The rats were killed at various times after the last treatment.
[b] These figures indicate the amounts of each THM administered once daily. The total dose volumes were as follows: $CHCl_3$, 119.4×5 mg/kg; $CHCl_2Br$, 163.8×5 mg/kg; $CHClBr_2$, 208.3×5 mg/kg; $CHBr_3$, 253×5 mg/kg.
[c] Figures in parentheses indicate the number of animals examined.
[d] Not including the cells with gaps. Values indicate the mean and standard deviation of the results from 3 rats.
* Significantly different from untreated control at $P < 0.05$.
** Significantly different from untreated control at $P < 0.01$.

In conclusion, chloroform did not produced chromosomal rearrangements in any of the aberrant cells, the type of damage being largely limited to chromatid-type aberrations. The study shows a positive result at 119.4 mg/kg for 12 and 18h after last day of treatment.

Hoechst *et al.*, 1988.

- Chromosomal aberration assay.

Chloroform was evaluated for clastogenicity in Chinese Hamsters (5/sex/treatment group) exposed by oral gavage to single dose of 0 (solvent control), 40, 120, and 400 mg/kg bw with subsequent harvest, preparation and analysis of metaphase bone marrow cells (100 cells/animal) at 6 (high dose), 24 (all doses), and 48 (high dose) hours post-treatment.

Results are presented in Table 4.34. When male and female results are combined, the slight enhancement of chromosomal aberrations was statistically significant (Mann-Whitney-U-test) 6 and 24 hours after doses of 400 mg/kg, although the rate was still within the range of historical negative controls. In a second study, exposing groups of hamsters to doses of 0 (solvent control), 120, and 400 mg/kg bw, 24-hour cytogenetic assay again revealed a slight but statistically significant increase in chromosome aberrations in association with 400 mg/kg doses, failing again to demonstrate a dose-response relationship for rates of damage (chromosome breaks) beyond the range of historical controls. However, when the results are individually analysed for both sexes, no reproducible increase of chromosomal aberrations was observed.

The study authors noted an inference of chloroform mutagenicity, based on the nature of marked damage (multiple aberrations, chromosomal disintegration, and exchanges) associated with oral chloroform at doses of 120 and 400 mg/kg (6-, 24-, and 48-hour assessments). However, these "heavy" aberrations are not unusual (Engelhardt and Fleig, 1993) and were not regarded as treatment-related.

However, the authors concluded that chloroform can induce rare but heavy structural chromosome alterations as analysed in bone marrow cells of the Chinese hamster under the experimental conditions described in this report. Therefore a mutagenic potential of the test substance cannot be excluded.

Table 4.34

Dose mg/kg	Time (hours)	Aberration rate excluding gaps (%)
First experiment		
Negative control	24	1.3
Positive control (CPA, 30mg/kg)	24	9.7*
40	24	1.4
120	24	1.7
400	6 24 48	2.4* 1.6* 1.0
Second experiment		
Negative control	24	0.2
Positive control (CPA, 30mg/kg)	24	11.4*
120	24	0.6
400	24	0.9*

*Significantly different from control, $p<0.05$.

Micronucleus assays

Robbiano *et al.*, 1998:

- Oral micronuclei evaluation in kidney cells.

The frequency of micronucleated kidney cells was evaluated in rats exposed to 6 halogenated anaesthetics including Chloroform.

7 males Sprague-Dawley albinos rats per group were injected i.v with 250 mg/kg of folic acid to increase the proliferative activity of kidney cells induced by nephrectomy. Chloroform was dissolved in corn oil and administered as a single p.o. dose of 472 mg/kg bw/day in corn oil (which was half of the LD_{50} of chloroform) 2 days after folic acid injection. The dose was administered by gastric intubation in a volume of 0.01 ml/g. NDMA (20 mg/kg) was used as a positive control. Results are presented in Table 4.35.

Chloroform induced a statistically significant increase in the average frequency of micronucleated kidney cells. The mean frequency of micronucleated cells in rats was $1.33.10^{-3}$ for the negative control. The ratio treated/control being 3.32, and the ratio for positive control being 6.52.

This test was conducted according to OECD guideline 474 with the following deviations:
- The study was realized on kidney cells instead of erythrocytes but kidney is the target organ
- Only one concentration was tested: 472 mg / kg bw/day whereas according to OECD guideline 474, three doses are recommended.

Table 4.35 Frequency of micronucleated kidney cells in rats treated with chloroform.

Treatment conditions	N° of cells scored	Frequency ($x10^{-3}$) of micronucleated cells	Frequency ($x10^{-2}$) of binucleated cells
Control	37046	1.33 ± 0.41	1.91 ± 0.37
Chloroform 4 mmol/kg	15995	4.42 ± 1.16*	2.15 ± 0.55
NDMA 20mg/kg	9038	8.68 ± 2.69*	1.62 ± 0.61

*Significantly different from the control group at p< 0.001 as determined by the Wilcoxon's two sample (two tail test).

<u>Gocke *et al.*, 1981:</u>

- <u>Intraperitoneal mice bone-marrow micronucleus assay.</u>

This study consisted in a micronucleus assay in bone marrow cells in male and female NMRI mice treated with chloroform.

Male and female NMRI Mice were injected intraperitoneally with 0, 238, 476 and 952 mg/kg in olive oil at 0 and 24 h with a sacrifice at 30 h. Results are presented in Table 4.35. This study was conducted according to OCDE guideline 471, no deviation was noted.

Table 4.36 Results of the micronucleus test on mouse bone marrow.

Compound	Surviving / treated mice	Dose mg/kg	Route of application	Micronucleated PE (‰)
Chloroform	4/4	2 x 952	ip	2.2
	4/4	2 x 476	ip	2.6
	4/4	2 x 238	ip	2.2
	4/4	0	ip	1.2

Hydroquinone	8/8 8/8 4/4 4/4	2 x 110 2 x 55 2 x 22 0	ip ip ip ip	10.0** 3.5 1.4 1.1

** Significantly different from control, p<0.01.

No statistically significant dose-related increase in micronuclei formation was observed with chloroform.

Tsuchimoto & Matter, 1981:

- Intraperitoneal bone marrow micronucleus assay.

Activity of chloroform in the micronucleus test was assessed in male and female CD1 mice. Each group consisted of two males and two females.

Chloroform was administered i.p twice with 0, 0.015, 0.03 and 0.06 ml/kg (equivalent to 0, 22, 44 and 89 mg / kg bw/day) in DMSO, 24 h apart. The animals were killed 6 h after the second application. Femoral bone marrow cells were obtained and smears were prepared. The number of micronucleated polychromatic erythrocytes (MPE) were counted, but not the number of micronuclei per cell.

The data obtained were evaluated on the basis of the following criteria:

- Two or more mice per group with MPE frequencies above 0.40%
- One or more treated groups with mean MPE frequencies above 0.30%
- Statistical significance in one or more treated group.

This study was conducted according to OCDE guideline 471.

Results were presented in Table 4.37.

Table 4.37 Frequencies of micronucleated polychromatic erythrocytes.

Compound	Doses	Micronucleated polychromatic erythrocytes (%)
Chloroform	0 ml/kg 0.015 ml/kg 0.03 ml/kg 0.06 ml/kg	0.12 0.08 0.08 0.07
2-acetylaminofluorene	0 mg/kg 280 mg/kg 560 mg/kg 1120 ml/kg	0.08 0.70* 0.65* 0.45*

* Significantly different from control, p<0.05.

A test substance was judged positive when all three of these criteria were met. The mutagenic compound 2-acetylaminofluorene was considered as positive.

In the conditions of this study, the authors concluded that no micronucleus formation was observed whatever the concentration of chloroform tested.

Shelby & Witt 1995:

Tests for the induction of micronuclei (MN) in bone marrow cells of mice have been conducted on 65 chemicals including chloroform.

- Micronucleus assay in bone marrow cells by intraperitoneal route.

Groups of 5 or more male B6C3F1 mice were injected intraperitoneally (i.p.) chloroform at 200, 400, 600 and 800 mg/kg bw/day three times at 24 h intervals with the test chemical dissolved in corn oil (CO) in two independent trials. The total dosing volume per mouse was 0.4 ml (chloroform or solvent control). A concurrent positive control group (including benzene, acrylamide and phenol) of mice was included in each of the micronucleus tests (data not presented). Twenty-four hours after the final injection, smears of the bone marrow cells from femurs were prepared and 2000 polychromatic erythrocytes (PCE) were scored per animal for frequency of micronucleated cells. The percentage of PCE among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of toxicity (see Table 4.38). This study was conducted according to OCDE guideline 474, no major deviation was noted.

Table 4.38 Percentage of PCE among the total erythrocyte population

Chloroform (CAS No. 67-66-3) (MN+/ABS−)

Test[a] (solvent)	Tissue	Trend P value	Dose (mg/kg)	MN-PCE/1,000	Survival (No. scored)
Micronucleus (CO)	BM	0.011*	0	2.40 ± 0.45	10/10
			200	3.00 ± 0.39	10/10
			400	3.50 ± 0.72	10/10
			800	4.20 ± 0.47	10/10
		0.001*	0	2.10 ± 0.29	5/5
			400	4.00 ± 0.72*	5/5
			600	4.75 ± 1.20*	4/5

One trial gave a non statistically significant increase in MN but with a dose-response trend and the second trial gave a statistically significant dose-related increase in MN, although the highest effects observed were only about 2 times control value. The results of this study were considered as positive.

Salamone *et al.*, 1981:

- Intraperitoneal bone marrow micronucleus assay.

This study consisted in micronucleus assay in bone marrow cells in B6C3F1 mice treated with chloroform.

B6C3F1 mice were injected intraperitoneally with 80% of the LD50 of chloroform (exact dose not specified) as follow:

- P1: 2 treatments with 80% of LD50 at 0 and 24 h, sampling times: 48, 72 and 96 h.
- P2: 1 treatment with 80% of LD50, sampling times 36,48, 60 and 72 h.
- CT: 1 treatment with 80% of LD50, sampling time: 60h.

Results were presented in Table 4.39. Micronuclei formation was observed at 60 h for chloroform with a concentration of 80 % of LD50. 2-acetylaminofluorene, known to be a mutagenic compound, was used as positive control. This study was conducted according to OECD guideline 471 with minor deviations:

- Only one concentration was tested for chloroform.
- This concentration was described as 80% LD50 but numerical data was not indicated.
- 500 PCE were counted per mouse instead of 1000.

Table 4.39 Number of micronuclei/500 PCE for a single mouse for each compound. Statistically significant positive groups are underscored.

Chemical	Phase P1, P2 or CT	Dose % LD_{50}	N° of treatments	Sampling time					
				30	36	48	60	72	96
Chloroform	P1	80	2			0,0,0,0		0,1,0,0	0,1
	P2	80	1		0,0,0		2,3	0,2	
	CT	80	1				0,0,1,1,1,1		
2-acetylaminofluorene	P2	50	1	0,2		1,0,1	5,2,11		
		50	1				0,0,0,0,1,2,3 3,4,6,8		
	CT	25	1				0,1,2,2,4		
		12.5	1				0,1,1,2,4		

In conclusion, as only 2 animals presented micronuclei formation in first experiment, which was not confirmed in the second trial. The results of this study were considered as negative.

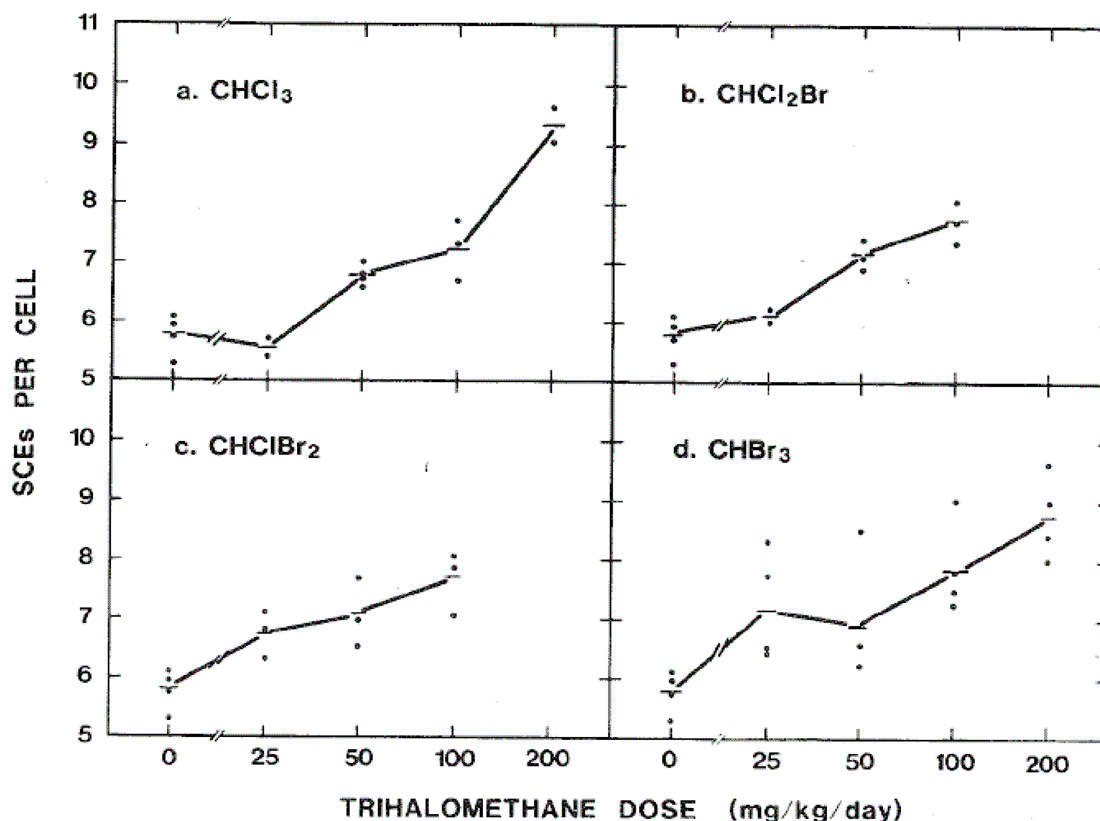
Primary DNA damage assays

Morimoto & Koizumi, 1983:

- Sister chromatide exchange (SCEs).

Trihalomethanes (THMs) including chloroform have been investigated for their ability to induce sister chromatid exchanges (SCEs) in mouse bone marrow cells in vivo.

Chloroform, dissolved in olive oil, was administered orally to male ICR/SJ mice (0, 25, 50, 100, 200 mg/kg /day) once a day for 4 days (see Figure 4.9). In bone marrow cells, an increase in SCE frequencies was observed from 50 mg/kg with a significant increase in the SCE frequency ($P< 0.05$). Administration of 200 mg/kg of chloroform led to an increase of about 3 SCEs per cell above the control value.



The frequencies of SCEs in bone marrow cells from mice orally ingesting each of the trihalomethanes for 4 days. Each point represents the mean SCE frequency of 25 second-division cells from each animal. The bar indicates the average of the mean SCE frequencies in each dose group.

Figure 4.9 SCE frequencies in mouse bone marrow cells

The authors suggest that the formation of SCE after chloroform exposure could be due to the formation of phosgene described as the major toxicologically relevant metabolite of chloroform (Gemma et al., 2003; Golden et al., 1997; Pohl and Krishna, 1978). Indeed, chloroform is known to be metabolically converted into trichloromethanol Cl3OH and then converted into phosgene COCl2, by mixed-function oxidases (MFOs). Phosgene is thus believed to be an active metabolite that might be responsible for the toxicity of chloroform.

Pereira *et al.*, 1982 :

- DNA binding.

Trihalomethanes as initiators and promotors of carcinogenesis were evaluated in this study. The authors attempted to determine whether chloroform increases the incidence of cancer in the NCI bioassay by genetic, epigenetic or both mechanisms. The authors evaluated namely the DNA binding of chloroform.

Male Sprague-Dawley rats and female B6C3/F1 mice were administered intragastrically 14C-chloroform (47.2 mg / kg bw for rats and 118 mg/kg bw for mice) dissolved in corn oil. The animals were sacrificed by cervical dislocation 16-18 hr later.

In rat liver and kidney, a definite peak of radioactivity representating chloroform was found associated with the ultraviolet-absorbing peak containing the DNA, whereas no association was found for chloroform in mouse liver.

Chloroform was demonstrated to bind rat liver and kidney DNA but there was no evidence for binding to mouse liver DNA within the sensitivity of the assay. The binding index of chloroform to rat liver and kidney DNA was 0.017 and 0.0055, respectively, which represents 0.05-0.15% the binding index for DMN (11.4) used as positive control.

The low level of DNA binding by chloroform indicated that the contribution of the genetic or initiating component of the carcinogenicity of the chloroform was much less than the genetic component of DMN.

Diaz-Gomez and Castro, 1980:

- Binding to DNA, RNA or nuclear proteins.

This work aims to find evidence of covalent binding of chloroform or its metabolites to rat or mouse liver DNA, RNA or nuclear proteins.

Male strain A/J mice or Sprague-Dawley male rats were injected i.p with [14C]CHCl3 22.72µCi/ml (spec. act. 5.4 Ci/mol) (estimated to 4.96 mg/kg bw/ day) and toxic dose (spec. act. 13.15 µCi/mmol, conc 10% in olive oil) (estimated to 730 mg/kg/day). Mice were sacrificed 6h after the last chloroform injection and their liver processed for DNA or RNA isolation, purification and counting. Results are presented in Table 4.40 for covalent binding to mouse liver DNA or RNA.

Table 4.40 Studies on possible covalent binding of 14C from [14C]CHCl3 to mouse liver DNA or RNA.

Experimental conditions	^{14}C from [^{14}C]$CHCl_3$ in dpm/mg	
	DNA	RNA
Control	12 ± 3	11 ± 3
Phenobarbital	8 ± 2	20 ± 6
3-Methylchloanthrene	13 ± 3	15 ± 4
730 mg/kg 1 admin.	16 ± 4	15 ± 4
730 mg/kg x 4 days	6 ± 2	9 ± 3
730 mg/kg x 2 weeks	3 ± 1	8 ± 3

Under the experimental conditions, results failed to detected any significant covalent binding of CHCl3 or its reactive metabolites to DNA or RNA in mouse liver. However, positive controls (phenobarbital and 3-methylcholanthrene) did not showed high DNA or RNA binding.

Rats were sacrificed 6h after the last chloroform injection and their liver processed for separation of nuclear protein fraction. Details of protocol were not described in the study.

14C from [14C]CHCl3 was detected in all fractions of nuclear protein analysed. The authors concluded that nuclear protein covalently binds 14C from 14CHCl3 and that all the fractions isolated (acidic, histone, deoxyribonucleo-protein and residual) participated in the interaction.

Reitz *et al*., 1982:

- DNA binding/DNA repair *in vivo* assay.

The potential of chloroform to induce genetic damage and/or organ toxicity at the site where tumors have been observed (liver and kidney) in the various bioassays was evaluated in male B6C3F1 mice and male Sprague-Dawley rats.

To evaluate DNA binding, male mice (B6C3F1 strains) were exposed to 14C-chloroform (240 mg/kg bw, Per Os).

The capacity of 14C-chloroform binding to DNA isolated from the liver and kidneys of B6C3F1 mice was represented by a Chemical Binding Index (CBI) of 1.5 µmol/mol DNA. This CBI was slightly increased with chloroform administration when compared to chemical compounds which strongly bind to DNA such as aflatoxine (CBI=17,000 µmole/DNA) or dimethylnitrosamine (CBI=6,000 µmole/mole DNA).

DNA repair was estimated by administering non-radioactive chloroform to animals and subsequently determining the rate of incorporation of 3H-thymidine into DNA in animals receiving doses of hydroxyurea sufficient to depress normal DNA synthesis. Details of this procedure were not described in the study. Results are presented in Figure 4.10.

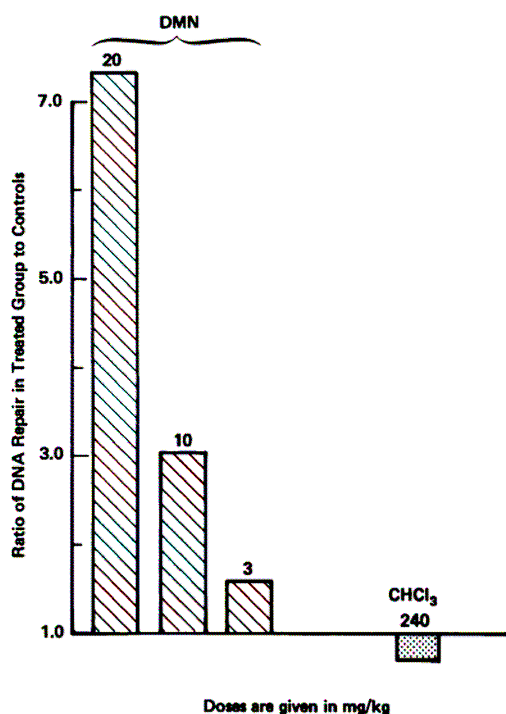


FIGURE 1. DNA repair in the liver of mice treated with dimethylnitrosamine (DMN) or chloroform ($CHCl_3$) relative to control groups.

Figure 4.10 DNA repair in the liver of mice treated with dimethylnitrosamine (DMN) or chloroform (CHCl3) relative to control group.

Intraperitoneal administration of dimethylnitrosamine (DMN) cause a large increases in DNA repair in the liver of B6C3F1 mice, but chloroform was inactive in this system. Thus these data fail to indicate any significant repair of DNA (estimated as hydroxyurea-resistant incorporation of 3H-thymidine into DNA) for orally administered chloroform.

Potter *et al.*, 1996:

- Induction of DNA strand breaks.

Effects of four trihalomethanes including chloroform on DNA strand breaks in kidneys were evaluated in male F-344 rats by an alkaline unwinding procedure.

Male F-344 rats were administered chloroform daily by oral gavage equimolar doses (0.75 or 1.5 mmole / kg body weight equivalent to 88.5 mg / kg bw or 177 mg / kg bw respectively) in vegetable oil for 7 days. Induction of DNA strand break was evaluated by the fraction of double stranded DNA. The decrease of this fraction suggests the induction of DNA strand break as observed for positive controls diethylnitrosamine and dimethylnitrosamine.

Results are presented in Table 4.41.

Table 4.41 DNA strand break induction by THMs.

Treatment	Fraction of double stranded DNA remaining after 45 min unwinding
Vehicle control	0.83 ± 0.02
Chloroform	0.87 ± 0.01
Diethylnitrosamine	0.79 ± 0.003*
Dimethylnitrosamine	0.55 ± 0.02*

* Significantly different from control, $p<0.05$.

The fraction of double stranded DNA for chloroform was equivalent to fraction observed for negative control which suggest that chloroform did not induce DNA strand breaks in rat kidneys.

Mirsalis *et al*., 1982:

- UDS assay.

Unscheduled DNA synthesis (UDS) was evaluated in hepatocytes of male Fischer 344 rats orally administered with a single dose of 0, 40 or 400 mg/kg of chloroform. Rats were treated at 0h and sacrificed at 2 and/or 12h. This study was conducted according to OECD guideline 486 without major deviations; except that the cells were stained with solution of methyl-green Pyronin Y. Results were presented in Table 4.42.

Table 4.42 Induction of UDS by chemicals in the in vivo – in vitro hepatocyte DNA repair assay.

Chemical	Dose mg/kg	Sacrifice Time (h)	Number of treated animals	NG ± SE
Corn oil		2	7	-5.1 ± 0.5
		12	13	-4.4 ± 0.5
DMN	10	2	4	55.8 ± 3.3
CCl_3	40	2	3	-4.1 ± 0.4
	400	2	3	-4.4 ± 0.8
	400	12	3	-2.7 ± 0.3

Net Grain (NG) formation was not observed in chloroform treated cells by comparison to negative control. Positive control (DMN) leads to a significant increase in Net Grain formation.

Cell proliferation

Larson e*t al*., 1994:

- Regenerative cell proliferation in livers and kidneys.

This study was designed to determine the dose-relationships for chloroform-induced cell proliferation in the male F-344 rat kidney and liver. The labeling index (LI) was evaluated as the percentage of S-phase cells in livers and kidneys of male F-344 rats given chloroform by gavage or in drinking water.

In the gavage study: (i) in kidney, an increase of labelling index was observed only with 180 mg/kg bw/day at 4 days; (ii) in liver, an increase of labelling index was detected from 90 mg/kg bw/day at 4 days and with 180 mg/kg bw/day after 3 weeks of treatment.

In the drinking water study, chloroform exposure caused no increase in LI in any region of the kidney at any exposure either at 4 days or 3 weeks. The range of exposure in drinking water was lesser (0-90 mg/kg bw/ day) than exposure by gavage.

The authors concluded that dose-dependent increases in cell proliferation were associated with the mild hepatotoxic effects of chloroform administered in corn oil.

This study described the regenerative cell proliferation in liver and kidney of rats and the relevance of the results presented in this study to evaluate the mutagenicity of chloroform is unclear.

Table 4.43 Summary of keystudies

Species	End Point	Doses	Exposure	Vehicle	Route of administration	Results	Reliability	Guideline Deviations	References
Micronucleus assay									
Sprague Dawley rat	MN Kidney	472 mg / kg bw / d	Single dose	Corn oil	Oral	+ 472 mg /kg bw/d	2	OCDE 471 Rat kidney cells instead of erythrocytes	Robbiano *et al.*, 1998
Mice	MN Bone marrow	0; 238; 476; 952 mg / kg bw	Treatment at 0 and 24 h	Olive oil	i.p	-	2	OCDE 471	Gocke *et al.*, 1981
Male and female mice	MN Bone marrow	0; 22; 44; 89 mg / kg bw	2 treatments at 24 h sacrifice 6 h after the final injection	DMSO	i.p	-	2	OCDE 471 Route of administration was not adequate	Tsuchimoto and Matter, 1981
B6C3F1 mice	MN Bone marrow	200, 400, 800 mg / kg bw	3 daily inject	Corn oil	i.p	+	2	OCDE 474 No deviation	Shelby and Witt 1995
B6C3F1 mice	MN Bone marrow	80% of LD_{50}	½ daily doses	DMSO	i.p	+/- 60 h	2	Only one concentration was tested (80% LD_{50}) 500 PCE counted per mouse	Salamone *et al.*, 1981
Chromosomal aberration									
B6C3F1 mice	CA Bone marrow	200, 400, 800 mg / kg bw	single injection	Corn oil	i.p	-	2	OCDE 475 no major deviation	Shelby and Witt 1995
Long Evans rat	CA Bone marrow	1.2, 11.9 and 119.4 mg / kg bw	5 days	Distilled water	Oral	+ 119 mg / kg	2	OCDE 475 no deviation	Fujie *et al.*, 1990

Species	End Point	Doses	Exposure	Vehicle	Route of administration	Results	Reliability	Guideline Deviations	References
Long Evans rat	CA Bone marrow	1.2, 11.9 and 119.4 mg / kg bw	Treatment at 0h, sacrifice at 6, 12, 18 or 24 h	Distilled water	i.p	+ 1.2mg / kg	2	OCDE 475 no deviation	Fujie *et al*., 1990
Male and female hamsters	CA Bone marrow	0; 40; 120; 400 mg / kg bw	6, 24, 48 h	Paraffin oil	Oral	+/- 400 mg / kg bw	1	OCDE 475 No deviation	Hoechst *et al*, 1988 Not published
Sister chromatide exchange –									
ICR/SJ mice	SCE Bone marrow	25, 50, 100, 200 mg / kg bw	4 days	Olive oil	Oral	+ ≥ 50 mg /kg bw / d	2	OCDE 479 No deviation	Morimoto and Koizumi 1982
Mutations									
B6C3F1 mice	Mutation Liver	0; 50; 166; 500 mg / kg bw	6h / 7 days Sacrifice at 24 after treatment	Unspecified	Inhalation	-	2	No guideline	Butterworth *et al*., 1998
DNA damage – DNA binding									
Sprague Dawley rat	DNA binding Liver, kidney	47.2 mg / kg bw /d	Single dose	Corn oil	Oral	+/- 47.2 mg /kg bw/d	2	No Guideline	Pereira *et al*., 1982
B6C3F1 mice	DNA binding Liver, kidney	118 mg / kg bw / d	Single dose	Corn oil	Oral	-	2	No Guideline	Pereira *et al*., 1982
B6C3F1 mice	DNA binding Liver, kidney	240 mg / kg bw / d	Single dose	Unspecified	Oral	+/- 240 mg / kg bw / d	2	No Guideline	Reitz *et al*., 1982
B6C3F1 mice	DNA repair Liver, kidney	240 mg / kg bw / d	Single dose	Unspecified	Oral	-	2	No Guideline	Reitz *et al*., 1982
F-344 rats	DNA strand break Kidney	88.5 ; 177 mg /kg bw /d	7 days	Vegetable oil	Gavage	-	2	No guideline	Potter *et al*., 1996

Species	End Point	Doses	Exposure	Vehicle	Route of administration	Results	Reliability	Guideline Deviations	References
Male F-344 rats	UDS DNA repair Liver	0; 40; 400 mg / kg bw /d	Single dose	Corn oil	Gavage	-	2	OCDE 486 No deviation	Mirsalis *et al.*, 1982
Male A/J mice	DNA binding Liver	Up to toxic dose	Single or once daily for 4 days or twice a week for 2 weeks	Olive oil	i.p	-	2	No guideline	Diaz-Gomez and Castro, 1980

30 in vivo studies are available on chloroform, 16 studies were described in this paper and summarized in the above Table 4.43. Vogel and Nivard, (1993); Gocke et al., (1981), Vogel et al., (1981) were not described because these studies were realized in Drosophila Melanogaster. Le Curieux et al., (1995); Fernandez et al., (1993) described study conducted in Larvae of pleurodeles, these studies were not taken in account.

The other studies have not been retained because of their weak reliability (3 or 4), these studies are summarized in Table 4.44 in order to be exhaustive.

Table 4.44 Summary of non reliable studies conducted in rats or mice.

Species	End Point	Doses	Exposure	Vehicle	Route of administration	Results	Reliability	Guideline	References
Lacca mice	Chromosomal aberration	0, 100, 200 mg/kg	Treatment at 0h, sacrifice at 6, 12 and 24 h at 100 mg/kg	ND	s.c	+	3	No	Sharma and Anand, 1984
Albino mice	Micronucleus in bone marrow cells	0, 100, 200, 400, 600, 700, 800, 900 mg/kg	No data	ND	No data	+	3	No	San Augustin and Lim-Sylianco, 1978
Male F-344 rats	Micronucleus in hepatocytes	0, 100, 200, 400 mg/kg	No data	ND	i.p	+	4	No	Sasaki *et al.*, 1998
ICR mice	Sister chromatid exchange	0, 1665 mg / kg bw /day	Up to 6 h	ND	inhalation	+	4	No	Iijima *et al.*, 1982

Species	End Point	Doses	Exposure	Vehicle	Route of administration	Results	Reliability	Guideline	References
Male Wistar rats and Balb/c mice	Binding to DNA, RNA and proteins	500 µci/ kg bw	Treatment at 0h sacrifice at 22h	ND	i.p	+	3	No	Colacci *et al*., 1991

Summary of Data

In vitro, positive results appear sporadically and are outnumbered by negative results in other tests in the same system.

In vivo, studies conducted to evaluate DNA binding suggest that chloroform or its metabolites does not bind or slightly bind to DNA (Pereira et al., 1982; Reitz et al., 1982; Butterworth et al., 1998; Mirsalis et al., 1982; Diaz-Gomez and Castro, 1980; Rosenthal et al., 1987).

Chloroform is able to induce micronucleus formation or chromosomal aberrations when the compound was orally administered in rats and mice (Robbiano et al., 1998; Morimoto and Koizumi, 1983; Fujie et al., 1991) but not in hamster (Hoechst et al., 1988). By i.p route, chromosomal aberrations were induced in rats (Fujie et al., 1990). In mice, no effect was induced in studies at low dose (Tsuchimoto and Matter, 1981) or with single administration (Shelby and Witt, 1995; Gocke et al., 1981) but a positive effect was seen after repeated administration of high doses in Shelby and Witt (1995). The increase for micronucleus formation was about 3.3 fold and 50 % of positive control in Robbiano et al., (1998) and about 1.75 fold in Shelby and Witt, (1995), no information is available on positive control. The increase of micronucleus formation after treatment with chloroform was between 1.75 and 3.32 fold when compare to negative control.

The chromosomal aberration formation was increased about 6 and 8.5 fold in Fujie et al., (1990) by oral and intraperitoneal route, respectively.

No DNA strand breaks were observed in F-344 rats treated with 88.5 or 177 mg / kg bw during 7 days (Potter et al., 1996).

Metabolism of chloroform

Chloroform can undergo both oxidative and reductive metabolism in the human liver (Figure 4.11), depending on oxygen and substrate concentration. The required step for CHCl3-induced toxicity is the cytochrome P450 (P450)-mediated bioactivation to reactive metabolites. Extensive in vitro and in vivo studies on rodents have demonstrated that chloroform may be metabolized oxidatively to trichloromethanol, which spontaneously decomposes to the electrophilic phosgene (COCl2). COCl2 is highly reactive and binds covalently to cell components containing nucleophilic groups, including proteins, phospholipid's polar heads, and reduce gluthatione (Gemma et al., 2003).

At low levels, reflecting human exposure through the use of chlorinated waters, CHCl3 is metabolized primarily to phosgene by CYP2E1. When the CYP2E1-mediated reaction is saturated the predominant role in phosgene production is for CYP2A6, efficient even in highly hypoxic conditions (1% pO2). Phosgene is the major toxicologically relevant metabolite produced by the human liver (Gemma et al., 2003; Golden et al., 1997).

At high concentrations, chloroform is believed to increase the half-life of phosgene with the electrophilic chlorine atoms of chloroform. The stabilisation could prevent a direct reaction with water and allow phosgene to reach more reactive compounds (Potts et al., 1949) such as glutathione and other critical cell components.

Moreover, the reductive metabolism of chloroform produces CHCl2 which is highly reactive and then could lead to lipid peroxidation. The lipid peroxidation could also contribute to radical peroxide formation.

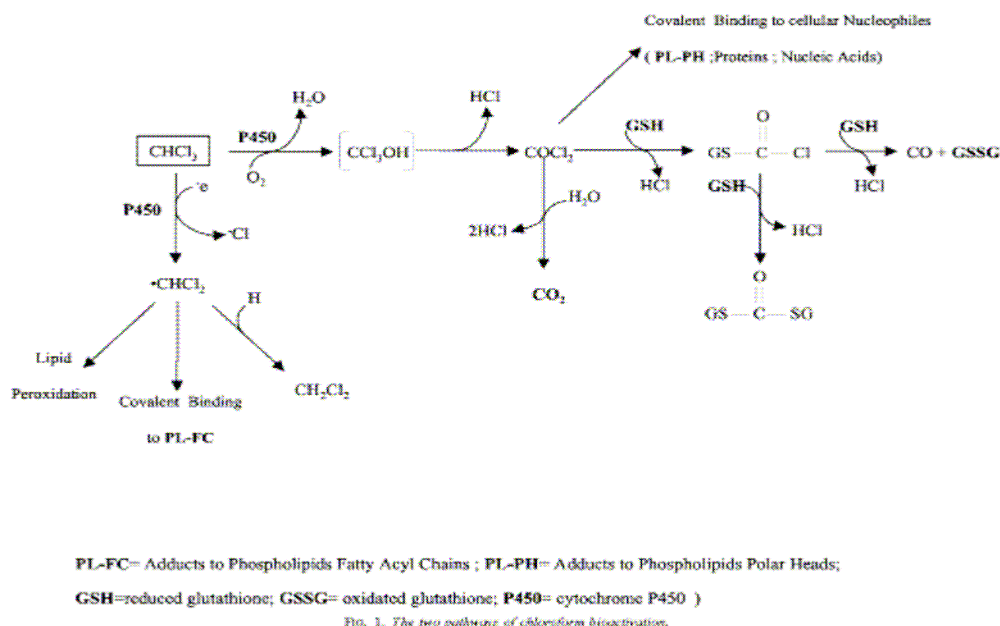


Figure 4.11 The two pathways of chloroform bioactivation.

Glutathione.

Acute chloroform toxicity is associated with glutathione depletion (Brown et al., 1974; Steven and Anders, 1981), and it has been reported that glutathione levels decrease in a dose dependent manner prior to microscopic evidence of liver pathology (Brown et al., 1974; Docks and Krishna, 1976).

Ammann et al., (1998) demonstrated that chloroform as well as phosgene induce a moderate glutathione (GSH) depletion, (Sciuto et al., 2004; Jaskot et al., 1991). GSH is produced by cells for its antioxidant properties but this function could be saturated. The decrease of GSH levels by chloroform and / or phosgene will decrease protective levels of GSH. This could increase oxidative stress and probably reactive oxygen species production. These free radicals generation could bind to DNA and contribute to genotoxicity at high or repeated dose.

Role of vehicle

The results of some animal studies have suggested that the vehicle used to administrate chloroform may affect the toxicity (EPA report 2001). Indeed, Larson et al., (1994) indicated that dose-related increases renal damage were observed in male rat F-344 administered with chloroform in corn oil and not with chloroform in drinking water. However, the range of exposure in drinking water (0-90 mg / kg bw/ day) was lower than the exposure in corn oil (0-180 mg / kg bw / day). However, from the results presented in this report, this hypothesis was not confirmed. Indeed, Fujie et al., (1990) observed chromosomal aberration when chloroform was administered in distilled water whereas, Pereira et al., (1982), Potter et al., (1996), Gocke

et al., 1981 and Mirsalis et al., (1982) presented negative results while chloroform was administered in oil.

Role of phosgene

ILSI (1997) noted that phosgene is highly reactive and might be expected to have the capacity to interact directly with DNA, but that phosgene has not been tested in any standard mutagenicity test system. The committee also noted that, because of its high reactivity, phosgene formed in the cytosol following chloroform metabolism would likely react with cellular components prior to reaching the cell nucleus, and concluded that direct effects on DNA would be unlikely. However, it is contradictory with a recent finding of Fabrizi et al., (2003) which demonstrated that phosgene is able to reach cell nucleus, since phosgene can react with the N-terminus of human histone H2B, especially with proline and serine residues. Histone H2B is one of the 5 main histone proteins involved in the structure of chromatin in eukaryotic cells. Representated by a main globular domain and a long N terminal tail H2B is involved with the structure of the nucleosomes of the 'beads on a string' structure. Histone plays a role in chromatine folding, stabilization of DNA and double DNA strand breaks repair. Moreover, Diaz-Gomez et al., (1980) demonstrated that chloroform or its metabolites is able to bind to nuclear protein such as histone.

Mechanistic hypothesis

The data presented herein indicate that chloroform does not bind to DNA. Previously studies (Brown et al., 1974; Gopinath and Ford, 1975; Constant et al., 1999; Pohl and Krishna, 1978) and results presented in this report support the conclusion that metabolism of chloroform is required for toxicity (CYP P450 (1)).

Data indicates that chloroform as well as phosgene induce glutathione (GSH) depletion (2) which could contribute to oxidative stress (3). Moreover, it was shown by Fabrizi et al., (2003) that phosgene could react with Histone H2B (4) which could lead to disturbance of DNA repair. These results are summarized in Figure 4.12.

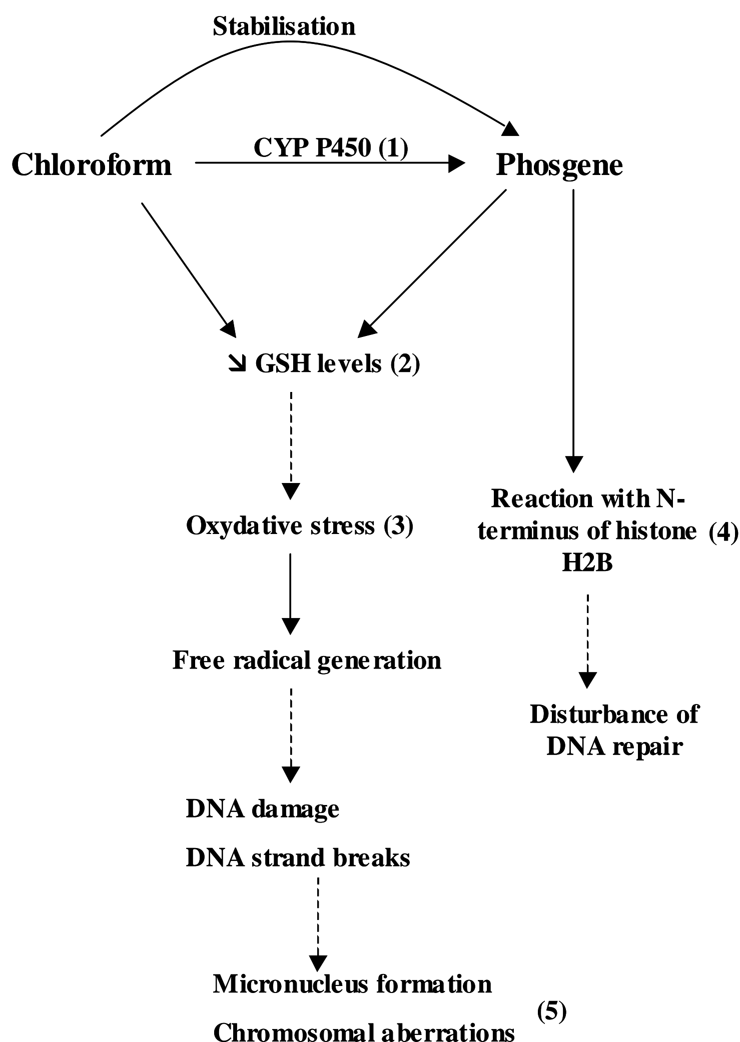


Figure 4.12 Hypothesis for micronucleus formation and chromosomal aberration after exposure to chloroform

4.1.2.7.3 Summary of mutagenicity

Reviews by other groups:

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:

The International Life Sciences Institute (ILSI, 1997) performed a review of the available data on the mutagenicity of chloroform. ILIS committee concluded that no subset of observations points unequivocally to a specific genotoxic mode of action associated with chloroform, and that the preponderance of the evidence indicates that chloroform is not strongly mutagenic. The conclusion of IARC study on carcinogenic chemicals (1999) is that no data were available on the genetic and related effects of chloroform in humans. There is weak evidence for the genotoxicity of chloroform in experimental systems in vivo and in mammalian cells, fungi and yeast in vitro. It was not mutagenic to bacteria.

US EPA (2001) concluded that the weight of evidence indicates that even though a role for mutagenicity cannot be excluded with certainty, chloroform is not a strong mutagen and that neither chloroform nor its metabolites readily bind to DNA.

CICAD (2004) based on Environment Canada (2001) source document, concluded that most studies did not identify genotoxic potential for chloroform. Results from a few, non-standard studies indicate the possibility of a weak positive response in rats. Overall, however, the weight of evidence indicates that chloroform does not have significant genotoxic potential.

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.

These studies suggest that chloroform is a slightly genotoxic compound in vivo and requires the classification as mutagenic compound category 3.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

In vivo studies

Inhalation

Yamamoto et al. (2002) conducted a carcinogenicity study in BDF1 mice and F344 rats (50 animals/sex/dose). Inhalation exposure concentrations to chloroform were 5, 30 or 90 ppm for mice and 10, 30 or 90 ppm for rats, 6h/day, 5days/week, for 104 weeks. Due to the acute lethality of the 30 and 90 ppm concentrations in mice, an adaptation period with lower doses was performed. Mice in the 30 and 90 ppm groups were first exposed to 5 ppm for two weeks then 10 ppm for two weeks (then 30 ppm for two weeks in the 90 ppm group) before the 30 and 90 ppm concentrations were maintained. Statistically significant increases in the incidence of overall renal cell adenomas and carcinomas were observed in the male mice exposed to 30 and 90 ppm (see table below; control, 0/50; 5 ppm, 1/50; 30 ppm, 7/50; 90 ppm, 12/48). The incidence rates of renal cell carcinoma were statistically increased in male mice in the 90 ppm group when compared with controls (control, 0/50; 90 ppm, 11/48). There were no statistically significant changes in tumor incidence for female mice or for rats of either sex in any exposure group. Nasal lesions including thickening of the bone and atrophy and respiratory metaplasia of the olfactory epithelium were observed for rats of both sexes and female mice exposed to 5 ppm and above. The NOAEC for the kidney adenoma/carcinoma was identified at 5 ppm in mice, for nasal lesions a LOAEC of 5 ppm was determined. (**Considered as key study for risk characterisation).**

Table 4.45 Incidences of neoplastic lesions in the mice and rats exposed to chloroform vapor at different concentrations for 104 weeks (Yamamoto et al., 2002)

(A) Mice

Group	Male				Peto	Female				Peto
	Control	5 ppm	30 ppm	90 ppm		Control	5 ppm	30 ppm	90 ppm	
Number of animals examined	50	50	50	48		50	49	50	48	
Liver										
Hepatocellular adenoma	5	7	6	8		1	1	4	3	
Hepatocellular carcinoma	10	0**	7	10	↑	1	1	0	3	↑
Hepatocellular adenoma + carcinoma	14	7	12	17	↑	2	2	4	6	↑↑
Hemangioma	0	0	1	0		0	0	0	0	
Hemangiosarcoma	3	0	2	1		2	0	0	1	
Histiocytic sarcoma	2	0	0	0		0	0	1	0	
Kidneys										
Renal cell adenoma	0	0	3	1		0	0	0	0	
Renal cell carcinoma	0	1	4	11**	↑↑	0	0	0	0	
Renal cell adenoma + carcinoma	0	1	7*	12**	↑↑	0	0	0	0	

(B) Rats

Group	Male				Peto	Female				Peto
	Control	10 ppm	30 ppm	90 ppm		Control	10 ppm	30 ppm	90 ppm	
Number of animals examined	50	50	50	50		50	50	50	49	
Liver										
Hepatocellular adenoma	0	0	0	0		1	0	2	1	
Kidneys										
Renal cell adenoma	0	0	0	0		0	0	0	1	
Pituitary gland										
Adenoma	22	23	21	17		24	20	18	11*	

*: P ≤ 0.05, **: P ≤0.01 Fisher Exact Test, ↑: P ≤ 0.05, ↑↑: P ≤ 0.01 Peto's Test

As part of a combined inhalation and oral carcinogenicity study (Nagano et al., 2006), groups of 50 male F344 rats were exposed by inhalation to 0 (clean air), 25, 50, or 100 ppm (v/v) of chloroform vapour-containing air for 6 h/d and 5 d/wk during a 104 weeks period. There were no statistically significant changes in kidney tumor incidence in any exposure groups.

Dermal

No data available

Oral

The carcinogenic potential of chloroform was evaluated by NCI (1976 in IARC, 1999) in Osborne-Mendel rats and B6C3F1 mice via oral gavage for 78 weeks. Administered chloroform concentrations in corn oil were 90 or 180 mg/kg bw/d (male), 100 or 200 mg/kg bw/d (female) for rats and 138 or 277 mg/kg bw/d (male), 238 or 477 mg/kg bw/d (female) for mice. In rats, a statistically significant increase (24%) in the incidence of kidney epithelial tumors was observed in males in the high-dose group when compared with males in the control group (control, 0/99; matched controls, 0/19; low-dose, 4/50; high-dose, 12/50). In mice, the incidence of hepatocellular carcinomas was significantly increased in males and females in both the low- and high-dose groups when compared to controls (male control, 5/77; matched controls, 1/18; 138mg/kg bw/d, 18/50; 277mg/kg bw/d, 44/45; female control, 1/80; matched controls, 0/20; 238mg/kg bw/d, 36/45; 477mg/kg bw/d, 39/41). Many of the

male mice in the low-dose group that did not develop hepatocellular carcinoma had nodular hyperplasia of the liver. The incidence of thyroid tumors was increased by treatment in the female rats, however this increase was not statistically significant.

Roe et al. (1979) reported three experiments in different mouse strains and genders, 10-week-old mice were administered chloroform by gavage 6d/week for 80 weeks. There were no statistically significant differences in survival, body weight, or food consumption between chloroform-treated and control groups in any of the experiments. A slight increase in moderate to severe fatty degeneration of the liver was seen and kidney tumors (adenomas and carcinomas) were statistically higher in high-dose male ICI mice (60 mg/kg/day), than in controls. Treatment with chloroform was associated with increased incidence of moderate to severe kidney lesions in CBA and CF/1 mice. (**Considered as key study for risk characterisation).**

Table 4.46 Incidence of renal tubule adenomas and carcinomas in ICI mice exposed orally to chloroform (Roe et al., 1979 in IARC, 1999)

Treatment	Sex	Incidence of renal tumors
First Study		
Vehicle Control (toothpaste)	Male	0/72
17 mg/kg bw/day $CHCl_3$		0/37
60 mg/kg bw/day $CHCl_3$		8/38
Vehicle Control (toothpaste)	Female	0/59
17 mg/kg bw/day $CHCl_3$		0/35
60 mg/kg bw/day $CHCl_3$		0/38
Second study		
Control	Male	1/48
Vehicle control (toothpaste)		6/237
60 mg/kg bw/day $CHCl_3$		9/49
Third Study		
Control	Male	0/83
Vehicle control (toothpaste)		1/49
Vehicle control (arachis oil)		1/50
60 mg/kg bw/day (toothpaste) $CHCl_3$		5/47
60 mg/kg bw/day (arachis oil) $CHCl_3$		12/48

Jorgenson et al. (1985) exposed male Osborne-Mendel rats and female B6C3F1 mice to chloroform in drinking water for 104 weeks. The time-weighted average doses, based on measured water intake and body weights, were 0, 19, 38, 81, or 160 mg/kg/day for rats and 0, 34, 65, 130, or 263 mg/kg/day for mice. A statistically significant dose-related increase in the incidence of kidney tumors (tubular cell adenomas and adenocarcinomas) was observed in male rats in the high-dose group (control, 2% [5/301]; matched controls, 2% [1/50]; 19mg/kg/d, 2% [6/313]; 38mg/kg/d, 5% [7/148]; 81mg/kg/d, 6% [3/48]; 160mg/kg/d, 14% [7/50]). Chloroform in the drinking water did not increase the incidence of hepatocellular carcinomas in female B6C3F1 mice. The combined incidence of hepatocellular adenomas and carcinomas was 2% in the high-dose group compared with 6% in the control groups. The authors speculated that the differences observed between this study and the NCI (1976) bioassay may be related to differences in the mode of administration (in drinking water versus in corn oil by gavage). (Jorgenson et al., 1985 as cited in US EPA, 2001)

Kidney tissue from a carcinogenicity bioassay of chloroform in Osborne-Mendel rats (Jorgenson et al., 1985) was re-evaluated for histological evidence of compound-induced cytotoxicity and cell turnover. All rats treated with 1800 ppm (160 mg/kg/day, highdose group) in the drinking water for 2 years and half the rats treated with 900 ppm (81 mg/kg/day) had mild to moderate changes in proximal convoluted tubules in the mid to deep cortex indicative of chronic cytotoxicity. Tubule alterations specifically associated with chronic chloroform exposure included cytoplasmic basophilia, cytoplasmic vacuolation, and nuclear crowding consistent with simple tubule hyperplasia. Occasional pyknotic cells, mitotic figures in proximal tubules, and prominent karyomegaly of the renal tubule epithelium were present. These alterations were not present in control groups or at the 200-ppm (19 mg/kg/day) or 400-ppm (38 mg/kg/day) dose levels. This information adds substantially to the weight of evidence that the key events in chloroform-induced carcinogenicity in rat kidney include sustained cellular toxicity and chronic regenerative hyperplasia (Hard et al., 2000)

Combined inhalation and oral exposure

Effects of combined inhalation and oral exposures to chloroform on carcinogenicity and chronic toxicity in male F344 rats were examined by Nagano et al. (2006). A group of 50 male rats was exposed by inhalation to 0 (clean air), 25, 50, or 100 ppm (v/v) of chloroform vapour-containing air for 6 h/d and 5 d/wk during a 104 w period, and each inhalation group was given chloroform-formulated drinking water (1000 ppm w/w) or vehicle water for 104 wk, *ad libitum*. Renal-cell adenomas and carcinomas and atypical renal-tubule hyperplasias were increased in the combined inhalation and oral exposure groups, but not in the oral- or inhalation-alone groups. The results from this study revealed that renal tumors found in the combined-exposure groups were greater in size (16-17 mm in average size, with a maximum of 40-50 mm) and incidence than those reported previously in gavage-only or drinking water-only administration studies. It was concluded that combined inhalation and oral exposures markedly enhanced carcinogenicity and chronic toxicity in the proximal tubule of male rat kidneys, suggesting that carcinogenic and toxic effects of the combined exposures on the kidneys were greater than the ones that would be expected under an assumption that the two effects of single route exposures through inhalation and drinking were additive.

Table 4.47 Dose-Response Relationships for the Incidences of Renal Tumors Induced by Chloroform Exposures in the Male Rat Study (Nagano et al., 2006).

Drinking-water exposure 1000 ppm (Estimated uptake)	Inhalation exposure	Estimated amount of chloroform uptake (mg/kg/d)	Renal tumor incidence[a]
0	0		0/50
0	25 ppm	20	0/50
0	50 ppm	39	0/50
0	100 ppm	78	1/50 (2%)
45 mg/kg/d	0	45	0/49
53 mg/kg/d	*25 ppm*	*73*	*4/50 (8%)*
54 mg/kg/d	*50 ppm*	*93*	*4/50 (8%)*
57 mg/kg/d	*100 ppm*	*135*	*18/50 (36%)**

Note. Data in the combined-exposure groups are indicated in italics.
[a] Incidence of renal-cell adenoma and carcinoma.
* significantly different from the untreated control group, the oral-alone group, and each inhalation-alone group with matching concentrations, respectively, at $p \leq 0.05$ by Fisher's exact test.

In vitro studies

No data available.

4.1.2.8.2 Studies in humans

In vivo studies

Inhalation

Heineman et al., (1994) evaluated chlorinated aliphatic hydrocarbons (CAHs) as potential risk factors for astrocytic brain tumors. Job-exposure matrices for six individual CAHs and for the general class of organic solvents were applied to data from a case-control study of brain cancer among white men. The matrices indicated whether the CAHs were likely to have been used in each industry and occupation by decade (1920-1980), and provided estimates of probability and intensity of exposure for "exposed" industries and occupations. Exposure to chloroform or methyl chloroform showed little indication of an association with brain cancer.

Dermal

No data available.

Oral

In a cohort study following-up 14553 male and 16227 female residents over 25 years of age, Wilkins and Comstock (1981) assessed the cancer incidence in two subcohorts: people exposed to chlorinated surface water (average chloroform concentration 107µg/l) and users of water from deep wells with no chlorination. Risk ratios were calculated by contrasting the two cohorts, with various adjustments (age, marital status, education, smoking, church attendance, adequacy of housing and persons per room). The only significant excess risk was reported for death from breast cancer (RR, 2.7; 95% CI, 1.2-4.9), an excess of borderline significance were found for liver cancer (RR, 3.0; 95% CI 0.92-15). A complementary mortality study also suggested an association of chlorinated water with cancer of the liver and urinary tract.

Morris et al. (1992) conducted a meta-analysis which attempted to integrate quantitatively the results of previously published studies in which individual exposures were evaluated (i.e. case control and cohort studies). The authors identified increased rates of bladder and colo-rectal cancer in individuals exposed to chlorinated surface water, which appeared to exhibit a dose-related trend. Although this study was confounded by substantial differences in exposure variables that occur in different water supplies. Higher risk rates were estimated when the analysis was restricted to studies judged to have the highest quality exposure assessments. Because of the confounding of these results by chlorine residual levels and a multiplicity of other animal carcinogens/mutagens chemicals, none of the drinking-water studies specifically implicate chloroform as a human carcinogen.

McGeehin et al. (1993) conducted a population-based case-control study of bladder cancer and drinking water disinfection methods, during 1990-1991 in Colorado. After adjustment for cigarette smoking, tap water and coffee consumption, and medical history factors by logistic regression, years of exposure to chlorinated surface water were significantly associated with risk for bladder cancer ($p = 0.0007$). The odds ratio for bladder cancer increased for longer durations of exposure to a level of 1.8 (95% confidence interval 1.1-2.9) for more than 30

years of exposure to chlorinated surface water compared with no exposure. The increased bladder cancer risk was similar for males and females and for nonsmokers and smokers.

In a population-based case-control study, King and Marrett (1996) examined the relationship between bladder cancer and exposure to chlorination by-products in public water supplies in Canada. Exposures were estimated for the 40-year period prior to the interview, using 696 cases diagnosed with bladder cancer between 1 September 1992 and 1 May 1994 and 1,545 controls with at least 30 years of exposure information. Odds ratios (OR) adjusted for potential confounders were used to estimate relative risk. Those exposed to chlorinated surface water for 35 or more years had an increased risk of bladder cancer compared with those exposed for less than 10 years (OR = 1.41, 95% confidence interval [CI] = 1.10-1.81). Those exposed to an estimated THM level ≥ 50 µg/l for 35 or more years had 1.63 times the risk of those exposed for less than 10 years (CI = 1.08-2.46).

In a cohort study, Doyle et al., (1997) assessed the association of drinking water source and chlorination by-product exposure with cancer incidence. Exposure to chlorination by-products was determined from statewide water quality data. A cohort of 28,237 Iowa women reported their drinking water source. In comparison with women who used municipal ground-water sources, women with municipal surface water sources were at an increased risk of cancer of the colon, lung and skin melanoma. A clear dose-response relation was observed between four categories of increasing chloroform levels in finished drinking water and the risk of colon cancer and all cancers combined. No consistent association with either water source or chloroform concentration was observed for other cancer sites.

In vitro studies

No data available.

4.1.2.8.3 Summary of carcinogenicity

According to US EPA, (2001) 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. Two studies showed nasal lesion in rats or mice due to chloroform inhalation exposure. "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. The persistent cell proliferation presumably would lead to higher probabilities of spontaneous cell mutation and subsequent cancer (US EPA, 2001)."

There have been no reported studies of toxicity or cancer incidence in humans chronically exposed to chloroform (alone) via drinking water. Chlorinated drinking water typically contains chloroform, along with other trihalomethanes and a wide variety of other disinfection by-products. It should be noted that humans exposed to chloroform in drinking water are likely to be exposed both by direct ingestion and by inhalation of chloroform gas released from water into indoor air.

Although some studies have found increased risks of bladder cancer associated with long-term ingestion of chlorinated drinking-water and cumulative exposure to trihalomethanes, results were inconsistent between men and women and between smokers and non-smokers.

Moreover, relevant studies contain little information on specific exposure, and it is not possible to attribute any excess risk specifically to chloroform. Specific risks may be due to other disinfection by-products, mixtures of by-products, other water contaminants, or other factors for which chlorinated drinking-water or trihalomethanes may serve as a surrogate (WHO, 2004; IARC, 1999).

IARC, (1999) concluded there is inadequate evidence in humans for the carcinogenicity of chloroform but sufficient evidence in experimental animals for the carcinogenicity of chloroform. To conclude, the current human data are insufficient to establish a causal relationship between exposure to chloroform in drinking water and increased risk of cancer.

The NOAEC via inhalation for the kidney adenoma/carcinoma was identified at 5 ppm in mice, for nasal lesions a LOAEC of 5 ppm was determined (Yamamoto et al., 2002). Oral treatment with chloroform was associated with increased incidence of moderate to severe kidney lesions in CBA and CF/1 mice. NOAEL= 17 mg/kg bw (Roe et al., 1979). These values are considered as starting point for risk characterisation. **Considered as key studies for risk characterisation.**

Based on animal results the current classification for carcinogenicity of chloroform should be maintained: Category 3 with the risk phrases R40 limited evidence of carcinogenic effects.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Available data on the potential fertility toxicity of the chloroform include, on the one hand, reproductive toxicity studies on mice, and on the other hand, epidemiological studies (occupational exposures and case studies).

Studies in animals

One pair-based study is available. Chapin *et al.* (1997, in US EPA, 2004) exposed albino mice (20 mated pairs/group) to 8, 20 and 50 mg/kg-day chloroform by gavage, in a corn oil vehicle, for 31 weeks. Due to the volatilization of chloroform, the actual doses administered were 6.6, 15.9 and 41.2 mg/kg-day. No death occurred in relation with the treatment. Food and water consumptions were not affected by the treatment. Reduced maternal body weight was observed at the delivery of the 4th litter and on PND 14 of the 5th litter for 41.2 mg/kg-day group. No treatment related effect was observed on any endpoint of reproductive function. Absolute and relative liver weights were significantly higher in chloroform-exposed females than in controls ($p<0.01$), associated with dose related histopatholgic changes, described as degeneration of hepatocytes. Concerning males, only absolute and relative weights of the right epididymis were increased in high dose treated animals (+ 7%, $p<0.05$). Sperm mobility, density and percent of abnormal sperm were not affected by the treatment. Epididymal lesions rated as “minimal” were identified in 3/20 control mice, and 6/20 in high dose treated mice; two additional treated mice had epididymal lesions classified as “mild.” The nature of these lesions is described as “vacuolar degeneration of ductal epithelium in the cauda epididymis. (**Considered as key study for risk characterisation).** For effects on fertility**,** the estimated NOAEC is 15.9 mg/kg.

Table 4.48 Absolute and adjusted epididymal weights of F1 males (mean + SD) after exposure to chloroform by gavage (Chapin et al., 1997 in US EPA, 2004)

Dose (mg/kg-day)	Number per group	Body weight (g)	Right epididymis weight (mg)	Adjusted right epididymis weight (mg)
0	20	33.686 + 0.536	44.685 + 1.087	44.736 + 0.949
41.2	29	33.789 + 0.570	47.725 + 1.078*	47.674 + 0.949*

* Significant difference from controls at $p < 0.05$

Land *et al.* (1979, in US EPA, 2004) exposed male C57B1/C3H mice (control n=15, 800 ppm n=9) to an air concentration of 800 ppm chloroform, 4 hr/day, for five days. A significant increase in the frequency of abnormal sperm morphology was found: 2.76% in the treated group vs. 1.42% in controls, p<0.05. In 1981, these authors conducted an expansion of the experiment described above (Land *et al.*, 1981) with mice (n=4)exposed to 400 ppm chloroform: a significant increase in the percent of abnormal sperm was found as well (1.88% in treated group vs. 1.42% in controls, p<0.01).

In the US EPA (1980) 90-day subchronic toxicity study detailed in 4.1.2.6.1, for male rats no effect was reported on kidneys, testes, prostate and seminal vesicles except one case of testicular hyperplasia and one interstitial cell hyperplasia for animals exposed to 900 ppm, after 30 days of treatment (chloroform in drinking water at concentrations 0, 200, 400, 600, 900 or 1800 ppm). In mice receiving 600-900-1800 or 2700 ppm chloroform in drinking water, no effect was observed on ovaries and uteri.

In the Heywood *et al.* (1979, in US EPA, 2001) study detailed in 4.1.2.6.1, beagle dogs were exposed to 15 or 30 mg/kg-day chloroform in a toothpaste base, orally in the form of gelatin capsules, 6 d/week for 7.5 years, followed by a 20-24 week recovery period. No effect was observed on liver, brain, kidneys, testes and prostate or ovaries and uteri. Ectopic testes with inhibition of spermatogenesis were observed in one control, one dog at 15 mg/kg-day and 2 dogs at 30 mg/kg-day. Nodular hyperplasia of the mammary gland was observed for one control, five vehicle controls and 3 females at 15 mg/kg-day. These latter findings were not considered to be related to the treatment.

Studies in humans

One case study of occupational exposure to chloroform and its effect on male reproductive toxicity was available (Chang *et al.*, 2001 in US EPA, 2004). A 34-year-old male laboratory worker was exposed to solvents at work for 8 months (August 1996 to April 1997), due to the shutdown of the ventilation system. Before the exposure, a complete fertility test was performed on May 1996 in a local hospital.. The patient had normal semen appearance, volume, and sperm count. Ninety-two percent of sperm were normal in morphology. At 30 min after ejaculation, 95% of sperm were motile at a normal speed, and at 60 min, 30% were motile. After the exposure, asthenospermia was diagnosed (Table 4.49). An investigation was hence performed to determine the worker's possible exposure level to chemical hazards: the worker was exposed to chloroform levels approximately 10 times higher than the permissible exposure limit of 50 ppm (US EPA, 2004) and 50 times higher than the threshold limit value of 10 ppm (ACGIH, 2001), during 8 months. The worker was also exposed to other chemicals like isooctane and tetrahydrofuran but no study of male reproductive effects in association with exposure to isooctane was identified and no adverse effect of tetrahydrofuran on male fertility was reported in studies.

Table 4.49 Semen analysis after 8 months (August 1996 to April 1997) exposure (Chang *et al.*, 2001 in US EPA, 2004)

Parameters	July 1997	August 1997	October 1997
Volume (ml)	4	5.5	3
Count (million/ml)	68.6	73.8	90.6
Motility 30 min after ejaculation:			
rapid	17%	10%	32%
medium	6%	1%	6%
slow	3%	0%	2%
static	74%	89%	30%
Path velocity (m/sec)	35	40	50

Dahl *et al.* (1999) found no association between dental workplace exposure (number of root fillings with chloroform based root canal sealing material placed by week) and effect on fertility in female dental surgeons.

A case report cited in Reprotext 2004 (Tylleskar-Jensen, 1967 in US EPA, 2004) described two women with eclamspia who had worked in laboratories, exposed to concentrations of 100-1000 ppm chloroform (recommended exposure limit 50 ppm), in comparison to a background incidence in the population of 1 case per 4000 pregnancies.

4.1.2.9.2 Developmental toxicity

Available data on the potential developmental toxicity of the chloroform include, on the one hand, developmental toxicity studies in the rat, both by inhalation and oral routes, in the mouse by the inhalation route and in the rabbit by the oral one, and on the other hand, epidemiological studies (occupational study, case-control studies, retrospective cohort and prospective cohort studies). All these studies are summarized below.

<u>Studies in animals</u>

Inhalation route

Time mated Sprague-Dawley rats were exposed to chloroform by inhalation, 7 hr/day on each gestation days 6 through 15, at concentration levels of 30, 100 or 300 ppm; a starved control group (restricted to 3.7 gfood/day on gestation days 6-15) was also added to the experiment due to the marked anorexia observed (Schwetz *et al.*, 1974 in US EPA, 2004). No dams died during the study but statistically significant decreases of percent pregnant, maternal weight gain and food consumption were observed (see Table 4.50).

Table 4.50 Main maternal parameters following exposure to chloroform by inhalation (Schwetz *et al.*, 1974 in US EPA, 2004)

Parameters	control	control	30 ppm	100 ppm	300 ppm

		starved			
% pregnant	88	100	71	82	15*
body weight (g) ± SD					
GD 6	275 ± 21	274 ± 13	266 ± 14	274 ± 17	284 ± 9
GD 13	310 ± 17	223 ± 13*	280 ± 14*	274 ± 18*	192 ± 9*
GD 21	389 ± 28	326 ± 24*	381 ± 23*	365 ± 22*	241 ± 29*
feed (g/day)					
GD 6-7	19 ± 3	starved	5 ± 3*	13 ± 4*	1 ± 1*
GD 12-13	22 ± 2	starved	20 ± 1	15 ± 2*	1 ± 1*
GD 18-19	26 ± 3	24 ± 8*	29 ± 5	33 ± 3*	not done

* statistically different from controls at p<0.05

Changes in serum glutamic-pyruvic transaminase (SGPT) were measured as a mean of evaluating liver function and to assess the degree of liver toxicity in rats. No statistically difference was observed between controls and rats exposed to 300 ppm of chloroform. In addition, livers for pregnant and nonpregnant rats, evaluated 6 days after the cessation of the treatment, were considered to have a normal appearance. Relative liver weights were affected only in the 300 ppm group of nonpregnant rats, showing a significant increase in comparison to the controls (p<0.05). Considering pregnant rats, relative liver weights were increased over control values at 100 and 300 ppm of chloroform, and in starved control (p<0.05).

In the 300 ppm group, only three dams out of 20 were found to be pregnant; one of these pregnant females showed total litter resorption and the two remaining had reduced litter size and increased incidence of resorptions. (see Table 4.51).

Table 4.51 Main fetal parameters following exposure to chloroform by inhalation (Schwetz *et al.*, 1974 in US EPA, 2004)

Parameters	control	control starved	30 ppm	100 ppm	300 ppm
Number of mated females	77	8	31	28	20
Number of litters	68	8	22	23	3
Mean number of live foetus/litter	10 ± 4	10 ± 4	12 ± 2	11 ± 2	4 ± 7*
Mean Implantation sites/litter	11 ± 3	11± 4	13 ± 2	12 ± 2	11± 4
resorptions/implantation	8%	7%	8%	6%	61%*
litters with total resoption	0	0	0	0	1
litters with resorptions	57%	25%	68%	52%	100%
sex ratio M:F	53:47	45:55	53:47	55:45	34:66*
mean fetal weight/litter (g)	5.69 ± 0.36	5.19 ± 0.29*	5.51 ± 0.2	5.59 ± 0.24	3.42 ± 0.02*
CRL (mm)	43.5 ± 1.1	42.1 ± 1.1*	42.5 ± 0.6*	43.6 ± 0.7	36.9 ± 0.2*
Gross anomalies	Percent of litters affected (No. of litter)				
acaudia (short tail)	0	0	0	13(3)*	0
imperforate anus	0	0	0	13(3)*	0
Skeletal anomalies					
total skeletal anomalies (% affected litters)	68%	38%	90%*	74%	100%
delayed ossification, skull	21(14)	0	73(16)	30(7)	50(1)
missing ribs	0	0	0	13(3)*	0
wavy ribs	0	0	18(4)*	0	0
split sternebrae	1.5(1)	0	9(2)	9(2)	50(1)
delayed ossification, sternebrae	22(15)	38(3)	0	74(17)*	100(2)
Soft tissue anomalies					
total soft tissue anomalies (% affected litters)	48%	38%	45%	65%	100%
subcutaneous odema	34(23)	38(3)	41(9)	61(14)*	100(1)

* statistically different from controls at $p<0.05$
CRL: crown-rump length

At a concentration of 100 ppm, three out of 23 litters showed gross malformations, 3/23 had fetuses with acaudia or short tail and 3/23 had fetuses with imperforate anus: as the control malformation rate was 1/68, the increase was significant over the control. Otherwise, it is not stated how many fetuses were affected among the litters or if the same fetuses were affected by the anomalies. At 30 ppm, skeletal malformations were increased with delayed ossification of the skull (16/22), wavy ribs (4/22) and split sternebrae (2/22). The number of affected fetuses was not clearly reported. A LOAEC of 30 ppm was selected, based on reduced maternal body weight and a developmental LOAEC of 30 ppm was based on increased skeletal anomalies.

Murray *et al.* (1979, in US EPA, 2004) exposed CF-1 mice (34-40/group) to 0 or 100 ppm of chloroform by inhalation, 7 hr/day, on each gestation days 1-7, 6-15 or 8-15. Except one dam exposed to 100 ppm, which died on gestation day 18, consequently to extreme starvation, no clinical sign was reported during the study. Feed and water consumptions and body weight gain (on gestation days 1-7 or 8-15) were reduced in treated animals. Relative maternal liver weights were increased over controls, on gestation days 6-16 or 8-15, in association with an increase in SGPT activity, indication of some hepatic toxicity.

Fetal data are reported in Table 4.52.

Table 4.52 Fetal data from mice exposed to chloroform by inhalation (Murray et al., 1979 in US EPA, 2004)

Parameters	GD 1-7 0 ppm	GD 1-7 100 ppm	GD 6-15 0 ppm	GD 6-15 100 ppm	GD 8-15 0 ppm	GD 8-15 100 ppm
% pregnant	74	44	91	43	65	60
No. Litters	22	11	29	12	24	18
Live Fetuses/litter	10 ± 3	13 ± 2	12 ± 3	10 ± 4	12 ± 3	11 ± 3
Resorptions/litter	2 ± 2	4 ± 5*	2 ± 2	1 ± 1	2 ± 2	2 ± 2
Fetal weight (g)	1.02 ± 0.1	0.92 ± 0.07*	0.99 ± 0.11	0.95 ± 0.13	1 ± 0.12	0.85 ± 0.17*
CRL (mm)	24.7 ± 1	23.6 ± 1.2*	23.7 ± 1.3	23.2 ± 1.1	24.1 ± 1.1	22.9 ± 2.2*
Cleft palate /litter affected	3/1	-	-	-	1/1	10/4* [a]

* statistically different from controls, $p<0.05$
a six fetuses in one litter exhibited cleft palate

The number of pregnant females was significantly lower in treated groups exposed to chloroform from days 1 through 7 or 6 through 15 of gestation.

Frequencies of external malformations were not affected by the treatment.

Cleft palate was observed at a high incidence in 4 litters when animals were given 100 ppm from GD8 to 15. No other type of major malformation was observed. Only single incidents of missing testicles were reported for treated groups exposed on gestation days 1-7 or 8-15. Examination of the skeleton showed an increased occurrence of some minor skeletal variants: delayed ossification of skull bones was significantly increased among all exposed groups while delayed ossification of sternebrae was observed among fetuses exposed on gestation

days 1-7 or 8-15. It is difficult to establish a relationship between maternal toxicity and the fetal findings as the level of maternotoxicity, (body and food consumptions) is not reported.

Baeder and Hoffman (1988) exposed time mated Wistar rats (20-23/groups) to chloroform 7 hr/day on each day of gestation 7-16, at concentration levels of 0, 30, 100 or 300 ppm. No behavioral alteration or clinical symptom was induced in dams by treatment, and all females survived until the end of the study. Concentration-dependant reductions in feed consumption and body weight gain were observed. No effect was observed on kidneys, liver and spleen.

Litters were completely resorbed in two dams at 30 ppm, in three at 100 ppm and in eight at 300 ppm (Table 4.53). Fetal weight was significantly lower than controls at 300 ppm (-6%, p<0.05). CRL was minimally but significantly lower in all treated groups when compared to controls (around -6%, p<0.05).

There were no fetal external, soft tissue or skeletal observations that were considered related to the treatment. A LOEC of 30 ppm was based on maternal reduced body weight on gestation day 17 and a LOAEC of 30 ppm was based on increase in completely resorbed litters.

Table 4.53 Main fetal parameters following inhalation exposure to chloroform (Baeder and Hoffman, 1988 in US EPA, 2004)

Parameters	0	30 ppm	100 ppm	300 ppm
N lost litters	0	2	3	8
N live litters	20	18	17	12#
Resorptions/live litters	0.75	0.22	0.53	0.92
Live fetuses/litter	12.4	12.8	12.8	13.4
Fetal weight (g)	3.19 ± 0.3	3.16 ± 0.19	3.13 ± 0.21	3 ± 0.19*
Fetal CRL (cm)	3.52 ± 0.17	3.38 ± 0.12*	3.39 ± 0.1*	3.39 ± 0.12*

* statistically different, p<0.05
# statistically different, p<0.005

In addition to this first study, Baeder and Hoffman (1991) exposed Wistar rats (groups of 20 time-mated) to chloroform by inhalation at concentration of 0, 3, 10 or 30 ppm, 7 hr/day, daily on each gestation days 7-16. As in the previous study, concentration-dependant reductions in food consumption (for all doses) and in body weight gain (only for 10 and 30 ppm) were observed. At necropsy, maternal animals showed moderate to severe unilateral or bilateral renal pelvic dilatation in one dam at 3 ppm, in 3 dams at 10 ppm and in 4 dams at 30 ppm. In addition, kidney weights were higher in high dose treated animals than in controls (p<0.05). No effect was observed on heart, liver or spleen.

Table 4.54 Maternal feed consumption and body weight[a] after inhalation exposure to chloroform (Baeder and Hoffman, 1991 in US EPA, 2004).

Parameter	0	3 ppm	10 ppm	30 ppm
N	20	20	20	19
feed, gd 7-14*	8.03 + 0.68	7.19 + 0.66#	6.45 + 0.70#	5.60 + 0.75#
feed, gd 14-17*	7.07 + 0.32	7.16 + 0.59	7.12 + 0.67	6.52 + 0.67#
feed, gd 17-21*	6.63 + 0.40	6.49 + 0.61	6.91 + 0.33	7.25 + 0.52#
bw (g), gd 0**	193.3 + 12.2	197.5 + 7.7	192.2 + 6.4	200.0 + 7.4
bw (g), gd 7**	226.0 + 14.7	220.9 + 11.0	222.9 + 8.2	230.6 + 10.6
bw (g), gd 14**	255.8 + 16.2	253.6 + 13.7	237.1 + 10.4	237.3 + 12.3
bw (g), gd 17**	269.1 + 17.0	260.2 + 13.7	255.2 + 12.4	253.4 + 16.3
bw (g), gd 21**	321.9 + 22.5	319.1 + 21.1	308.0 + 17.5	308.7 + 18.5
weight gain, gd 0-7	32.7 + 9.5	31.4 + 9.1	30.7 + 3.5	30.6 + 7.3
weight gain, gd 7-14***	29.8 + 10.5	24.7 + 6.3	14.3 + 8.2	6.7 + 8.8
weight gain, gd 14-17***	13.3 + 4.6	14.6 + 5.7	16.1 + 5.0	16.1 + 6.7
weight gain, gd 17-21***	52.9 + 6.5	50.9 + 11.5	52.9 + 11.7	55.3 + 7.8
weight gain, gd 0-21***	120.6 + 17.8	121.6 +21.0	115.9 + 16.2	108.7 + 16.7

a mean + SD
* g feed consumed per 100 g body weight
# significant difference from controls at $p < 0.05$

Except one dam at 30 ppm, all dams carried live fetuses to term; numbers of corpora lutea and implantations, resorption frequency and live litter size were not affected by the treatment. According to the text of Baeder and Hoffman (1991), mean fetal body weights and lengths did not differ significantly among groups. Tabulated data in the report marks both fetal weight and CRL as significantly lower than controls for the 30 ppm group (see Table 4.55). In the case of fetal weight, however, both the mean weight and the standard deviation (SD) for all treated groups are identical, with N for the 30 ppm group being 19, rather than 20 litters. In any event, the text notes that fetuses with body weights of less than 3.0 g were more common in the 10 and 30 ppm groups than in the control and 3 ppm groups (24% and 26.9%, respectively, as opposed to 3.2% and 14.2%, respectively). Only mean fetal weight and CRL of the top dose treated animals were significantly lower than the controls (US EPA, 2004).

Table 4.55 Mean fetal parameters (Baeder and Hoffman, 1991 in US EPA, 2004).

Parameters	0	3 ppm	10 ppm	30 ppm
N lost litters	0	0	0	1
N live litters	20	20	20	19
Resorptions/live litters	0.55	0.4	0.75	0.84
Live fetuses/litter	12.4	12.4	12.9	12.5
Fetal weight (g)	3.4 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	3.2 ± 0.3*
Fetal CRL (cm)	3.58 ± 0.2	3.55 ± 0.21	3.44 ± 0.26	3.4 ± 0.19*
poorly ossified cranial bones $	42/14	47/17	48/16	60*/17
ossification of less than 2 caudal vertebrae $	4/3	14*/5	16*/6	14*/8
non or weakly ossified sternebrae $	7/3	32*/13	35*/14	18*/11
wavy or thickened ribs $	10/6	11/5	22*/10	15/4

* statistically different, p<0.05

$ number affected fetuses/number litters with affected fetuses

One incident of internal hydrocephalus was observed in a live fetus of the 3 ppm group. No other gross malformations were reported in any group.

The frequency of fetuses with poorly ossified cranial bones was significantly ($p < 0.05$) higher in the 30 ppm chloroform group than among controls (Table 4.55). The frequency of litters having fetuses with poorly ossified cranial bones did not differ significantly among groups. All three treated groups had significantly ($p < 0.05$) higher frequencies of poor ossification of the caudal vertebrae and sternebrae than did control fetuses, when considered as total numbers of affected fetuses per group. When considered on a per litter basis, as litters containing at least one affected fetus, sternebral ossification alone was significantly affected ($p < 0.05$). The frequency of fetuses with wavy and/or thickened ribs was greater in the 10 ppm group than among controls ($p < 0.05$). This difference was not significant when considered on a per litter basis. Other skeletal and ossification variations were observed sporadically across all groups (US EPA, 2004).

US EPA, (2001) determined a NOAEC of 10 ppm (50 mg/m^3) for developmental effects from this study. A LOEC of 10 ppm was based on apparent reduced maternal body weight and weight gain. A NOAEC of 10 ppm was based on decreased fetal weight & CRL (**Considered as key study for risk characterisation).**

Oral route

Male and female albino ICR mice were given 31.1 mg/kg-day chloroform by gavage three weeks before being co-housed for mating. The vehicle used was a solution of one part “Emulphor” and eight parts saline (0.9%). Treatment continued through the mating period for

males, and throughout mating, gestation, and lactation for females. Five treated and five vehicle-control litters were used for the study; litters (5were culled to no more than eight pups by random selection on the day of birth. On postnatal day seven, and for the remainder of the study, all pups were given either 31.1 mg/kg-day chloroform, or the vehicle, by gavage (Burkhalter and Balster, 1979 in US EPA, 2004).

Each day 3 pups per litter were tested for: righting reflex, forelimb placing response, forepaw grasp, rooting reflex, cliff drop aversion, auditory startle response, bar-holding ability, and eye opening. Motor performance was tested in 15 mice randomly selected from both groups on postnatal day 17. On days 22 and 23, 15 mice randomly selected from both groups were tested for passive avoidance learning.

Mean litter size did not differ between groups, nor did mean pup body weights (taken daily on postnatal days 7-21). Weight gain over days 7-21 was significantly lower in chloroform-exposed animals ($p < 0.01$). Righting reflex, forelimb placing response, forepaw grasp, cliff drop aversion, auditory startle response, bar-holding ability, and eye opening all showed progressive increases in scale scores over the days of testing. Rooting reflex increased up to about days 8-10, and then was lost by day 14. While there were scattered significant differences between the chloroform and control groups on specific days, chloroform showed no overall tendency to retard neurobehavioral development of mouse pups. The one exception was forelimb placement, for which the chloroform group had lower scores on each of days 5-8, with significant differences ($p < 0.05$) on days 5 and 7.

The inverted-screen climbing test of motor performance showed no significant difference between groups. In the test of passive avoidance, all animals learned the task as demonstrated by increased latency in the second and third trials ($p < 0.05$). There were no differences between chloroform-treated animals and the control group for latencies across the three trials, nor did the groups differ with respect to the effects of shock (US EPA, 2004).

Following the National Toxicology Program's Continuous Breeding protocol, male and female CD1 mice (20 mated pairs/dose group, 40 mated pairs/control) were exposed to chloroform by gavage for seven days prior to first mating, as well as during a subsequent 98-day cohabitation period (Chapin et al., 1997; NTP, 1988 in US EPA, 2004). Actual doses administered were closer to 6.6, 15.9, and 41.2 mg/kg, due to volatilization of the chloroform. No treatment-related changes were identified in any of the evaluated endpoints of reproductive function. No significant differences were observed among groups for the number of litters per pair, litter size, proportion of live pups, sex ratio, or pup weight at birth. Inter-litter intervals were considered to be essentially identical across all groups. Neither the proportion of stillbirths nor postnatal survival differed among groups. Pup weights did not differ among groups at any of the time points evaluated. The NOAEL for reproductive toxicity is > 41.2 mg/kg.

Two studies by the oral route were reported. In the first, Sprague-Dawley rats (25/group) were given twice daily gavage dosings of chloroform to total daily doses of 0, 20, 50 or 126 mg/kg/day, on each gestation days 6-15. Control were given equivalent daily doses of the vehicle. (Thompson *et al.*, 1974). All dams survived to the treatment. Reduced weight gain was observed for dams of the 50 and 126 mg/kg-day groups, feed consumption was reduced for all groups. No spontaneous deaths occurred during this study and no effect was observed on liver or kidneys. Among fetal parameters, only implantation frequency was significantly higher at 126 mg/kg-day than the controls and fetal weight was significantly lower ($p<0.05$). Males and females were affected similarly. Sex ratio were not altered by treatment. (Table 4.56).

Table 4.56 Litter data

Dose (mg/kg-day)	Implants	Corpora lutea	Resorptions	Live fetuses	Fetal weight (g)	M:F
0	11.5 ± 2.4	13.1 ± 1.4	1 ±2.9	10.6 ± 3.9	4 ± 0.3	52:48
126	13.5 ± 1.1*	14.2 ± 1.2	1.2 ± 2.6	12.3 ± 3.1	3.7 ± 0.4*	56:44

* statistically different from controls, p<0.05

Minor visceral and skeletal fetal abnormalities such as dilated renal pelves, distended ureters, unossified and malaligned sternebrae, incompletely ossified vertebral centra and skull bones occurred sporadically and were not increased significantly among fetuses or litters.

In the second study, Sprague-Dawley rats (15/group) received 0, 100, 200 or 400 mg/kg-day of chloroform by oral intubation, in a corn oil vehicule, on each gestation days 6-15 (Ruddick *et al.*, 1983). In all treated groups, maternal body weight decreased; maternal liver weight increased at all dose levels while kidneys'one increased only at the top dose (p<0.05). Otherwise, no histopathological abnormality was observed in these organs. Clinical and chemical maternal parameters were affected by the treatment: decreasing hemoglobin, hematocrit and serum sorbitol dehydrogenase for all doses, decreasing red blood cell counts at 400 mg/kg-day and increased serum inorganic phosphorus and cholesterol at 200 and 400 mg/kg-day.

While resorption frequency and liver litter size were unaffected by the treatment, mean fetal weight was decreased (-19%, p<0.05) and associated with an increase of runts. The frequency of sternebral aberrations was increased in fetuses exposed to the highest dose of chloroform (Table 4.57).

Table 4.57 Data from rat fetuses exposed orally to chloroform

Parameters	0	100 mg/kg-day	200 mg/kg-day	400 mg/kg-day
Number of litters	14	12	10	8
Litter size	11.2 ± 0.2	11.8 ± 0.6	12.5 ± 0.7	10.9 ± 1.1
Fetal weight (g)	5.4 ± 0.8	5.3 ± 0.1	5 ± 0.1	4.4 ± 0.3*
Sternebral aberrations[1]	0/0	1/1	5/3	14/8
Runts[2]	1/1	2/1	0/0	11/3
Runts[3]	0/0	1/1	0/0	26/8

* statistically different form controls, p<0.05
[1] fetuses/litters
[2] among fetuses preprared for skeletal examination, fetuses/litters
[3] among fetuses preprared for visceral examination, fetuses/litters

Thompson *et al.* (1974) exposed rabbits (15/group) to 0, 20, 35 or 50 mg/kg-day of chloroform, in corn oil by gavage, daily on gestation days 6-18. Seven dams died during the study and deaths in the high dose group were attributed to hepatotoxicity. Body weight gain decreased in dams of the top dose group. Complete abortions were seen in all groups (3 in the control group, 2 at 20 mg/kg-day, 1 at 35 mg/kg-day and 4 at 50 mg/kg-day). Mean fetal weights were significantly lower than controls for the 20 and 50 mg/kg-day groups. No

external or visceral malformation was observed while incomplete ossification of skull bones was observed in all groups with fetal incidence significant at 20 and 35 mg/kg-day ($p<0.05$). LOAEL= 20 mg/kg/day (**Considered as key study for risk characterisation).**

Studies in humans

Only one study studied exposure to chloroform in laboratory or non-laboratory department for 1 year, in association with pregnancy outcomes (Wennborg *et al.*, 2000). A cohort of Swedish women (n=697, births=1417), born in 1945 or later, was studied. No association was reported between laboratory work and reported spontaneous abortion, small gestation age or variations in birth weight. However, limitations are various: lack of exposure measurements, possible exposure to other solvents, long time between pregnancies and administration of the questionnaire.

As chloroform is a water disinfection byproduct, many studies have examined the relation between trihalomethanes (THMs), including chloroform, in drinking water and pregnancy outcomes.

A population-based case-control study was conducted in Iowa, between 1987 and 1990, to evaluate the relation between exposures to chloroform via drinking water and low birth weight (case=159, controls=795), prematurity (case=342, controls=1710) and intrauterine growth retardation (case=187, controls=935) (Kramer *et al.*, 1992). The result showed that exposure to chloroform at concentration ≥ 10 µg/l was associated with an increase risk of intrauterine growth retardation (odd ratio = 1.8, 95% CI, 1.1 – 2.9).

King *et al.* (2000) conducted a retrospective cohort study to determine the association between exposure to specific disinfectant by-products, including chloroform, and the risk of stillbirth, in Nova Scotia between 1988 and 1995 (perinatal database n= 49842). Exposure of chloroform ≥ 100 µg/l leads to a relative risk for stillbirth about 1.56; the risk estimate was higher for asphyxia-related deaths and increased with increasing levels of chloroform exposure. However, the lack of individual data on chloroform exposure could be a limitation of this study.

Dodds and King (2001) conducted a retrospective cohort study to determine the association between exposure to chloroform and birth defects, in Nova Scotia between 1988 and 1995 (perinatal database n= 49842). An increased risk of chromosomal abnormalities was observed with exposure to chloroform at levels 75-99 µg/l (relative risk = 1.9) and at levels ≥ 100 µg/l (relative risk = 1.4). An increased risk of cleft defects was reported too for exposure to chloroform ≥ 100 µg/l (relative risk = 1.5).

Dodds *et al.* (2004) conducted a case-control study to identify the association between exposure to THMs, including chloroform, in public water supplies and the risk of stillbirth. This study was performed in Nova Scotia and Eastern Ontario, between 1999 and 2001 (cases=112, controls=398). The results showed that the odds ratios for stillbirths were increased at the 1-49 µg/l level (OR=1.8, 95% CI, 1.1 – 3.0) and at the ≥ 80 µg/l level (OR=2.2, 95% CI, 1.0 – 4.8). There was no evidence of a monotonic increase.

Wright *et al.* (2004) conducted a retrospective cohort study to determine the effect of maternal third trimester exposure to chloroform on birth weight, gestational age, small for gestation age and preterm delivery. This study was based on birth certificate data from 1995-1998 (n=196000) in Massachusetts. Reductions in mean birth weight were observed for chloroform concentrations > 20 µg/l. In addition, exposure to chloroform was associated too with an increase in mean gestational age and a decreased risk for preterm delivery.

4.1.2.9.3 Summary of toxicity for reproduction

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). **Considered as key studies for risk characterisation.**

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. They are summarized in the following table.

Table 4.58 Developmental toxicity data on different species

Reference	Protocol	Doses	Maternal effects	Developmental effects
Schwetz *et al.*, 1974	Sprague-Dawley rats *Inhalation* 0, 30, 100, 300 ppm 7 hr/day, gd 6-15	30 ppm	Reduced food consumption on gd 6-7 LOAEC =30 ppm based on reduced maternal body weight	Increased skeletal anomalies LOAEC =30 ppm based on increased skeletal anomalies
		100 ppm	Decreased body weight Reduced food consumption, increased relative liver weight	Increased gross anomalies
		300 ppm	Reduced food consumption, increased relative liver weight	Reduced pregnancy rate, decreased litter size, increased resorptions, altered sex ratio and decreased fetal weight and CRL
Baeder & Hoffman, 1988	Wistar rats *Inhalation* 0, 30, 100, 300 ppm 7 hr/day, gd 7-16	All concentrations	Reduced food consumption, reduced body weight LOEC = 30 ppm	Increased in completely resorbed litters, decreased CRL LOAEC = 30 ppm Decreased fetal weight (300 ppm only)
Baeder & Hoffman, 1991	Wistar rats *Inhalation*	3 ppm	Reduced food consumption	Increased ossification variations

Reference	Protocol	Doses	Maternal effects	Developmental effects
	0, 3, 10, 30 ppm 7 hr/day, gd 7-16	10 ppm	Reduced body weight LOEC = 10 ppm	**NOAEC = 10 ppm based on decreased fetal weight & CRL**
		30 ppm		Decreased fetal weight and CRL
Thompson *et al.*, 1974	Sprague-Dawley rats Gavage 0, 20, 50, 126 mg/kg-day gd 6-15	50 mg/kg-day	Decreased food consumption, decreased weight gain	
		126 mg/kg-day		Increased implantations, decreased fetal weight
Ruddick *et al.*, 1983	Sprague-Dawley rats Intubation 0, 100, 200, 400 mg/kg-day gd 6-15	All doses	Decreased body weight, increased liver weight, decreased hematocrit, hemoglobin and red blood cells count	
		400 mg/kg/d	Increased kidney weight	Decreased fetal weight, increased of sternebrae aberrations and runting
Murray *et al.*, 1979	CF-1 mice *Inhalation* 0, 100 ppm 7 hr/day, gd 6-15, 1-7 or 8-15		Decreased weight gain, gd 1-7 or 8-15 Increased relative liver weight, gd 6-15 or 8-15	Decreased pregnancy rate, gd 1-7 or 6-15 Increased resorptions, gd 1-7 Decreased fetal weight and CRL, gd 1-7 or 8-15 Increased cleft palate, gd 8-15 Increased delayed ossification of sternebrae, gd 1-7 or 8-15
Thompson *et al.*, 1974	Rabbits Gavage 0, 20, 35, 50 mg/kg/d gd 6-18	All doses		Complete abortions
		20 mg/kg-day		**Decreased fetal weight LOAEL = 20 mg/kg/day**
		50 mg/kg-day	Death, decreased body weight gains	
Burkhalter & Balster, 1979	ICR mice 0, 31.1 mg/kg-day 3 weeks prior to mating, through mating, gestation and lactation, directly to weaned pups		Not discussed	Reduced postnatal weight gain Lower scores for forelimb placement on postnatal days 5 and 7

Reference	Protocol	Doses	Maternal effects	Developmental effects
Chapin et al., 1997 NTP, 1988	Mice, continuous breeding study by gavage 0, 6.6, 15.9, 41.2 mg/kg-day		Reduced bw observed at the delivery of the 4th litter and on PND 14 of the 5th litter for 41.2 mg/kg-day group	No significant differences observed among groups for the number of litters per pair, litter size, proportion of live pups, sex ratio, or pup weight at birth

References in bold are selected as a starting point for risk characterisation

Based on the data available for fertility, effects are not sufficiently severe to justify a classification

Based on the data available for developmental toxicity, chloroform should be classified as Category 3 with the risk phrase R63 possible risk of harm to the unborn child

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

[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.

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 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 4.59.

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 4.59 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 scenarios 1, 2 and 3.1 (Swimming instructor/lifeguard in a swimming pool) is limited to the dermal and the inhalation routes of exposure.

Chloroform is also a by-product chemical associated with disinfection of swimming pool water; chloroform is originated by the reaction of disinfecting agents with organic substances and not intentionally used. Consequently, it was agreed that the Risk Characterisation of chloroform as a by-product chemical should not be presented in the Chloroform risk assessment but rather than in the Sodium Hypochlorite RAR. Any risk identified in scenario 3 for workers as swimming instructors, lifeguards, competitive swimmers and for consumers as child swimmers and adult swimmers should be addressed in the Sodium Hypochlorite RAR (results of RC for scenario 3 are presented in Annex 1 for information).

Table 4.60 Summary of Workers Reasonable Worst Case exposure and Total systemic dose.

Scenario	RWC Inhalation exposure	RWC Dermal exposure	RWC Ingestion exposure
1. Manufacture of chloroform and HCFC 22 (closed continuous process)	1.15 ppm 5.6 mg/m3	16.8 mg/person/day 0.24 mg/kg/day	0
2. Chloroform as intermediate or solvent in the synthesis of chemicals (closed batch process)	2 ppm 10 mg/m3	16.8 mg/person/day 0.24 mg/kg/day	0

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)
1. Manufacture of chloroform and HCFC 22 (closed continuous process)	1.25*8*5.6*0.8/70 = 0.64	16.8*0.1/70 = 0.024	0	0.66
2. Chloroform as intermediate or solvent in the synthesis of chemicals (closed batch process)	1.25*8*10*0.8/70 = 1.14	16.8*0.1/70 = 0.024	0	1.164

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 4.62 and compared with Reference MOS reported in Table 4.61.

Table 4.61 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 1, while conclusion **iii** is reached for scenario 2.

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 4.60) to calculate the MOS. Calculated MOSs are compared with Reference MOS in Table 4.62.

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 4.60 to calculate the MOS. Calculated MOSs are compared with Reference MOS in Table 4.62.

For acute toxicity by combined exposure, conclusion ii is reached for scenario 3, while for scenario 1 and 2, conclusion iii is drawn.

Table 4.62 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/ m3	mg/ m3			mg/kg	mg/kg			mg/kg /day	mg/kg		
Production												
Scenario 1:Chloroform used as intermediate(closed batch process)	5.6	249	44	**ii**	0.024	3.56	148	**ii**	0.66	3.56	5	**iii**
Scenario 2:Chloroform used as solvent in the synthesis of chemicals (closed batch process)	10	249	25	**iii**	0.024	3.56	148	**ii**	1.164	3.56	3	**iii**

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. Dermal exposure to irritating concentrations of chloroform is considered to occur only accidentally if the required protection is strictly adhered to. It is assumed that existing controls (i.e., engineering controls and personal protective equipment based on classification and labelling with R38) are applied. Therefore, it is concluded that chloroform is of no concern for workers with regard to effects as a result of dermal exposure for scenarios 1 and 2 in which irritating concentrations of chloroform are handled (**conclusion ii**).

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. Based on this result, it is concluded that chloroform is of concern for workers with regard to effects as a result of eye exposure. However, ocular exposure can be excluded as effective use of personal protective equipment for the eyes (based on classification and labelling with R36) is assumed in all scenarios. Therefore, it is concluded that the substance is of no concern for workers with regard to effects as a result of eye exposure (**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).

The LOAEC of 50 mg/m^3 is used with exposure estimations to calculate the MOS (Table 4.64) and then compared to Reference MOS reported in Table 4.63.

Table 4.63 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 4.64 Occupational risk assessment for respiratory irritation

	Inhalation			
	Exposure	N(L)OAEC	MOS	Conclusion
	mg/m^3	mg/m^3		
Production				
Scenario 1: Chloroform used as intermediate(closed batch process)	5.6	50	10	**iii**
Scenario 2: Chloroform used as solvent in the synthesis of chemicals (closed batch process)	10	50	5	**iii**

For respiratory irritation **conclusion iii** is reached for scenarios 1 and 2.

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 4.67) and then compared to Reference MOS reported in Table 4.65.

Table 4.65 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 4.66.

Table 4.66 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 iii** is reached for scenario 1 and 2.

Table 4.67 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/m^3	mg/m^3			mg/m^3	mg/m^3		
Production								
Scenario 1:Chloroform used as intermediate(closed batch process)	5.6	10	2	**iii**	5.6	25	4.5	**iii**
Scenario 2:Chloroform used as solvent in the synthesis of chemicals (closed batch process)	10	10	1	**iii**	10	25	2.5	**iii**

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.

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

sRV = standard respiratory volume

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

[1] TGD 2005 Appendix VIII, part 2 B4

$ABS_{derm - Human} = 10\%$

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 4.60) to calculate the MOS.

Table 4.68 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 4.69.

For repeated dose toxicity by dermal route, **conclusion ii** is reached for scenario 1and 2.

Table 4.69 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		
Production								
Scenario 1: Chloroform used as intermediate(closed batch process)	0.024	8.2	342	**ii**	0.66	8.2	12	**iii**
Scenario 2: Chloroform used as solvent in the synthesis of chemicals (closed batch process)	0.024	8.2	342	**ii**	1.164	8.2	7	**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)

Table 4.70 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 4.69.

For combined exposure **conclusion iii** is reached for scenarios 1 and 2.

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.

[1] TGD 2005 Appendix VIII, Part 2 B7

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.

TCNES did not succeed in taking a decision on a conclusion on the endpoint mutagenicity as for a conclusion (ii) or (iii) there was not enough evidence which could be supported by the majority of the member states and for a conclusion (i) no test proposal could be supported. Therefore the risk assessment of chloroform cannot be finalized under the ESR program.

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 4.71. Results and conclusions are presented in Table 4.72.

Table 4.71 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 4.72 Occupational risk assessment for local carcinogenicity

	Inhalation (local)			
	Exposure	N(L)OAEC	MOS	Conclusion
	mg/m^3	mg/m^3		
Production				
Scenario 1: Chloroform used as intermediate(closed batch process)	5.6	25	4	**iii**
Scenario 2: Chloroform used as solvent in the synthesis of chemicals (closed batch process)	10	25	3	**iii**

For inhalation (local), **conclusion iii** is reached for scenario 1 and 2.

<u>Inhalation (systemic)</u>

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 4.73. Results and conclusions are presented in Table 4.76.

For inhalation, **conclusion iii** is reached for scenario 1 and 2.

Table 4.73 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.

$$\text{corrected dermal N(L)OAEL} = \text{inhalatory N(L)OAEC} \times sRV_{mouse} \times \frac{ABS_{inh\text{-}mouse}}{ABS_{derm\text{-}human}}$$ [1]

sRV = standard respiratory volume

ABS $_{inh - mouse}$ = 80%

ABS $_{derm - Human}$ = 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 4.60) to calculate the MOS.

Table 4.74 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

[1] TGD 2005 Appendix VIII, part 2 B4

Calculated MOSs are compared with Reference MOS in Table 4.76.

For dermal route **conclusion ii** is reached for scenario 1 and 2.

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.

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

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)

Table 4.75 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 scenarios 1 and 2.

[1] TGD 2005 Appendix VIII, Part 2 B7

Table 4.76 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/m³	mg/m³			mg/kg/day	mg/kg			mg/kg/day	mg/kg		
Production												
Scenario 1:Chloroform used as intermediate(closed batch process)	5.6	25	4	**iii**	0.024	8.2	342	**ii**	0.66	8.2	12	**iii**
Scenario 2:Chloroform used as solvent in the synthesis of chemicals (closed batch process)	10	25	2	**iii**	0.024	8.2	342	**ii**	1.164	8.2	7	**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 4.80 and compared to Reference MOS given in Table 4.77.

Conclusion ii is reached for all occupational scenarios.

Table 4.77 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.

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

$ABS_{\text{oral–mouse}} = 100\%$

$ABS_{\text{derm–Human}} = 10\%$

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 4.60) to calculate the MOS.

Table 4.78 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 4.80.

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.

[1] TGD 2005 Appendix VIII, Part 2 B5

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

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

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

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

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

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

bw = 70 kg (worker body weight)

Table 4.79 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 iii is reached for scenarios 1 and 2.

Table 4.80 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/m3	mg/m3			mg/kg	mg/kg			mg/kg/day	mg/kg		
Production												
Scenario 1:Chloroform used as intermediate(closed batch process)	5.6	2000	357	**ii**	0.024	16	667	**ii**	0.66	16	24	**iii**

[1] TGD 2005 Appendix VIII, Part 2 B7

Scenario 2:Chloroform used as solvent in the synthesis of chemicals (closed batch process)	10	2000	200	**ii**	0.024	16	667	**ii**	1.164	16	14	**iii**

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 4.84 and compared to Reference MOS given in Table 4.81.

Table 4.81 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 iii** is reached for scenario 1 and 2.

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.

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

sRV = standard respiratory volume

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

$ABS_{derm-Human} = 10\%$

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 4.60) to calculate the MOS.

Table 4.82 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 4.84.

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)

[1] TGD 2005 Appendix VIII, Part 2 B7

Table 4.83 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 iii is reached for scenarios 1 and 2.

Table 4.84 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/ m^3	mg/ m^3			mg/kg	mg/kg			mg/kg /day	mg/kg		
Production												
Scenario 1:Chloroform used as intermediate(closed batch process)	5.6	50	9	**iii**	0.024	13.6	567	**ii**	0.66	13.6	21	**iii**
Scenario 2:Chloroform used as solvent in the synthesis of chemicals (closed batch process)	10	50	5	**iii**	0.024	13.6	567	**ii**	1.164	13.6	12	**iii**

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**
Scenario1: Chloroform used as intermediate (closed batch process)	MOS	44	148	5	10				2 (local) **4.5 (syst)**	342	12		4 427 16	357 667 24	9 567 21
	Concl.	**ii**	**ii**	**iii**	**iii**			**ii**	**iii**	**ii**	**iii**	**i**	**iii inh local** **iii inh** **ii dermal** **iii combi**	**ii inh** **ii dermal** **iii combi**	**iii inh** **ii dermal** **iii combi**
Scenario2: Chloroform used as solvent in the synthesis of chemicals (closed batch process)	MOS	25	148	3	5				1 (local) **2.5 (syst)**	342	7		3 427 9	200 667 14	5 567 12
	Concl.	**iii**	**ii**	**iii**	**iii**			**ii**	**iii**	**ii**	**iii**	**i**	**iii inh local** **iii inh** **ii dermal** **iii combi**	**ii inh** **ii dermal** **iii combi**	**iii inh** **ii dermal** **iii 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.

Chloroform is also a by-product chemical associated with disinfection of swimming pool water; chloroform is originated by the reaction of disinfecting agents with organic substances and not intentionally used. Consequently, it was agreed that the Risk Characterisation of chloroform as a by-product chemical should not be presented in the Chloroform risk assessment but rather than in the Sodium Hypochlorite RAR. Any risk identified in scenario 3 for workers as swimming instructors, lifeguards, competitive swimmers and for consumers as child swimmers and adult swimmers should be addressed in the Sodium Hypochlorite RAR (results of RC for scenario 3 are presented in Annex 1 for information).

4.1.3.4 Humans exposed via the environment

The estimation of the indirect exposure of humans via the environment is presented in the EUSES calculation file. The total daily intake based on the local environmental concentrations due to production and the different uses are presented in Table 4.85.

Table 4.85 : Total daily intake due to local environmental exposures

Scenario	DOSE TOT (MG/KG BW/DAY)
Production :	
Site A :	6.73 E^{-3} $mg.kg^{-1}.d^{-1}$
Site B :	9.87 E^{-5} $mg.kg^{-1}.d^{-1}$
Site C :	5.55 E^{-4} $mg.kg^{-1}.d^{-1}$
Site D :	3.68 E^{-3} $mg.kg^{-1}.d^{-1}$
Site E :	2.65 E^{-3} $mg.kg^{-1}.d^{-1}$
Site F :	1.96 E^{-3} $mg.kg^{-1}.d^{-1}$
Site G :	5.75 E^{-4} $mg.kg^{-1}.d^{-1}$
Site H :	7.93 E^{-4} $mg.kg^{-1}.d^{-1}$
Site I :	2.66 E^{-4} $mg.kg^{-1}.d^{-1}$
Site J :	5.19 E^{-3} $mg.kg^{-1}.d^{-1}$
HCFC Production	**5.49 E^{-3} $mg.kg^{-1}.d^{-1}$**
Dyes and Pesticide Production	1.17 E^{-3} $mg.kg^{-1}.d^{-1}$
Other applications	2.24 E^{-3} $mg.kg^{-1}.d^{-1}$
Uses as a solvent	**5.48 E^{-2} $mg.kg^{-1}.d^{-1}$**
Losses as a by-product during chemical manufacturing	1.71 E^{-2} $mg.kg^{-1}.d^{-1}$

The highest indirect exposure is estimated for the use for HCFC production and its use as a solvent. The human intakes via different routes due to the use of chloroform as a solvent are presented in Table 4.86.

Table 4.86 : Different routes of intake from human exposure via the environment due to local and regional exposure

	Local exposure due to the use of chloroform as a solvent		Regional exposure	
	Predicted concentration	Estimated daily dose (mg/kg bw/d)	Predicted concentration	Estimated daily dose (mg/kg bw/d)
Drinking water	0.239 mg/L	0.00682	5.49×10^{-4} mg/L	1.57×10^{-5}
Fish	6.2 mg/kg	0.0102	10.8×10^{-3} mg/kg	1.77×10^{-5}
Leaf crops	1.75×10^{-3} mg/kg	0.00003	1.93×10^{-6} mg/kg	3.38×10^{-8}
Root crops	4.25×10^{-3} mg/kg	0.00002	1.09×10^{-3} mg/kg	6×10^{-6}
Meat	6.88×10^{-5} mg/kg	< 0.00001	1.14×10^{-7} mg/kg	4.92×10^{-10}
Milk	2.33×10^{-4} mg/kg	< 0.00001	3.88×10^{-7} mg/kg	3.11×10^{-9}
Air	0.132 mg/m^3	0.0377	0.145 µg/m^3	4.13×10^{-5}
Total daily dose (mg/kg bw/d)		0.0548		8.07×10^{-5}

The highest exposures are to be expected through intake of drinking water, intake of fish and through intake of air.

4.1.3.4.1 Exposure via air

In the EUSES calculations the local exposure due to the use of chloroform as a solvent is estimated at 0.132 mg/m^3 (estimated daily dose 0.0377 mg/kg bw/d) following production, whereas the regional exposure is 0.145 µg/m^3 (estimated daily dose 4.13×10-5 mg/kg bw/d).

There are no concerns for sensitisation and therefore conclusion (ii) is reached for this endpoint. Skin and eye irritation are irrelevant to indirect exposure via the environment and hence conclusion (ii) is also reached for these endpoints.

Respiratory tract

The starting point for the risk assessment is the rat inhalatory LOAEC of 50 mg/m^3 (Templin et al., 1996a). Taking into account intra- and interspecies differences, a minimal MOS of 75 (factors of 10 for intra- and 2.5 for interspecies differences, 3 for LOAEC to NOAEC Extrapolation) is applicable. A margin of safety (MOS) of 379 can be calculated for the local production scenario (**conclusion ii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>3.4E+5), and a conclusion ii can be drawn.

Repeated dose toxicity by inhalation (local)

The starting point for the risk assessment is the rats LOAEC of 10 mg/m^3 (2 ppm) (Templin et al., 1996a). Taking into account intra- and interspecies differences, a minimal MOS of 150 (factors of 10 for intra- and 2.5 for interspecies differences, 2 duration of the study, 3

extrapolation LOAEC to NOAEC) is applicable. A margin of safety (MOS) of 76 can be calculated for the local production scenario (**conclusion iii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>6.8E+4), and a conclusion ii can be drawn.

Repeated dose toxicity (systemic)

The starting point for the risk assessment is the mouse inhalatory NOAEC of 25 mg/m^3 (Templin et al., 1998; Yamamoto et al., 2002). Taking into account intra- and interspecies differences, a minimal MOS of 25 (factors of 10 for intra- and 2.5 for interspecies differences) is applicable. A margin of safety (MOS) of 189 can be calculated for the local production scenario (**conclusion ii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>1.7E+5), and a conclusion ii can be drawn.

Mutagenicity

Conclusion i applies with regard to mutagenicity of chloroform.

Carcinogenicity

The starting point for the risk assessment is the mouse inhalatory NOAEC of 25 mg/m^3 (Yamamoto et al., 2002). Taking into account intra- and interspecies differences, a minimal MOS of 25 (factors of 10 for intra- and 2.5 for interspecies differences) is applicable. A margin of safety (MOS) of 189 can be calculated for the local production scenario (**conclusion ii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>1.7E+5), and a conclusion ii can be drawn.

Reproductive toxicity

The starting point for the risk assessment of fertility is the mouse oral NOAEL of 16 mg/kg (Chapin et al., 1997). Assuming an oral absorption value of 100% for mice, this NOAEL corresponds to an internal no-effect dose of 16 mg/kg bw/day. Taking into account intra- and interspecies differences, a minimal MOS of 175 (factors of 10 for intra- and 17.5 (7*2.5) for interspecies differences) is applicable. A margin of safety (MOS) of 424 can be calculated for the local production scenario (**conclusion ii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>3.8E+5), and a conclusion ii can be drawn.

The starting point for the risk assessment of development is the rat inhalatory NOAEC of 50 mg/m^3 (Baeder & Hoffman, 1991). Taking into account intra- and interspecies differences, a minimal MOS of 25 (factors of 10 for intra- and 2.5 for interspecies differences) is applicable. A margin of safety (MOS) of 379 can be calculated for the local production scenario (**conclusion ii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>3.4E+5), and a conclusion ii can be drawn.

4.1.3.4.2 Exposure via food and water

In this section a combined risk characterisation was conducted for food and water with air included. When a concern has been identified for the combined exposure, the risk characterisation was performed for food and water only.

As far as the exposure to chloroform via drinking water, in the EU risk assessment of sodium hypochlorite (E.C., 2002), chloroform concentration in drinking water due to water chlorination was reported to be in the range of 11.7 – 13.4 µg/l (see section 3.1.1.3.2.1. of this report). IARC studies with chlorinated drinking water gave no evidence for carcinogenicity of chloroform in humans. A drinking-water guideline value of 200 mg/litre for an excess lifetime cancer risk of 10^{-5} has been recommended for chloroform by the World Health Organisation in 1993 and confirmed in the 2000 edition of the quality standards for drinking water (WHO, 2000).

In the EU Drinking Water Directive (Council Directive 98/83/EC), a guideline value of 100 mg trihalomethanes/litre is given for an excess lifetime cancer risk of 10^{-6}. On this basis a 70 years exposure of human to a drinking water containing 100 mg chloroform/litre could lead to one additional cancer for each 1,000,000 persons. This value, which corresponds to an acceptable daily intake of about 5.7 mg/kg/d, is considerably higher than the chloroform concentration measured in drinking water and even in surface water. Consequently the exposure to chloroform via drinking water can be considered as negligible.

In the EUSES calculations the local total daily intake (external exposure) is estimated at 54.8 µg/kg bw/day following production, whereas the regional total daily intake is 0.087 µg/kg bw/day.

Repeated dose toxicity

The starting point for the risk assessment is the mouse inhalatory NOAEC of 25 mg/m^3 (Templin et al., 1998; Yamamoto et al., 2002). Assuming an inhalation absorption value of 80% for mice, this NOAEC corresponds to an internal no-effect dose of 8.2 mg/kg bw/day. Taking into account intra- and interspecies differences, a minimal MOS of 175 (factors of 10 for intra- and 17.5 (7*2.5) for interspecies differences) is applicable. A margin of safety (MOS) of 150 can be calculated for the local production scenario (**conclusion iii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>1E+5), and a conclusion ii can be drawn.

A margin of safety (MOS) of 480 can be calculated for the local production scenario, taking in account the estimated daily dose resulting from food and water only (0.0548 - 0.0377 = 0.0171 mg/kg bw/d).

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.

TCNES did not succeed in taking a decision on a conclusion on the endpoint mutagenicity as for a conclusion (ii) or (iii) there was not enough evidence which could be supported by the majority of the member states and for a conclusion (i) no test proposal could be supported. Therefore the risk assessment of chloroform cannot be finalized under the ESR program.

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

Carcinogenicity

The starting point for the risk assessment is the mouse inhalatory NOAEC of 25 mg/m^3 (Yamamoto et al., 2002). Assuming an inhalation absorption value of 80% for mice, this NOAEC corresponds to an internal no-effect dose of 8.2 mg/kg bw/day. Taking into account intra- and interspecies differences, a minimal MOS of 175 (factors of 10 for intra- and 17.5 (7*2.5) for interspecies differences) is applicable. A margin of safety (MOS) of 150 can be calculated for the local production scenario (**conclusion iii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>1E+5), and a conclusion ii can be drawn.

A margin of safety (MOS) of 480 can be calculated for the local production scenario, taking in account the estimated daily dose resulting from food and water only (0.0548 - 0.0377 = 0.0171 mg/kg bw/d).

Reproductive toxicity

The starting point for the risk assessment of fertility is the mouse oral NOAEL of 16 mg/kg (Chapin et al., 1997). Assuming an oral absorption value of 100% for mice, this NOAEL corresponds to an internal no-effect dose of 16 mg/kg bw/day. Taking into account intra- and interspecies differences, a minimal MOS of 175 (factors of 10 for intra- and 17.5 (7*2.5) for interspecies differences) is applicable. A margin of safety (MOS) of 292 can be calculated for the local production scenario (**conclusion ii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (2E+5), and a conclusion ii can be drawn.

The starting point for the risk assessment of development is the rat inhalatory NOAEC of 50 mg/m^3 (Baeder & Hoffman, 1991). Assuming an oral absorption value of 80% for rats, this NOAEC corresponds to an internal no-effect dose of 13.6 mg/kg bw/day. Taking into account intra- and interspecies differences, a minimal MOS of 100 (factors of 10 for intra- and 10 (4*2.5) for interspecies differences) is applicable. A margin of safety (MOS) of 248 can be calculated for the local production scenario (**conclusion ii**). Because the estimated human daily intake doses via food, water and air are lower for the other local scenarios it can be concluded that Chloroform is of negligible risk for man exposed indirectly via the environment. For the regional scale the MOS is even higher (>1.6E+5), and a conclusion ii can be drawn.

4.1.3.4.3 Summary of risk characterisation for exposure via the environment

	N(L)OAEL	Local scale		Regional scale	
		MOS	Conclusion	MOS	Conclusion
Exposure via air					
Respiratory tract	50 mg/m^3	379	**ii**	>$3.4\times10^{+5}$	**ii**
RDT (local)	10 mg/m^3	76	**iii**	>$6.8\times10^{+4}$	**ii**
RDT	25 mg/m^3	189	**ii**	>$1.7\times10^{+5}$	**ii**
Carcinogenicity	25 mg/m^3	189	**ii**	>$1.7\times10^{+5}$	**ii**
Reproductive toxicity fertility	16 mg/kg	424	**ii**	>$3.8\times10^{+5}$	**ii**
Reproductive toxicity developement	50 mg/m^3	379	**ii**	>$3.4\times10^{+5}$	**ii**
Exposure via food and water					
RDT	25 mg/m^3	150	**iii**	>$1\times10^{+5}$	**ii**
Carcinogenicity	25 mg/m^3	150	**iii**	>$1\times10^{+5}$	**ii**
Reproductive toxicity fertility	16 mg/kg	292	**ii**	$2\times10^{+5}$	**ii**
Reproductive toxicity developement	50 mg/m^3	248	**ii**	>$1.6\times10^{+5}$	**ii**

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

Chloform is not flammable (no flash point). It has no explosive or oxidising properties.

The vapour pressure (209 hPa) being higher than 0.01 kPa at 293.15 K, chloroform could be considered as a Volatile Organic Compound (VOC). Therefore, the inhalation route is taken into account for the human risk assessment.

It can be concluded that there is no concern for human health with regard physico-chemical properties (conclusion ii).

5 RESULTS [1]

5.1 INTRODUCTION

5.2 ENVIRONMENT

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) is applied to the use of chloroform as a solvent for all compartments. Conclusion (iii) is also applied to production sites A, C, E and J, to all uses and to unintended releases for the sewage compartment.

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) is applied to all levels of the life cycle of chloroform (except the use as a solvent) for the following compartments: aquatic, sediment, atmosphere, terrestrial and non-compartment specific effects relevant to the food chain.

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 1, Manufacture of chloroform and HCFC 22 for acute toxicity (inhalation and dermal), sensitisation, RDT (dermal), carcinogenicity (dermal), fertility (inhalation and dermal) and development (dermal).
- Scenario 2, Chloroform as intermediate or solvent in the synthesis of chemicals for acute toxicity (dermal), sensitisation, RDT (dermal), carcinogenicity (dermal), fertility (inhalation and dermal) and development (dermal).

[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.

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 1, Manufacture of chloroform and HCFC 22 for acute toxicity (combined), irritation, RDT (inhalation and combined), carcinogenicity (inhalation and combined), fertility (combined) and development (inhalation and combined).
- Scenario 2, Chloroform as intermediate or solvent in the synthesis of chemicals for acute toxicity (inhalation and combined), irritation, RDT (inhalation and combined), carcinogenicity (inhalation and combined), fertility (combined) and development (inhalation and combined).

5.3.1.2 Consumers

Conclusion for Consumers are reported in Annex 1

5.3.1.3 Humans exposed via the environment

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:

- Human exposed via the environment for exposure via air, food and water.

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:

- Human exposed via the environment at local scale for RDT (local) via air; RDT and carcinogenicity via air, food and water.

5.3.2 Human health (risks from physico-chemical properties)

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.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw*, *b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests

EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient

Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$

pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative

vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

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