

# Chloroform

## Toxicological overview

### Key Points

#### *Kinetics and metabolism*

- Chloroform is readily absorbed from the lungs, gastrointestinal tract and skin
- Chloroform undergoes metabolism via an oxidative or a reductive pathway
- Chloroform is mainly excreted via the lungs unchanged or as the main metabolite carbon dioxide

#### *Health effects of acute exposure*

- Harmful, irritant and possibly carcinogenic after prolonged exposure
- Acute inhalation of chloroform can cause systemic effects such as excitement, nausea, vomiting followed by dizziness, ataxia and drowsiness. Convulsions, coma and death may occur following substantial exposures
- Delayed effects of chloroform exposure include renal and hepatic damage (up to 48 hours post exposure)
- Local effects are observed following inhalation (irritation of the nose and throat), ingestion (burning sensation of the mouth and throat), ocular (stinging) and dermal exposure (irritation and redness)

#### *Health effects of chronic exposure*

- Chronic inhalation or ingestion of chloroform may cause hepatic damage
- The International Agency for Research on Cancer (IARC) classified chloroform as a category 2B carcinogen i.e. possibly carcinogenic to humans

## Toxicological Overview

### *Summary of Health Effects*

Local effects following inhalation of chloroform include shortness of breath and irritation of the nose and throat. Acute inhalation can cause systemic effects such as, excitement, nausea and vomiting followed by ataxia, dizziness, drowsiness. Exposure to high concentrations may cause convulsions, coma and death due to respiratory failure or cardiac arrhythmias. Individuals who survive an acute exposure to chloroform may develop hepatic dysfunction and renal damage several days later. Chronic inhalation of chloroform may cause hepatic damage.

Following acute ingestion of chloroform, systemic effects as seen following inhalation may occur as well as a burning sensation in the mouth and throat, nausea and vomiting. Hepatic toxicity has been reported following chronic ingestion of chloroform.

Following acute dermal exposure to chloroform, local effects may include irritation and redness. Prolonged contact may result in systemic toxicity, dermatitis and burns.

Acute ocular exposure to chloroform may cause a stinging sensation and exposure to chloroform liquid can cause irritation of the conjunctival tissue, corneal necrosis and ulcers.

Chloroform does not have any significant mutagenic properties.

The IARC has concluded that there is inadequate evidence in humans for the carcinogenicity of chloroform but sufficient evidence in experimental animals for the carcinogenicity of chloroform, and it is therefore classified as possibly carcinogenic to humans (Group 2B). The tumours seen in animal bioassays were only at dose levels producing chronic cytotoxicity in the target organs (liver and kidney) and were believed to be secondary to the sustained cell proliferation that this induced.

Data from experimental studies in animals indicates that adverse effects on development would not be expected at exposures below those producing overt toxicity in the maternal animals. Similarly no adverse effects on fertility were seen at dose levels below those producing maternal systemic toxicity (reduction in weight gain and hepatotoxicity).

### ***Kinetics and Metabolism***

Chloroform is readily absorbed from the lungs, gastrointestinal tract and skin [1, 2]. Approximately 60 – 80% of inhaled chloroform is absorbed [1].

Following absorption, chloroform is distributed throughout the body. Human and animal inhalation and oral exposure studies have recorded high chloroform concentrations in adipose tissue, brain, liver, kidneys, adrenals and blood [1, 2].

Chloroform is metabolised in humans and animals by oxidative or reductive cytochrome P450 dependent pathways. It has been suggested that chloroform metabolism mainly occurs via the oxidative pathway [3, 4].

In the presence of oxygen (oxidative pathway) chloroform undergoes oxidative dechlorination to form trichloromethanol, which spontaneously dehydrochlorinates to form phosgene. Subsequent hydrolysis of phosgene forms hydrochloric acid and the main metabolite carbon dioxide [3, 4]. Phosgene may also react with cellular macromolecules (such as enzymes, proteins or the polar heads of phospholipids), leading to the formation of covalent adducts. The adducts can interfere with molecular function which may result in loss of cellular function and cell death [3].

In the absence of oxygen (reductive pathway), the main metabolite is dichloromethyl free radical, which is extremely reactive and forms covalent adducts with microsomal enzymes and fatty acid tails of phospholipids. This may result in the loss of microsomal enzyme activity and can also lead to lipid peroxidation [3].

Almost all tissues are capable of metabolising chloroform, the highest levels of metabolism occur in the liver, kidney and nasal mucosa.[3]. Chloroform is excreted via the lungs unchanged or in the form of the metabolite carbon dioxide, with small amounts of either detectable in the urine and faeces. [2].

### ***Sources and Route of Exposure***

Exposure to chloroform may occur via inhalation (contaminated air), ingestion (contaminated food, beverages and water) and possibly through dermal contact (showering, cleaning and swimming) [1, 2, 4].

Chloroform may be released into the environment from its use, production and transport. It is also indirectly formed as a result of the reaction of chlorine with organic compounds [1]. Processes known to contribute to the indirect formation and emission of chloroform include paper bleaching with chlorine and chlorination of municipal water, swimming pools and waste water [1, 2]. Under certain conditions some bacteria can dehalogenate carbon tetrachloride to release chloroform [2].

The majority of chloroform that enters the environment will eventually enter the atmosphere, due to its volatility [1, 2]. The degradation of chloroform involves a reaction with hydroxyl radicals; the half-life for degradation is reported to be approximately 100 – 180 days [1].

Chloroform is a by-product of water chlorination and is therefore present in drinking water. The drinking water quality guideline for chloroform is 0.2 mg L<sup>-1</sup> [5]. It has also been detected in sea, waste and ground waters [1].

Atmospheric chloroform levels in remote, urban and source-dominated areas have been reported to range from 0.1 – 0.25, 0.9 – 9.9 and 4.1 – 110  $\mu\text{g m}^{-3}$ , respectively. The indoor air concentration of chloroform can be up to ten-fold higher than outdoor air concentrations. The use of chlorinated water in homes is thought to significantly contribute to levels of chloroform in indoor air [1]. Concentrations of chloroform may exceed 1000  $\mu\text{g m}^{-3}$  in a shower cubical, as a result of volatilisation from hot water [2].

Exposure to chloroform may also occur in the workplace. Individuals that work at or near facilities that manufacture or use chloroform (e.g. drinking water-plants, waste water-treatment plants and pulp and paper plants) may be exposed to considerably higher levels of chloroform than the general population [2].

## Health Effects of Acute / Single Exposure

### Human Data

#### General toxicity

The main target organs of chloroform-induced toxicity are the central nervous system and the liver. The main symptoms of acute chloroform poisoning depend upon the concentration of chloroform absorbed, rather than the route of exposure [6]. Older clinical reports involving patients exposed to chloroform as a method of anaesthesia, have reported that exposure to 40,000 ppm chloroform ( $195,600 \text{ mg m}^{-3}$ ) for several minutes may be lethal [2, 7]. Dizziness and vertigo were observed in humans exposed to 920 ppm chloroform ( $4498 \text{ mg m}^{-3}$ ) for 3 minutes [2]. Chloroform can cause symptoms of illness at  $2490 \text{ mg m}^{-3}$  and discomfort at levels below  $249 \text{ mg m}^{-3}$  [1].

Chloroform also causes progressive central nervous system depression. Initial symptoms include excitement, nausea and vomiting followed by ataxia, dizziness, drowsiness, convulsions and coma [6, 8]. In severe cases paralysis of the medullary respiratory centre may lead to respiratory failure and sudden death [6].

Early death following exposure to high levels of chloroform is often due to cardiac arrhythmias. Chloroform may also cause hypotension [1, 2, 6].

In the past, chloroform was extensively used to induce and maintain medical anaesthesia. Its use as an anaesthetic was abandoned because it caused hepatic damage and deaths due to respiratory and cardiac arrhythmias and failure [1]. Chloroform levels of 3,000 – 30,000 ppm ( $14,670 - 146,700 \text{ mg m}^{-3}$ ) were used to induce anaesthesia [2].

#### Inhalation

Inhalation of chloroform can cause severe acute toxicity, as described in the general toxicity section. Inhalation of concentrated chloroform vapour causes irritation of exposed mucous membranes, including the nose and throat [7]. Shortness of breath may also occur [6].

Other effects reported following the use of chloroform as an anaesthetic include hypothermia, depression of gastrointestinal tract motility, respiratory acidosis, hyperglycaemia, ketoacidosis, constriction of the spleen and an increase in leukocyte count [1, 6].

#### Ingestion

Ingestion of chloroform can cause severe acute toxicity, as described in the general toxicity section. Local effects following ingestion of chloroform include gastrointestinal irritation with abdominal pain, nausea, vomiting and diarrhoea [6].

There are considerable inter-individual differences in susceptibility to chloroform following acute ingestion. Serious illness has been reported following ingestion of 7.5 g chloroform. Fatal doses have been reported to be as low as 14.8 g, whereas other individuals have

survived a dose of 270 g chloroform [1, 2]. The mean lethal dose for adults is estimated to be approximately 45 g [1].

### **Dermal / ocular exposure**

Chloroform may be absorbed across the skin and prolonged exposure may result in systemic toxicity, as described in the inhalation section. Skin exposure causes irritation and redness at the site of contact, especially sensitive areas such as the eyelids and neck [6]. Prolonged contact may result in burns and dermatitis [7].

Liquid chloroform splashed in the eye causes immediate burning pain, tearing and reddening of the conjunctiva. The corneal epithelium is usually injured but regeneration is prompt and as a rule the eye returns to normal within 1 - 3 days [9].

### **Delayed effects following an acute exposure**

Individuals who survive an acute exposure to chloroform may develop hepatic dysfunction several days later. Symptoms include prostration, nausea, vomiting, jaundice, coma and in some cases death [1]. Necrosis of the liver may occur, resulting in increased concentrations of serum bilirubin and transaminases [6].

Renal damage is less common than hepatic damage but it may occur following acute exposure to chloroform. Renal tubular necrosis and renal dysfunction (anuria, proteinuria and uraemia) have been reported in individuals who were exposed to chloroform as an anaesthetic [4].

## ***Animal and In-Vitro Data***

### **General toxicity**

The acute toxic effects of chloroform in animals are similar to those observed in humans. The main target organs are the liver, kidneys and the central nervous system.

### **Inhalation**

Exposure to chloroform at concentrations in the range of 10 – 100 g m<sup>-3</sup> resulted in anaesthesia in mice, rabbits, guinea pigs and cats. Cardiac effects including decreased diastolic pressure, reduction of stroke volume and decreased cardiac output were reported in rabbits exposed to 224 mg m<sup>-3</sup> chloroform for one minute. Liver toxicity was observed in mice and rats exposed to 490 and 1410 mg m<sup>-3</sup> chloroform, respectively. Kidney effects including necrosis of the proximal and distal tubules were reported in male mice exposed to 3400 – 5400 mg m<sup>-3</sup> chloroform for 1 – 3 hours [1].

### **Ingestion**

Oral administration of chloroform can result in anaesthesia. The ED<sub>50</sub> in mice for acute neurological effects (ataxia, incoordination and anaesthesia) was 484 mg kg<sup>-1</sup> bw. Hepatic

effects (centrilobular fatty infiltration) have been observed in mice at doses as low as 35 mg kg<sup>-1</sup> bw and chloroform administered at 250 mg kg<sup>-1</sup> bw by gavage has caused hepatic necrosis in mice [1, 4]. In rats, piloerection, sedation, flaccid muscle tone, ataxia, prostration, reduced food intake and kidney and liver effects following exposure to ≥ 546 mg kg<sup>-1</sup> bw [1].

The acute oral LD<sub>50</sub> for chloroform in mice ranges from 36 – 1366 mg kg<sup>-1</sup> bw and for rats it ranges from 450 – 2000 mg kg<sup>-1</sup> bw.

### **Dermal / ocular exposure**

Moderate necrosis, hyperaemia and eschar formation were observed when chloroform (1000 or 4000 mg kg<sup>-1</sup> bw) was applied under a patch to the skin of rabbits for 24 hours. Systemic effects including weight loss and degenerative changes in the tubules of the kidneys were also reported [1, 7].

Eye contact with chloroform liquid has resulted in corneal injury and conjunctivitis in rabbits [1, 7].

## Health Effects of Chronic / Repeated Exposure

### *Human Data*

#### **General toxicity**

Hepatic and central nervous system toxicity are the main effects following long term exposure to chloroform.

#### **Inhalation**

In an occupational study, workers exposed to 14 – 400 ppm (68 – 1956 mg m<sup>-3</sup>) developed toxic hepatitis and other effects, including jaundice, nausea and vomiting without fever [2, 7].

In another study, 68 workers were occupationally exposed to chloroform concentrations of 10 – 1000 mg m<sup>-3</sup> for 1 – 4 years. A higher frequency of hepatitis was found in the group of workers compared with city inhabitants. Seventeen of the workers had hepatomegaly, three of which developed hepatitis [1].

Chronic occupational exposure to 375 – 1330 mg m<sup>-3</sup> chloroform, with a peak concentration of 5680 mg m<sup>-3</sup>, for periods of 3 to 10 years was reported to cause lassitude, thirst, gastrointestinal distress, frequent and scalding urination, lack of concentration, depression and irritability in 8 exposed workers. Nine workers who were exposed to lower concentrations of chloroform (110 – 350 mg m<sup>-3</sup> for 10 – 24 months) also experienced the same effects, but to a lesser degree [1].

A case study of an individual who abused chloroform for approximately 12 years reported delusions, hallucinations, psychotic episodes and convulsions. Withdrawal symptoms, including dysarthria and ataxia have been reported following the abrupt discontinuation of chloroform use [2].

#### **Ingestion**

There is limited data available regarding the health effects of chronic exposure to chloroform in humans.

Hepatitis and nephrosis were observed in a man who ingested cough mixture containing 1.6 – 2.6 g of chloroform daily for 10 years [6]. No renal or hepatic effects were observed in humans who used ingested 0.34 – 0.96 mg kg<sup>-1</sup> bw day<sup>-1</sup> chloroform in mouthwash for up to 5 years [2].

#### **Genotoxicity**

There are currently no data available regarding the genotoxic effects of chloroform in humans.



## **Carcinogenicity**

Chlorinated drinking water typically contains chloroform, other trihalomethanes and a wide variety of other disinfection by-products. There has been considerable epidemiological research into the question of associations between chlorinated drinking-water and various diseases, and this research continues. The results have raised concern that chlorination by-products in drinking-water may increase the risk of certain cancers. However, no conclusions can be drawn specifically about chloroform from these studies.

The expert advisory Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) considered that the evidence was inconclusive, but advised that efforts to minimise exposure to chlorination by-products remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking-water (see the statement at <http://www.advisorybodies.doh.gov.uk/coc/drink.htm>) [10]. The committee will be reviewing these issues again shortly.

The International Agency for Research on Cancer (IARC) has concluded that there is inadequate evidence in humans for the carcinogenicity of chloroform but sufficient evidence in experimental animals for the carcinogenicity of chloroform. It is classified as possibly carcinogenic to humans (Group 2B) [11].

## **Reproductive and developmental toxicity**

There are no data available regarding the reproductive and developmental effects of chloroform *per se*.

There has been considerable epidemiological research into the question of associations between chlorinated drinking-water and various diseases, and this research continues. The results have raised concern that chlorination by-products in drinking-water may increase the risk of certain adverse reproductive outcomes. However, no conclusions can be drawn specifically about chloroform from these studies.

The expert advisory Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) considered that the evidence was inconclusive, but advised that efforts to minimise exposure to chlorination by-products remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking-water (see the statement at <http://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2004/chlorwater>) [12]. The committee will be reviewing these issues again shortly.

## **Animal and In-Vitro Data**

### **Inhalation**

The liver, kidneys and nasal cavity are primary targets for chloroform-induced toxicity following chronic exposure [1, 2, 4]. In a medium-term study, BDF<sub>1</sub> mice were exposed to chloroform 0, 59, 123, 245, 490, or 980 mg m<sup>-3</sup> 6 hours day<sup>-1</sup>, 5 days week<sup>-1</sup>, for 13 weeks. All the females survived but reduced growth and deaths occurred in males in all chloroform groups. At exposure levels of 59 mg m<sup>-3</sup> and above, male mice showed kidney (necrosis of the proximal tubules) and nasal cavity effects (bone thickening and degeneration of the

olfactory epithelium). Nasal cavity toxicity was observed in female mice at all exposure levels. Liver toxicity was not observed in either sex at up to 245 mg m<sup>-3</sup> chloroform, abnormal cells were seen in females at 490 mg m<sup>-3</sup> and swelling and necrosis at 980 mg m<sup>-3</sup> [4].

### **Ingestion**

Several studies have reported nephrotoxic and hepatotoxic effects in laboratory animals chronically exposed to chloroform [1, 2, 4]. In one study it was reported that chloroform administered by gavage in corn oil was significantly more hepatotoxic compared with chloroform administered in aqueous emulsion. Male and female B6C3F<sub>1</sub> mice were administered 0, 130 and 270 mg kg<sup>-1</sup> chloroform for 90 days. Liver body weight ratios were higher in all dose groups when chloroform was administered in corn oil. Disruption of hepatic architecture including cirrhosis was observed in the group administered the high dose of chloroform in corn oil. No pathological changes were observed in any of the animals administered chloroform in aqueous emulsion [1].

Hepatic damage was observed in beagle dogs administered 15 mg kg<sup>-1</sup> chloroform in toothpaste over a period of 7.5 years [1].

### **Genotoxicity**

The genotoxic potential of chloroform has been tested in several *in-vitro* and *in-vivo* assays. The majority of results for the Ames *Salmonella* assay and *Escherichia coli* test system were negative, both with and without metabolic activation. Tests for unscheduled DNA synthesis have produced negative results in human and animal cells. Chloroform did not induce chromosome aberrations in human lymphocytes in culture. Sister chromatid exchange assays using human lymphocytes gave mixed results. Chloroform also induced sister chromatid exchange in mouse bone marrow cells *in vivo* and chromosome aberrations in rat bone marrow *in vivo* [4]. Three out of four bone marrow micronuclei *in-vivo* studies in mice produced negative results, the fourth study gave a weekly positive result [1, 4]. Overall, the evidence suggests that chloroform does not have significant genotoxic potential.

### **Carcinogenicity**

The carcinogenicity of chloroform has been investigated in rodents. Chloroform produced renal adenomas and carcinomas in male mice exposed via inhalation or ingestion and in male rats following oral exposure. An increase in incidence of hepatocellular adenomas and carcinomas was observed in mice of either sex, exposed to chloroform by ingestion. Thyroid tumours were noted in female rats orally administered chloroform. The IARC has concluded that there is sufficient evidence in experimental animals for the carcinogenicity of chloroform [11, 13].

There is experimental evidence to suggest that kidney and liver tumours in rodents are secondary to the cytotoxic effects of metabolites (such as phosgene and hydrogen chloride) and persistent associated reparative cell proliferation [1, 4]. At the high dose levels used in the carcinogenicity bioassay in which tumours were produced, chloroform induced cytotoxicity and regenerative cell proliferation in the target organs for cancer consistent with the mode of action for tumourgenesis in the liver and kidney involving cytotoxicity [11].

### Reproductive and developmental toxicity

Developmental studies involving oral exposure in rats have reported effects on the fetus (reduced fetal body weight) but only at dose levels that were maternally toxic. No teratogenic effects were observed [4].

Few data were found regarding the effects of chloroform on reproduction.

In a continuous breeding reproductive toxicity study in CD-1 mice no effects were reported on fertility or reproduction in the F<sub>1</sub> generation. The mice had been exposed *in utero* and during lactation (as a result of maternal treatment) and then by gavage at 41 mg kg<sup>-1</sup> bw day<sup>-1</sup>, through to young adulthood. The dose level did not produce any effect on fertility, but did induce hepatotoxicity in the parent animals [4].

A three generation reproductive toxicity study has been reported using ICR mice. Chloroform was administered at 0.1, 1 or 5 mg ml<sup>-1</sup> in drinking water. The only statistically significant effects noted were reduced fertility, litter size, gestation index and viability index, at the highest dose level. Some evidence of hepatotoxicity was seen at all dose levels and mortality was seen at the highest dose.[1].

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