Chloroform

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Citation for most recent IARC review

IARC Monograph 73, 1999

Current evaluation

Conclusion from the previous Monograph:
There is *inadequate evidence* in humans for the carcinogenicity of chloroform. There is *sufficient evidence* in experimental animals for the carcinogenicity of chloroform. Chloroform is *possibly carcinogenic to humans (Group 2B)*.

Exposure and biomonitoring

Occupational exposure to chloroform (trichloromethane) may occur during its production and use as a solvent and chemical intermediate. The general population may be exposed as a result of the presence of chloroform in chlorinated drinking water, ambient air, and some foods.

Exposures to chloroform via showering, bathing, and swimming have been characterized much better since 1999, and these studies show that blood levels are usually higher via these routes than via drinking chlorinated water (Xu & Weisel, 2005; Caro & Gallego, 2007; Zwiener et al., 2007). Chloroform exposure has also been documented in various occupations (Ruder, 2006) and in microenvironments, such as restaurants (Loh et al., 2006). Chloroform exposure also may be occurring when people use triclosan-containing antibacterial soaps (Fiss et al., 2007).

Considerable efforts have been made to establish the levels of chloroform exposure in the general population using NHANES data (Riederer et al., 2009), and computational toxicology based on these data suggest that 95% of the U.S. population represented by NHANE III data had chloroform exposures ≤7 μg/L in tap water and ≤0.2 μg/L in ambient household air (Lyons et al., 2008). The levels of chloroform in alveolar air appear to be the most sensitive biomarker of exposure relative to ambient air levels; urinary levels of chloroform are less sensitive than breath levels (Caro & Gallego, 2008). Concentrations of chloroform in breath appear highly associated with levels in water, and blood and breath levels are also associated with each other (Gordon et al., 2006). New studies have attempted cancer risk assessments based on exposure assessments via oral and dermal/inhalation routes (Liao et al., 2007; Tan et al., 2006, 2007; Wang et al., 2007; Panyakapo et al., 2008; Hamidin et al., 2008).

A survey of levels of chloroform, as well as of other disinfection by-products (DBPs), in U.S. drinking water (Weinberg et al., 2002) showed that chloroform at high concentrations (>60 μg/L) likely reflects the presence of relatively high levels of other trihalomethanes and other DBPs. In contrast, low concentrations of chloroform (<20 μg/L) prevent any conclusions regarding the levels of other DBPs in the water. As indicated from some of the new epidemiology and mechanistic studies described below, chloroform is probably not an ideal

surrogate for assessing risk for bladder cancer for humans from chlorinated water. Other possibilities are total DBP, total trihalomethanes (THM), brominated THMs, or MX. The panel recommended future IARC evaluation of "water chlorination disinfection-by products."

Cancer in humans

(*inadequate*, Vol 73, 1999)
Drinking water is the main medium by which people are exposed to chloroform. However, drinking water is a complex mixture, and only a few epidemiology studies have had adequate exposure assessments to permit associations between cancer and specific compounds in the water.

Bladder cancer

Since IARC vol. 73, a number of epidemiological studies have been published on the association between water DBP exposure and the risk of bladder cancer, the two most important ones being a pooled analysis of previous case-control studies by Villanueva et al. (2004) (data from only one of the six pooled studies was included in IARC vol. 73), and a new case-control study from Spain (Villanueva et al., 2007). In both studies, interview data were collected on residential history and daily tap water consumption; and in the latter study data were also collected on bathing, showering, and swimming in pools. The investigators collected data on annual average THM levels, water source history, and year of start of chlorination from 123 municipalities. In addition, 113 chlorinated water samples were collected, and the THM levels were measured. The data sources were merged to create the exposure variables. In both studies there was a clear dose-response relationship between average residential THM levels and risk of bladder in men but not in women (see Table).

Average residential THM level µg/L*	Men		Women	
	Villanueva et al. (2004)	Villanueva et al. (2007)	Villanueva et al. (2004)	Villanueva et al. (2007)
0-1	1.00	1.00	1.00	1.00
>1-5/8.0	1.10 (0.92-1.31)		0.99 (0.72-1.36)	
>5/8.0 – 25/26.0	1.26 (1.05-1.51)	1.53 (0.95-2.48)	0.86 (0.63-1.18)	0.40 (0.13-1.27)
>25/26.0-50/49.0	1.25 (1.04-1.50)	2.34 (1.36-4.03)	1.04 (0.76-1.43)	1.14 (0.31-4.10)
>50/49.0	1.44 (1.20-1.73)	2.53 (1.23-5.20)	0.93 (0.67-1.28)	1.50 (0.26-8.61)
Trend p value	<0.001	<0.01	0.753	0.61

* Limits: Villanueva et al. (2004)/Villenueva et al. (2007)

Villanueva et al. (2007) reported detailed analysis of bladder cancer risk by average ingestion of THM, THM exposure in shower and bath, and life-time hours swimming in pools. Compared to men with the lowest level, men with the highest THM ingestion had a RR of 1.61 (95% CI 1.06-2.44), men with the highest shower/bath THM exposure had a RR of 2.01 (95% CI 1.23-3.28), and men swimming in pools most frequently had a RR of 1.59 (95% CI 1.01-2.51); all associations showed a dose-response pattern. For women the three relative risks were 0.47 (95% CI 0.15-1.51); 2.26 (95% CI 0.58-8.90); and 1.19 (95% CI 0.30-4.72);

with none of the associations showing a dose-response pattern. Further analysis showed that the dose-response relationship was found for persons with two deleted *GSTT1-1* alleles (-/-), but not for persons with at least one non-deleted *GSTT1-1* allele (+/+ or +/-) (Cantor et al., 2006). In all THM exposure categories, the risk of bladder cancer decreased with increasing water intake (Michaud et al., 2007).

Other cancer sites

Based on the observation of an increased risk of bladder cancer following dermal exposure in Spain, a re-analysis was undertaken of data from a case-control study on skin cancer from New Hampshire, U.S. High exposure to THM (>40 µg/L) versus low exposure (<1 µg/L) was associated with an increased risk of both basal cell carcinoma, OR 2.4 (95% CI 0.9-6.7) and squamous cell carcinoma, OR 2.1 (95% CI 0.7-7.0) (Karagas et al., 2008).

The risk of colon and rectal cancer in relation to THM exposure was investigated in a case-control study in southern Ontario, Canada. Interview data on residence history was collected and merged with water supply data. A positive dose-response association was found for THM-years and colon cancer in men; highest versus lowest, OR 1.74 (95% CI 1.25-2.43). No association was found for women, nor was there any association between THM-years and the risk of rectal cancer (King et al., 2000).

The risk of rectal cancer was studied also in Monroe County, Western New York State, U.S. No association was found between THM exposure (µg/L) and risk of rectal cancer, OR 1.01 (95% CI 0.98-1.03), while the risk associated with bromoform exposure (µg/L) was slightly increased, OR 1.20 (95% CI 1.05-1.35) (Bove et al., 2007).

A large Canadian case-control study showed a borderline significant increase in risk of chronic myelocytic leukemia with duration of exposure to chlorinated surface water. In contrast, this exposure tended to be protective against chronic lymphocytic leukemia and hairy cell leukemia (Kasim et al., 2006).

Cancer in experimental animals

(*sufficient*, Vol 73, 1999)

Two, 2-year rodent bioassays of chloroform had been performed at the time of the 1999 IARC monograph, and two additional studies have been published since then. Yamamoto et al. (2002) showed that inhalation exposure to chloroform was not carcinogenic to male or female F344 rats but did induce kidney tumors in male BDF mice and liver tumors in female mice. Nagano et al. (2006) found no increase in cancer at any organ in male F344 rats exposed to chloroform in their drinking water (1000 ppm w/w) or in rats exposed by inhalation to chloroform at 25, 50, or 100 ppm (v/v) for 6 h/day and 5 day/week. However, male F344 rats exposed by both routes had increased frequencies of renal-cell adenomas and carcinomas, atypical renal-tubule hyperplasia, increased cytoplasmic basophilia, increased dilated tubular lumens of the kidney, and increased urinary glucose. Thus, the combined exposure enhanced carcinogenicity and chronic toxicity in the proximal tubule of the male rat kidney.

In summary, four studies in rats showed that chloroform induced kidney tumors by gavage, kidney tumors by drinking water in one study but no tumors in another study, no tumors in

two studies by inhalation, and kidney tumors by a combined drinking water/inhalation exposure. Three studies in mice showed that chloroform induced liver tumors by gavage, liver and kidney tumors by inhalation, and no tumors by drinking water. Thus, chloroform induced kidney tumors in three studies in rat, liver tumors in two studies in mouse, and kidney tumors in one study in mouse. A small case-control study of dogs showed no association between chlorinated household water and risk of bladder cancer (Backer et al., 2008).

Mechanisms of carcinogenicity

Chloroform is an anomaly among the trihalomethanes in that its mechanism of action is entirely different from that of the other THMs. All of the other trihalomethanes are activated to mutagens by *GSTT1-1*, and as discussed below, there is emerging evidence that these other trihalomethanes (i.e., brominated THMs) be the relevant causative agents for the bladder cancer associated with chlorinated drinking water. In contrast, chloroform is not mutagenic, and a likely mechanism for its carcinogenicity has been summarized by Schoeny et al. (2006).

The postulated mechanism involves oxidative metabolism primarily by CYP2E1 to produce cytotoxic metabolites, especially phosgene. Although reductive metabolism could produce mutagenic metabolites, this pathway would likely operate only under conditions of high exposure, as in the chronic bioassay. These metabolites injure and kill cells, resulting in regenerative cell proliferation. If this tissue injury and consequent proliferation is sustained, as it is in the chronic rodent studies, mutations, epigenetic changes, etc. likely occur that, with selection, lead to the kidney tumors observed in rats.

The concept that increased cellular proliferation in response to cellular toxicity is enough to produce tumors is an oversimplification of other crucial events taking place within transformed or initiated cells. Events such as increased growth advantage of initiated cells either by increase in cellular proliferation or decreased apoptosis are important determinants in the pathway to carcinogenesis. For instance, at an approximately 18% increase in cellular proliferation, carbon tetrachloride produced an 85% hepatic tumor incidence in mice (Nagno et al., 2007). At a similar frequency of cellular proliferation, chloroform produced a 12% hepatic tumor incidence (Yamamoto et al., 2002). The difference in tumor incidence between the two chemicals suggests that cellular processes downstream of increased cellular proliferation may be crucial. Furthermore, several studies reviewed in IARC Vol 73 reported that chloroform has the ability to inhibit the development of tumors, although the exact mechanism is not known.

As noted by Schoeny et al. (2006), the postulated mechanism for chloroform described above is presumed to be relevant to humans because all three key steps (metabolism to phosgene, toxicity, and cell proliferation) are known to occur in humans. However, children are not expected to have increased susceptibility because CYP2E1 is expressed minimally in fetal and neonatal tissue, and the developing organism is not especially sensitive to cytotoxic agents at low doses.

Philip et al. (2006) showed that repeated exposure of Swiss Webster mice to 150 mg of chloroform/kg/day by gavage for 30 days, followed by a single, normally lethal dose of chloroform (750 mg/kg) by gavage 24 h after the last exposure, permitted 100% of the mice to

survive, whereas 90% of the mice given only the lethal dose died. The authors noted that the pre-exposure resulted in 40% lower chloroform levels in the blood and increased tissue repair in the kidney (but not the liver) and that the protective effect of pre-exposure was not associated with enhanced detoxification or decreased bioactivation of chloroform.

Fabrizi et al. (2003) found that phosgene, one of the metabolites of chloroform, forms a covalent bond with human histone H2B in vitro. Because modified histones could alter gene expression, among other cellular functions, this epigenetic process could play a role in the carcinogenic mechanism of chloroform.

Chloroform does not induce colorectal tumors in rodents, but some of the brominated trihalomethanes do. A mechanistic study by DeAngelo et al. (2002) in which mice were exposed to various trihalomethanes in drinking water for 13 weeks found that the brominated trihalomethanes, but not chloroform, induced aberrant crypt foci in the colon. These potentially pre-neoplastic cells may develop into colon tumors, and studies such as this and others suggest that chloroform is unlikely to be the cause of such tumors.

As noted above, chloroform is usually the most prevalent DBP in drinking water; however, it is not necessarily a surrogate for the levels or presence of other DBPs. In addition, other DBPs, most likely the brominated trihalomethanes, are more probable as potential causes of the bladder cancer associated with drinking water than is chloroform. Richardson et al. (2007) have summarized the carcinogenicity, occurrence levels, and mutagenicity of more than 80 DBPs, and their current hypothesis, which is based on a set of studies in transgenic bacteria, rodents, humans, and the epidemiology study of Villanueva et al. (2007), is that exposure to the brominated trihalomethanes via dermal/inhalation routes through swimming/showering/bathing may result in systemic distribution of the compounds via the blood, and in the bladder these could be activated to mutagens by *GSTT1-1* and initiate bladder cancer. In contrast, THMs consumed via the oral route would likely be inactivated in the liver by CYP2E1 (Richardson et al., 2007).

Research needs and recommendations

To date, neither chloroform nor any other DBP has been tested in animals for any health effects whatsoever, including cancer, via the dermal route. Given the extensive and well-documented exposure to DBPs, including chloroform, via this route, and the emerging evidence that dermal/inhalation exposure to trihalomethanes (especially the brominated ones) may be most associated with bladder cancer, this type of experiment in rodents is necessary to explore the role that route of exposure may play in carcinogenicity of chloroform.

Additional epidemiology studies are needed such as that of Villanueva et al. (2007) in which route of exposure is stratified. In addition, detailed DBP exposure assessment, especially of chloroform and the brominated trihalomethanes, is needed to explore better any link between chloroform in drinking water and the risk for bladder or colon cancer. A large New England bladder cancer case-control study is currently being undertaken. A pooled analysis is under way of bladder cancer case-control studies from Spain, France, and Finland, and of colorectal cancer case-control studies from Spain and Italy (Nieuwenhuijsen et al., 2009). Epidemiological studies of high-exposure groups, such as competition swimmers, are

warranted. Pool attendants and indoor life guards are other potentially exposed groups worth investigating. Finally, follow up is recommended of cohorts of nurses and doctors exposed to chloroform when it was used as an anesthetic gas.

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