CHLOROFORM

This substance was considered by previous working groups, in 1971 (IARC, 1972), 1978 (IARC, 1979) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

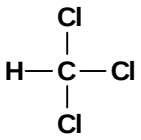
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 67-66-3
Deleted CAS Reg. No.: 8013-54-5
Chem. Abstr. Name: Trichloromethane
IUPAC Systematic Name: Chloroform
Synonyms: HCC 20; R 20; R 20 (refrigerant); trichloroform

1.1.2 *Structural and molecular formulae and relative molecular mass*



$CHCl_3$ Relative molecular mass: 119.38

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with characteristic odour (Budavari, 1996)
(*b*) *Boiling-point*: 61.1°C (Lide, 1997)
(*c*) *Melting-point*: –63.6°C (Lide, 1997)
(*d*) *Density*: 1.4832 g/cm^3 at 20°C (Lide, 1997)
(*e*) *Solubility*: Slightly soluble in water; miscible in ethanol and ethyl ether; soluble in acetone (Lide, 1997)
(*f*) *Volatility*: Vapour pressure, 21.3 kPa at 20°C; relative vapour density (air = 1), 4.12 (National Toxicology Program, 1991)
(*g*) *Octanol/water partition coefficient (P)*: log P, 1.97 at 20°C (Verschueren, 1996)
(*h*) *Conversion factor*: mg/m^3 = 4.88 × ppm

1.2 Production and use

Worldwide commercial production of chloroform amounted to about 440 000 tonnes in 1987. Significant amounts are also produced as by-products in the chlorination of water and in the bleaching of paper pulp (WHO, 1994). Information available in 1995 indicated that chloroform was produced in 19 countries (Chemical Information Services, 1995). The volume of commercial production of chloroform in the United States was approximately 229 000 tonnes in 1991 and 216 000 tonnes in 1993 (International Trade Commission, 1991, 1993).

Chloroform is used in pesticide formulations, as a solvent and as a chemical intermediate. Its use as an anaesthetic and in proprietary medicines is banned in some countries (WHO, 1994). Chloroform is used as a solvent for fats, oils, rubber, alkaloids, waxes, gutta-percha and resins; as a cleansing agent; in fire extinguishers to lower the freezing temperature of carbon tetrachloride; and in the rubber industry (Budavari, 1996). It is used in the manufacture of fluorocarbon plastics, resins, refrigerants and propellants. It has been used as an analgesic, muscle relaxant, carminative, flavouring agent, preservative, bactericide, heat-transfer medium and as a counter-irritant in liniments (National Toxicology Program, 1991).

1.3 Occurrence

1.3.1 *Natural occurrence*

Chloroform is not known to occur naturally.

1.3.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 1998), approximately 96 000 workers in the United States were potentially exposed to chloroform. Occupational exposure to chloroform may occur during its production and during its use as a solvent and chemical intermediate.

1.3.3 *Environmental occurrence*

The concentrations of chloroform found in the oceans in remote regions are typically several nanograms per litre. In coastal waters, inland rivers, lakes and groundwater, the concentrations range from several nanograms per litre in rural and remote areas up to 100 µg/L or more in areas directly affected by industrialized sources. In drinking-water treated by chlorination, the concentrations are typically 10–100 µg/L (WHO, 1994; National Library of Medicine, 1998a).

The reported concentrations of chloroform in ambient air range from < 1 µg/m^3 in remote regions to around 10 µg/m^3 in urban areas. Indoor air concentrations in residences and offices can be higher, and the average concentration in the air above indoor swimming pools has been reported to be about 100 µg/m^3 (WHO, 1994).

Chloroform has also been detected in a variety of foods at concentrations of < 1 to 90 µg/kg (WHO, 1994).

On the basis of estimates of mean exposure from various media, the general population is exposed to chloroform principally in food (approximately 1 μg/kg bw per day), drinking-water (approximately 0.5 μg/kg bw per day) and indoor air (0.3–1 μg/kg bw per day). The estimated intake from outdoor air is considerably less (0.01 μg/kg bw per day). The estimated total intakes of individuals living in dwellings supplied with tap-water containing relatively high concentrations of chloroform may be up to 10 μg/kg bw per day (WHO, 1994).

According to the United States Environmental Protection Agency Toxic Chemical Release Inventory for 1987, 12 000 000 kg of chloroform were released into the air, 560 000 kg were discharged into water, 7000 kg were disposed of by underground injection, and 18 000 kg were released onto the land from manufacturing and processing facilities in the United States. By 1996, 4 200 000 kg were released into the air, 150 000 kg were discharged into water, 21 000 kg were disposed of by underground injection, and 15 000 kg were released onto the land (National Library of Medicine, 1998b).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (1997) has recommended 49 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposure to chloroform in workplace air. Values of 9.8–240 mg/m^3 have been used as standards or guidelines in other countries (International Labour Office, 1991).

WHO (1993) has established an international drinking-water guideline for chloroform of 200 μg/L.

2. Studies of Cancer in Humans

2.1 Ecological studies

In some ecological studies, chloroform or total trihalomethane concentrations in drinking-water or surrogate exposure indicators, such as plain chlorination of drinking-water, were correlated with cancer mortality or incidence, but the site-specific excess risks were inconsistent across studies. The results of such studies are not presented here, as ecological studies suffer from several drawbacks, deriving from the impossibility of linking exposure to the end-point at the individual level; further limitations of ecological studies are described in the Preamble. In addition, exposure was usually assessed on the basis of cross-sectional sampling. Although the risk estimates derived in ecological studies are usually adjusted for some sociodemographic parameters at the geographical level, but ample possibility is left for uncontrolled confounding.

2.2 Cohort studies

These studies are summarized in Table 1.

Wilkins and Comstock (1981) followed-up 14 553 male and 16 227 female residents over 25 years of age of Washington County, MD (United States) using data from a census

Table 1. Cohort studies of chloroform exposure or water chlorination and cancer

Reference	Population/ follow-up	Sex	Exposure	Cancer site	RR	95% CI	Comments
Wilkins & Comstock (1981)	Washington County, MD, USA, 1963–75	14 583 men and 16 247 women	Surface and chlorination (average, 107 µg/L chloroform) versus deep well and no chlorination	*Incidence in men*			Adjustment for age, marital status, education, smoking, church attendance, adequacy of housing, persons per room
				Liver	0.71	0.19–3.5	
				Kidney	0.78	0.27–2.7	
				Bladder	1.8	0.80–4.8	
				Incidence in women			
				Liver	1.8	0.64–6.8	
				Kidney	1.0	0.26–6.0	
				Bladder	1.6	0.54–6.3	
				Mortality, men and women			
				Liver	3.0	0.92–15	
				Kidney	2.8	0.7–23	
				Breast	2.3	1.2–4.9	
				Bladder	2.2	0.7–9.4	
				Oesophagus	1.8	0.5–8.7	
				Cervix	1.7	0.6–6.7	
				Leukaemia	1.6	0.9–3.1	
				Rectum	1.4	0.7–3.2	
				Ovary	1.3	0.4–4.9	
				Lung	1.0	0.6–1.6	
				Colon	0.9	0.6–1.4	
				Prostate	0.9	0.4–2.1	
				Stomach	0.6	0.3–1.4	
Doyle *et al*. (1997)	Iowa Women's Health Study (28 237), 1963–75	Women	Chloroform concentration from statewide survey of water supplies	Colon	1.7	1.1–2.5	RR for highest categories (> 13 µg/L; p (trend) < 0.05); adjustment for a number of factors (see text)
				Lung	1.2	0.7–2.0	
				Melanoma	1.1	0.4–3.2	
				All cancers	1.2	1.0–1.5	

RR, relative risk; CI, confidence interval

conducted in 1963. Cancer incidence and mortality rates in 1963–75 were assessed in two subcohorts: people exposed to chlorinated surface water (average chloroform concentration, 107 μg/L) and users of water from deep wells with no chlorination. Risk ratios were calculated by contrasting the two cohorts, with adjustment for age, marital status, education, smoking, church attendance, adequacy of housing and number of persons per room. Incidence rates were reported for several subsites of cancer. No significant elevations or deficits were seen for cancers of the liver, kidney or urinary bladder in either men or women who drank chlorinated surface water. The only significant excess risk was reported for death from breast cancer (relative risk, 2.7; 95% confidence interval [CI], 1.2–4.9), and excesses of borderline significance were found for liver cancer (relative risk, 3.0; 95% CI, 0.92–15).

The cohort involved in the Iowa Women's Health Study in the United States, consisting of 41 836 women aged 55–69 in 1986, was followed-up for cancer incidence through 31 December 1993 (Doyle *et al*., 1997). The source of the drinking-water for each cohort member was assessed from a mail survey in 1989. Women who reported having drunk municipal or private well-water for less than the past 10 years were excluded. Data on 252 municipal water supplies in 1979 and 856 municipal water systems in 1986–87 were used to assess exposure. All women who lived in the same community and reported drinking municipal water were assigned the same concentration of trihalomethanes, including chloroform. The chloroform concentrations in 1986–87 were categorized as below the detection limit (reference) or 1–2, 3–13 and 14–287 μg/L. The incidences of a number of cancers were estimated in the categories. Significantly increasing trends over the categories were observed for cancers of the colon (risk ratio for highest versus lowest category, 1.7 [95% CI, 1.1–2.5]; $p < 0.01$ for trend), lung (1.8 [95% CI, 0.97–2.6]; $p = 0.025$ for trend), melanoma (3.4 [95% CI, 1.3–8.6]; $p = 0.049$ for trend) and all cancers (1.2 [95% CI, 1.0–1.5]; $p < 0.01$ for trend) after adjustment for some potential confounding variables. The excess of cancers at all sites was driven predominantly by excess incidences of cancers of the colon, lung and endometrium and malignant melanoma. All of the relative risks were adjusted for age, education, smoking, physical activity, fruit and vegetable intake, total energy intake, body mass index and waist-to-hip ratio.

2.3 Case–control studies

These studies are summarized in Table 2.

2.3.1 *Multiple sites*

Brenniman *et al*. (1980) studied 3208 deceased cases of gastrointestinal and urinary tract cancers and 43 666 non-cancer deaths as controls in Illinois (United States). All of the deaths were extracted from state death certificate tapes over the period 1973–76 and classified according to residence in communities served by chlorinated or unchlorinated groundwater on the basis of data obtained from water-treatment plants by referring to the 1963 inventory of municipal water facilities. Populations using surface water supplies and those living in Cook County (mostly Chicago) were excluded. Slight excesses (odds

Table 2. Case–control studies of chloroform exposure or water chlorination and cancer

Reference	Population	Sex	Exposure	Cancer site		Odds ratio	95% CI or *p* value	Comments
Multiple sites								
Brenniman *et al*. (1980)	Illinois, USA; 3208 deaths 1973–76 from gastrointestinal and urinary-tract cancers; 43 666 non-cancer controls	Men and women	Chlorinated/unchlorinated water supplies based on residence	Oesophagus	Men	0.9	NS	
					Women	1.1	NS	
				Stomach	Men	0.9	NS	
					Women	1.1	NS	
				Colon/rectum	Men	1.1	NS	
					Women	1.2	< 0.05	
				Colon	Men	1.0	NS	
					Women	1.2	NS	
				Rectum	Men	1.1	NS	
					Women	1.3	NS	
				Liver	Men	0.9	NS	
					Women	1.1	NS	
				Gall-bladder	Men	0.6	NS	
					Women	1.2	NS	
				Digestive (excl. liver)				
					Men	1.0	NS	
					Women	1.1	< 0.05	
				Urinary bladder	Men	1.1	NS	
					Women	0.7	NS	
				All gastrointestinal and urinary				
				Total	Men			
					Women	1.0	NS	
				SMSA	Men	1.1	NS	
					Women	1.0	NS	
				Urban	Men	1.3	< 0.05	
					Women	1.0	NS	
						1.3	< 0.25	
				Rural	Men	1.1	NS	
					Women	1.3	NS	

Table 2 (contd)

Reference	Population	Sex	Exposure	Cancer site		Odds ratio	95% CI or *p* value	Comments
Multiple sites (contd)								
Alavanja *et al*. (1980)	New York State, USA, 7 counties 3446 from gastro-intestinal and urinary-tract cancers; 3444 matched controls. Unknown number of cases of lung cancer	Men and women	Chlorination/no chlorination at usual place of residence	All gastrointestinal and urinary				
				Total	Men	2.1	< 0.005	
					Women	1.4	< 0.005	
				Urban	Men	3.6	< 0.005	
					Women	2.2	< 0.005	
				Lung				
				Total	Men	1.8	< 0.005	
					Women	1.5	NS	
				Urban	Men	3.2	< 0.005	
					Women	2.9		
				Oesophagus	Men	2.4	< 0.05	
					Women	1.3	NS	
				Stomach	Men	1.7	< 0.025	
					Women	2.2	< 0.025	
				Colon	Men	2.0	< 0.005	
					Women	1.3	NS	
				Rectum	Men	2.3	< 0.005	
					Women	1.3	NS	
				Liver and kidney				
					Men	2.8	< 0.005	
					Women	1.5	NS	
				Pancreas	Men	2.6	< 0.005	
					Women	1.3	NS	
				Urinary bladder	Men	2.0	< 0.005	
					Women	0.8	NS	
				Total	Men	2.1	< 0.005	
					Women	1.4	< 0.005	

Table 2 (contd)

Reference	Population	Sex	Exposure	Cancer site	Odds ratio	95% CI or *p* value	Comments
Multiple sites (contd)							
Gottlieb *et al*. (1981, 1982); Gottlieb & Carr (1982)	Louisiana, USA 11 349 deaths from cancer; 22 698 non-cancer deaths	Men and women	Chlorination/no chlorination of water source at death	Rectum	1.8	1.3–2.6	
				Lung	1.4	1.0–1.8	
				Breast	1.5	1.2–2.0	
			Chlorination level	Rectum	1.3	0.9–1.8	Low (< 1.09 ppm)
					1.7	1.2–2.4	High (> 1.09 ppm)
Kanarek & Young (1982)	Wisconsin, USA [numbers not given]	Women	Chlorinated/unchlorinated water supplies	Oesophagus	0.30	NS	Multiple adjustments
				Stomach	1.3	NS	
				Colon	1.5	< 0.02	
				Rectum	1.6	NS	
				Liver	1.3	NS	
				Pancreas	1.5	NS	
				Kidney	0.5	NS	
				Urinary bladder	1.4	NS	
				Lung	0.7	0.6	
				Brain	4.7	< 0.03	
				Breast	0.8	NS	
			Organic contamination and chlorination	Colon	1.8	0.03	
			Chlorinated surface water		2.8	0.01	
			Purified chlorinated		1.7	0.01	
			Unpurified chlorinated		1.5	0.05	
			Organic contamination and chlorination	Brain	6.9	0.03	
			Unpurified chlorinated		4.4	0.04	

Table 2 (contd)

Reference	Population	Sex	Exposure	Cancer site	Odds ratio	95% CI or *p* value	Comments
Multiple sites (contd)							
Siemiatycki (1991)	Montréal, Canada 99 oesophageal cancer, 251 stomach cancer, 497 colon cancer, 257 rectal cancer, 116 pancreatic cancer, 857 lung cancer, 449 prostate cancer, 484 bladder cancer, 177 kidney cancer, 103 skin melanoma, 215 non-Hodgkin lymphoma	Men	Chloroform, occupational	Prostate Lung	4.0 8.8	1.4–12 1.2–65	90% CI; multiple adjustments French Canadians
Colon/rectum							
Lawrence *et al*. (1984)	New York State (USA) teachers, deaths 395 cases, 395 controls	Women	Chlorinated/unchlorinated water supplies	Colorectal cancer	1.1	0.8–1.4	90% CI
Young *et al*. (1987)	Wisconsin, USA 347 cases; 639 cancer controls; 611 population controls	Men and women	Cumulative trihalomethane (mg), population controls	Colon *Over lifetime* < 100 100–300 > 300 *Over past 10 years* < 33 33–99 > 99	 1.0 1.1 0.73 1.0 0.9 1.0	 0.7–1.8 0.4–1.2 0.5–1.5 0.4–2.3	Response rate, 65%; results for general population controls are presented

Table 2 (contd)

Reference	Population	Sex	Exposure	Cancer site	Odds ratio	95% CI or *p* value	Comments
Urinary bladder							
Cantor *et al*. (1987)	USA; 2805 cases; 5258 population controls	Men and women	Years of residence with chlorinated surface drinking-water source	*Men, low consumption (below median)*			Newly diagnosed cases; interviews
				Never	1.0		
				≥ 60 years	0.8	0.5–1.5	*p* trend = 0.7
				Women, low consumption (below median)			
				Never	1.0		
				≥ 60 years	1.7	0.7–4.0	*p* trend = 0.4
				Men, high consumption (above median)			
				Never	1.0		
				≥ 60 years	1.2	0.7–2.1	*p* trend = 0.4
				Women, high consumption (above mean)			
				Never	1.0		
				≥ 60 years	3.2	1.2–8.7	*p* trend = 0.02
			Nonsmokers only	*Men, low consumption (below median)*			
				Never	1.0		
				≥ 60 years	1.3	0.4–4.4	*p* trend = 0.6
				Women, low consumption (below median)			
				Never	1.0		
				≥ 60 years	4.3	1.3–14	*p* trend = 0.02

Table 2 (contd)

Reference	Population	Sex	Exposure	Cancer site	Odds ratio	95% CI or *p* value	Comments
Urinary bladder (contd)							
Cantor *et al*. (1987) (contd)				*Men, high consumption (above median)*			
				Never	1.0		
				≥ 60 years	3.7	1.1–12	*p* trend = 0.02
				Women, high consumption (above median)			
				Never	1.0		
				≥ 60 years	3.6	0.8–15	*p* trend = 0.2
Zierler *et al*. (1988)	Massachusetts, USA; mortality; 614 cases, 1074 population controls	Men and women	Chlorine versus chloramine by residence	Crude analysis	1.3	1.1–1.7	Lifetime exposure
					1.2	1.0–1.5	Usual exposure
				Adjusted analysis	1.6	1.2–2.1	Lifetime exposure
					1.4	1.1–1.8	Usual exposure
							See text for adjustment
McGeehin *et al*. (1993)	Colorado; USA; 261 cases, 327 population controls	Men and women	Crude analysis by years of exposure to chlorinated water and by smoking status	*Nonsmokers*			Telephone interviews with subjects for residential and water source histories; water utility data
				0 years	1.0		
				1–11 years	0.8	0.2–2.4	
				12–34 years	0.9	0.3–2.3	
				> 34 years	2.9	1.2–7.4	
				Smokers			
				0 years	1.0		
				1–11 years	0.8	0.4–1.4	
				12–34 years	1.4	0.8–2.4	
				> 34 years	2.1	1.1–3.8	
			Adjusted analysis by years of exposure to chlorinated water	0 years	1.0		See text for adjustments
				1–10 years	0.7	0.4–1.3	
				11–20 years	1.4	0.8–2.5	*p* for trend < 0.01
				21–30 years	1.5	0.8–2.9	
				> 30 years	1.8	1.1–2.9	

Table 2 (contd)

Reference	Population	Sex	Exposure	Cancer site	Odds ratio	95% CI or *p* value	Comments
Urinary bladder (contd)							
Cantor *et al.* (1998)	Iowa, USA 1123 cases 1983 controls	Men and women	Total lifetime trihalomethanes (g) Highest category (≥ 2.42)	Men Women	1.8 0.6	1.2–2.7 0.3–1.4	Multiple adjustments *p* for trend = 0.05 *p* for trend, NS
			Lifetime average total trihalomethanes (μg/L) Highest category (≥ 46.4)	Men Women	1.5 0.6	1.0–2.4 0.3–1.3	*p* for trend = 0.02 *p* for trend = 0.33
Colorectal cancer							
Hildesheim *et al.* (1998)	Iowa, USA 560 colon cancers 537 rectal cancers 1983 controls with information on water use	Men and women	Total lifetime trihalomethanes (g) Highest category (≥ 2.42)	Colon Rectum	1.1 1.6	0.7–1.8 1.0–2.6	*p* for trend, NS *p* for trend = 0.08
			Lifetime average total trihalomethanes (g/L) Highest category (≥ 46.4)	Colon Rectum	1.1 1.7	0.7–1.6 1.1–2.6	*p* for trend, NS *p* for trend = 0.01
Brain cancer							
Heineman *et al.* (1994)	Southern Louisiana, New Jersey, Philadelphia, USA; 300 cases 320 controls	Men	Chloroform (occupational)	Astrocytic brain Duration of exposure Never 2–20 years ≥ 21 years	 1.0 0.8 2.3	 0.4–1.4 0.8–6.6	 *p* for trend, NS

Table 2 (contd)

Reference	Population	Sex	Exposure	Cancer site	Odds ratio	95% CI or *p* value	Comments
Brain cancer (contd)							
Heineman *et al*. (1994) (contd)				Cumulative exposure score			
				0			
				Low	1.0		
				Medium	0.8		0.4–1.6
				High	1.3		0.6–2.9
					1.8		0.4–7.8
							p for trend, NS
				Averate intensity			
				0	1.0		
				Low-medium	1.0		0.6–1.7
				High	1.4		0.4–5.2

CI, confidence interval; NS, not significant ($p > 0.05$); SMSA, standard metropolitan statistical area

ratio, 1.2, $p < 0.05$) were found in chlorinated communities for cancers of the colon and rectum combined and for cancers of the total digestive tract only in women. The odds ratios were adjusted for age, sex, urban or rural residence and standard metropolitan statistical area or other areas. The odds ratios for total gastrointestinal and urinary tract cancers were significantly elevated only among women in standard metropolitan statistical areas (odds ratio, 1.3; $p \leq 0.025$) and women in urban areas (odds ratio, 1.2; $p \leq 0.05$). [The Working Group noted that the chloroform concentrations in drinking-water were not given.]

Alavanja *et al*. (1980) reported on a case–control study of 3446 deaths from gastrointestinal and urinary tract cancers conducted in seven counties in New York State (United States) selected for a low rate of immigration and no major change in the source or distribution of water supply during the preceding 15 years. The counties were then classified according to whether the predominant source of drinking-water was chlorinated groundwater, chlorinated surface water or unchlorinated groundwater. The cases and 3444 matched controls and their 'usual place of residence' were identified from New York State death certificates for the period 1968–70. Each residence was classified into one of three exposure categories defined by the drinking-water source of the county. Raised odds ratios, most of which were significant, were observed for cancers of the oesophagus, stomach, large intestine, rectum, liver and kidney, pancreas and urinary bladder (see Table 2). The odds ratios consistently exceeded unity in people of each sex, with one exception: 0.82 for bladder cancer in women. Mortality from lung cancer was also studied [number of deaths not reported], and significantly raised odds ratios ($p < 0.005$) were found for men (odds ratio, 1.8) and in urban areas (odds ratio, 3.2). [The Working Group noted that it is not clear which confounders were adjusted for and that the chloroform concentrations in the drinking-water were not given.]

Gottlieb *et al*. (1981), Gottlieb and Carr (1982) and Gottlieb *et al*. (1982) conducted a multisite case–control study between 1960 and 1975 in parishes in South Louisiana (United States). Cancer deaths were compared with non-cancer deaths individually matched on age, sex, race and year of death. Use of a chlorinated water source at the residence at the time of death was the exposure indicator. Excesses were found for rectal cancer (odds ratio, 1.8; 95% CI, 1.3–2.6), lung cancer (odds ratio, 1.4; 95% CI, 1.0–1.8) and breast cancer (odds ratio, 1.5; 95% CI, 1.2–2.0) in association with concentrations of chlorine greater than or less than 1.09 ppm when compared with no chlorination. There was a suggestion of an exposure–response gradient for rectal cancer only. [The Working Group noted that the chloroform concentrations were not given.]

A multisite case–control study was conducted of deaths from cancer and other causes among white women who had lived for 15–20 years before their death in 28 counties in Wisconsin (United States). Counties with less than a 10% population increase attributed to immigration over the previous 20 years and with both chlorinated and unchlorinated water supplies were selected (Young *et al*., 1981; Kanarek & Young, 1982). Data from all 202 waterworks and the 1970 Wisconsin Waterworks Survey were used to obtain longitudinal data on chlorine concentrations and other water characteristics. Cases of cancers

of the oesophagus, stomach, colon, rectum, liver, pancreas, urinary bladder, kidney, lung, brain and breast and controls were ascertained during a follow-up period in 1972–77. The results were presented as contrasts between chlorinated and unchlorinated water; urbanization, marital status, occupation, age, organic contamination of water, water purification and source depth were included in logistic models. Significant ($p < 0.05$) excess odds ratios were found for cancers of the colon (odds ratio, 1.5; $p < 0.02$) and brain (odds ratio, 4.7; $p < 0.03$). For colon cancer, interactions were found between chlorination and water with organic contamination (odds ratio, 1.8; $p = 0.03$), surface water (odds ratio, 2.8; $p < 0.01$) and purified water (odds ratio, 1.8; $p < 0.01$). Similarly, excesses were observed for brain cancer in association with exposure jointly to chlorination and organic contamination (odds ratio, 6.9; $p = 0.03$) and unpurified water (odds ratio, 4.4; $p = 0.04$). [The Working Group noted that the chloroform concentrations in drinking-water were not given.]

A population-based case–control study of cancer associated with occupational exposure among male residents of Montréal, Canada, aged 35–70, included histologically confirmed cases of several types of cancer, newly diagnosed between 1979 and 1985 in 19 major hospitals (Siemiatycki, 1991). Interviews were carried out with 3730 cancer patients (response rate, 82%) and 533 age-stratified controls from the general population (response rate, 72%). The main cancer sites included were: oesophagus (99 cases), stomach (251 cases), colon (497 cases), rectum (257 cases), pancreas (116 cases), lung (857 cases), prostate (449 cases), bladder (484 cases), kidney (177 cases), skin melanoma (103 cases) and non-Hodgkin lymphoma (215 cases). For each site of cancer analysed, two controls were available: a population control and a control selected from among cases of cancer at the other sites. The interview was designed to obtain lifetime job histories and information on potential confounders. Each job was reviewed by a team of chemists and industrial hygienists who translated the jobs into occupational exposures using a checklist of 293 substances found in the workplace. Chloroform was one of the substances. About 0.7% of the subjects had ever been exposed to chloroform. Among the main occupations to which exposure to chloroform was attributed were nurses' aides and orderlies, dental prosthesis makers and laboratory technicians. No excess risks were seen for cancers at most of the sites examined; however, the odds ratio for prostate cancer, based on six exposed cases, was 4.0 (90% CI, 1.4–12), and the odds ratio for lung cancer among French Canadians, based on six exposed cases, was 8.8 (90% CI, 1.2–65). [The Working Group noted the limited power and the exploratory nature of the study.]

2.3.2 *Cancers of the colon and rectum*

Lawrence *et al*. (1984) reported the results of a study of 395 deaths from colorectal cancer (319 colon, 76 rectal) and 395 deaths from other causes that occurred during 1962–78 among white women teachers in New York State (United States). Cases and controls were matched on age and year of death. A crude analysis for the colorectal cancer risk of teachers who were exposed to chlorinated surface water or who were unexposed (exposed to groundwater containing little or no trihalomethanes) resulted in an odds ratio

of 1.1 (90% CI, 0.79–1.4). An additional analysis with adjustment for water source type, population density at place of residence, marital status and the matching variables gave a similar result.

On the basis of their previous findings of an excess risk for colon cancer associated with exposure to chlorinated water supplies (Kanarek & Young, 1982; see section 2.3.1), Young *et al*. (1987) conducted a case–control study of 347 incident cases of colon cancer and 639 cancer controls, excluding gastrointestinal and urinary tract cancers, identified in the Wisconsin cancer reporting system. A group of 611 population controls was also used. White men and women in whom colon cancer had been diagnosed when they were aged 35–90 were considered eligible. The overall response rate to a self-administered questionnaire on background variables, past water sources, water-drinking and bathing habits, home treatment of tap water, and medical, occupational, social and lifestyle histories was 65%. The exposure of each study subject to trihalomethanes was estimated from an algorithm based on the results of a survey of 81 Wisconsin water supplies, historical data from water facilities, the residential history of the subjects, data on individual water use and other information. Average trihalomethane concentrations of 10–40 μg/L or > 40 μg/L at the place of residence in 1951–81 were not associated with an excess risk for colon cancer, when compared with exposure to < 10 μg/L. A similar result was found with cumulative lifetime exposure to trihalomethanes (in milligrams) over the past 30, over the past 20 and over the past 10 years of exposure.

2.3.3 *Urinary bladder cancer*

Cantor *et al*. (1987) interviewed 2805 patients with urinary bladder cancer aged 21–84 years at the time of diagnosis and 5258 population controls in a case–control study in 10 geographical areas in the United States. The cases were newly diagnosed and histologically confirmed during 1977–78. Controls were frequency matched on sex, age and geographical area. All subjects were administered a questionnaire at home by trained interviewers, which included questions on consumption of tap water during a typical week one year before the interview. A lifetime residential history, with water sources, was ascertained. A total of 1102 water utilities were visited, and utility personnel were interviewed; the water sources were then categorized for chlorination status (chlorination/no chlorination) during various periods. The residential histories of the subjects were linked with year-by-year water source and data on treatment. Logistic regression models with adjustment for sex, age, study area, smoking, high-risk occupation and population size were used with various indicators of water quality. The only significant trends ($p = 0.02$) with duration of residence with a chlorinated surface drinking-water source were found for women whose tap-water consumption was above the median, for nonsmoking women whose tap-water consumption was below the median and for nonsmoking men whose consumption was above the median. [The Working Group noted that the chloroform concentrations in the drinking-water were not given.]

Zierler *et al*. (1988) reported the results of a study of 614 persons with urinary bladder cancer who had died after the age of 44 years during 1978–84 while resident in

Massachusetts (United States) and whose drinking-water had been disinfected with either chlorine or chloramine. Chloramination results in substantially lower concentrations of trihalomethanes than chlorination (Lykins & Koffskey, 1986) and has been used in Massachusetts since 1938. A total of 1074 controls were selected from among persons who had died from cardiovascular disease, cerebrovascular disease, chronic obstructive pulmonary disease or lymphatic cancer. Data on water treatment were obtained from the Massachusetts Water Resources Authority, the United States Environmental Protection Agency and the State Division of Water Supply in the Department of Environmental Quality Engineering. Residence in communities with chlorinated drinking-water was contrasted with residence in communities with chloraminated drinking-water. Information was obtained for each deceased person on his or her residential and smoking history, and mortality odds ratios were calculated. About one-half of the eligible cases and controls were excluded, predominantly because informants could not be located. The crude mortality odds ratio for bladder cancer associated with lifetime exposure to chlorine versus chloramine was 1.3 (95% CI, 1.1–1.7), and that for usual exposure to chlorine versus chloramine was 1.2 (95% CI, 1.0–1.5). Adjustment for age, sex, cigarette pack–years and residence in a community in which at least 3% of the working population had jobs in industries in which there is a high risk for bladder cancer resulted in an odds ratio of 1.6 (95% CI, 1.2–2.1) for lifetime and 1.4 (95% CI, 1.1–1.8) for usual exposure. [The Working Group noted that the concentrations of chloroform in the drinking-water were not given and the possibility of selection bias due to the exclusion of about one-half of the study subjects.]

A population-based study of 327 histologically verified cases of urinary bladder cancer from the State cancer registry matched to 261 controls with other cancers was conducted in Colorado (United States) during 1990–91 (McGeehin *et al.*, 1993). Telephone interviews were used to obtain individual residential and water source histories from living patients and controls; the responses were 78% and 75%, respectively. These data were linked to data from water utilities and the Colorado Department of Health records. More than 34 years of exposure to chlorinated water, contrasted with no such exposure, was associated with increased risks in both nonsmokers (crude odds ratio, 2.9; 95% CI, 1.2–7.4) and smokers (odds ratio, 2.1; 95% CI, 1.1–3.8). Adjustment for coffee consumption, smoking, tap-water intake, family history of urinary bladder cancer, sex and medical history of urinary bladder infection or kidney stones resulted in odds ratios that increased with lifetime years of exposure to chlorinated water, to 1.8 (95% CI, 1.1–2.9) for the highest category (> 30 years) of exposure. The total lifetime trihalomethane concentration was calculated for each subject as a time-weighted mean from data for each water system in Colorado in 1989. The mean lifetime concentration was 620 μg/L for cases and 420 μg/L for controls ($p < 0.001$). [The Working Group noted that the chloroform concentrations were not given.]

Cantor *et al.* (1998) reported on a case–control study of bladder cancer among residents of Iowa (United States) aged 40–85 years. Patients with histologically confirmed bladder cancer were identified through the State Health Registry of Iowa, supplemented

by a rapid reporting system, during 1986–89. Patients, controls and proxies were sent a questionnaire and interviewed by telephone in order to obtain demographic data, smoking history, occupational history, further indicators of lifestyle and medical conditions and the frequency of consumption as an adult inside and outside the home of beverages containing tap-water and other beverages. Lifetime residential histories were recorded, and the water source at each place was identified. For 10% of the patients and none of the controls, the interviews were conducted with proxies. All 280 Iowa water utilities that served at least 1000 persons were contacted for historical information, and at each utility an interviewer collected one or two samples from the clear well where the water enters the distribution system or from nearby in the system. The concentration of total trihalomethanes (grams) and the lifetime average trihalomethane concentration (μg/L) were calculated for 1123 cases and 1983 controls. The logistic regression models included adjustment for age, study period, level of education, high-risk occupation and cigarette smoking. The risk increased significantly with increasing total lifetime dose of trihalomethanes and lifetime average total trihalomethane concentration in men but not in women. The odds ratio for men in the highest total lifetime trihalomethane category (over 2.4 g) was associated with an odds ratio of 1.8 (95% CI, 1.2–2.7), the trend over six increasing categories being significant at $p = 0.05$; for the highest lifetime concentration (> 46 μg/L), the odds ratio was 1.5 (95% CI, 1.0–2.4; p for trend = 0.02). The corresponding odds ratios for women were 0.6 (95% CI, 0.3–1.4) and 0.6 (95% CI, 0.3–1.3).

2.4 Colorectal cancer

Hildesheim *et al.* (1998) reported on a case-control study of colon and rectal cancer among residents of Iowa, United States, aged 40–85 years. Patients with histologically confirmed cancers of the colon and rectum were identified through the State Health Registry of Iowa during 1986–87; the controls, the mailed questionnaire and the telephone interview were the same as those in the study of Cantor *et al.* (1998; see section 2.3.3). The concentration of total trihalomethanes (g) and the lifetime average trihalomethane concentrations (μg/L) were calculated for 560 colon cancer patients, 537 rectal cancer patients and 1983 controls from data on water samples and from interviews. About 15% of the patients were interviewed by proxy. The logistic regression models included adjustment for sex, age, study period, education, high-risk occupation and cigarette smoking. There was a suggestion of a trend of increasing risk for rectal cancer with lifetime concentration of trihalomethanes (odds ratio for the highest category (≥ 2.4 g), 1.6; 95% CI, 1.0–2.6; p for trend, 0.08) and for average lifetime concentration of trihalomethanes (odds ratio for the highest category (≥ 46 μg/L), 1.7; 95% CI, 1.1–2.6; p for trend, 0.01). No such trend was observed for colon cancer.

2.5 Brain cancer

Heineman *et al.* (1994) reported on occupational exposures to chlorinated aliphatic hydrocarbons and the risk for astrocytic brain cancer. A total of 300 cases of histologically

confirmed brain tumours in deceased white men and 320 deceased population controls were identified in southern Lousiana, New Jersey and Philadelphia (United States) during 1978–81. The controls were frequency matched with the cases by age and area of death. Lifelong occupational histories were obtained by interviewing next-of-kin. Each job title was converted into period-specific probabilities and intensities of exposures to seven chlorinated hydrocarbon compounds, including chloroform. The logistic regression models included adjustment for age, study area and employment in electronics-related occupations or industries. Analyses by duration of exposure, cumulative exposure score and average intensity showed little indication of an association between exposure to chloroform and brain cancer.

3. Studies of Cancer in Experimental Animals

Previous evaluation

Chloroform was tested in three experiments in mice and in one in rats by oral administration. It produced hepatocellular adenomas and carcinomas in mice, malignant kidney tumours in male rats and tumours of the thyroid in female rats. Chloroform was also tested in one experiment by subcutaneous injection and in one by intraperitoneal injection in mice: these experiments were considered to be inadequate (IARC, 1979).

New studies

3.1 Oral administration

Mouse: Groups of female B6C3F_1 mice, 8.5 weeks of age, were given chloroform (pesticide quality, distilled to separate out diethylcarbonate) in distilled drinking-water at concentrations of 0 (control), 0 (matched control), 200, 400, 900 or 1800 mg/L (ppm) for 104 weeks, the numbers of mice per group being 430, 50, 430, 150, 50 and 50, respectively. Analysis of chloroform in the drinking-water indicated that the concentrations were maintained to an average of 93% or more of the target concentration over a four-day interval; these concentrations resulted in time-weighted average doses of 0, 0, 34, 65, 130 and 260 mg/kg bw, respectively. Survival was affected at the higher doses, with a 25% incidence of early deaths at 900 and 1800 mg/L. Survival of animals beyond the initial period did not differ significantly between groups. Chloroform did not increase the tumour incidence (Jorgenson *et al*., 1985).

Groups of 35 male B6C3F_1 mice, approximately 30 days of age, were given chloroform [purity not specified] in deionized drinking-water at concentrations of 0, 600 or 1800 mg/L (ppm) until they were killed at 52 weeks. This treatment did not increase the incidence of liver or lung tumours (Klaunig *et al*., 1986). [The Working Group noted that these were the control groups for a two-stage initiation–promotion study.]

Groups of 52 male and 52 female ICI mice, not more than 10 weeks of age, were given chloroform in toothpaste by oral gavage at doses of 17 or 60 mg/kg bw per day for 80 weeks followed by a further 16–24 weeks without treatment. A vehicle control group

of 104 males and 104 females received 1 mL/kg bw per day of unflavoured toothpaste by oral gavage. The survival of males beyond 60 weeks decreased to 10–30%, and that of females beyond 50 weeks to 10–20%. Chloroform increased the incidence of renal tubule tumours (adenomas and carcinomas) in males at the high dose (Table 3). This experiment was repeated under the same conditions with a group of 52 male mice that received chloroform at 60 mg/kg bw per day; 52 males served as untreated controls, and a control group of 260 male mice received the toothpaste base. Chloroform again increased the incidence of renal tubule tumours (adenomas and carcinomas; Table 3). In a third experiment under the same conditions, groups of 52 male mice of the ICI, C57BL, CBA and CF1 strains received either 60 mg/kg bw per day of chloroform in toothpaste base by oral gavage or 1 mL/kg bw per day of toothpaste alone; an additional group of 100 ICI males was untreated, and groups of 52 males received chloroform at 0 or 60 mg/kg bw day in arachis oil. Chloroform produced renal tubule tumours (adenomas and carcinomas) only in ICI strain males, with the highest incidence in the group receiving chloroform in arachis oil (Table 3; Roe *et al*., 1979).

Rat: Groups of 25 male and 25 female Sprague-Dawley rats (males weighing 180–240 g and females weighing 130–175 g received chloroform at concentrations of 15, 75 or 165 mg/kg bw per day, on six days per week, in toothpaste base by oral gavage. A control group of 75 males and 75 females received 1 mL/kg bw per day of toothpaste

Table 3. Incidence of renal tubule adenomas and carcinomas in ICI mice exposed orally to chloroform

Treatment	Sex	Incidence of renal tumours
Toothpaste	Male	0/72
17 mg/kg bw per day chloroform		0/37
60 mg/kg bw per day chloroform		8/38
Toothpaste	Female	0/59
17 mg/kg bw per day chloroform		0/35
60 mg/kg bw per day chloroform		0/38
None	Male	1/48
Toothpaste (1)		6/237
Toothpaste (2)		2/51
60 mg/kg bw per day chloroform		9/49
None	Male	0/83
Toothpaste		1/49
Arachis oil		1/50
60 mg/kg bw per day chloroform in toothpaste		5/47
60 mg/kg bw per day chloroform in arachis oil		12/48

From Roe *et al.* (1979)

base. Because of poor survival in all groups due to intercurrent disease, the study was terminated after one year. There was no increase in tumour incidence. In a second experiment, groups of 50 male (weighing 180–240 g) and 50 female (weighing 130–175 g) Sprague-Dawley rats were given chloroform at a concentration of 0 or 60 mg/kg bw per day, on six days per week, in 1 mL/kg bw of toothpaste by oral gavage for 80 weeks and observed for a further 15 weeks. Chloroform did not increase the tumour incidence (Palmer *et al*., 1979).

Groups of 26 and 32 male and 22 and 45 female weanling Wistar rats [age unspecified] were given chloroform [purity not specified] in the drinking-water at a concentration of 0 or 2.9 g/L, respectively, for 72 weeks, when the dose was halved to 1.45 g/L for the remaining weeks because of increased consumption of water. The animals were necropsied when found dead or moribund. Controls of each sex survived for approximately 145 weeks, while those exposed to chloroform survived for approximately 185 weeks. Chloroform produced neoplastic nodules in the livers of 10 female rats, representing a 25% incidence compared with 0% in the control group (Tumasonis *et al*., 1987).

Groups of male Osborne-Mendel rats, seven weeks of age, were given chloroform (pesticide quality) in drinking-water at concentrations of 0 (control), 0 (matched control), 200, 400, 900 or 1800 mg/L (ppm) for 104 weeks, the numbers of rats per group being 330, 50, 330, 150, 50 and 50, respectively. Analysis of chloroform in the drinking-water indicated that the concentrations were maintained to an average of 93% or more of the target concentration over a four-day interval; these concentrations resulted in time-weighted average doses of 19, 38, 81 and 160 mg/kg bw, respectively. The survival of these groups at 104 weeks was 12, 25, 29, 60 and 66%, respectively. Chloroform produced a statistically significant ($p < 0.01$) increase in the incidence of renal tubule tumours (adenomas and carcinomas) at the highest dose: control, 5/301; matched controls, 1/50; 200 mg/L, 6/313; 400 mg/L, 7/148; 900 mg/L, 3/48; and 1800 mg/L, 7/50 (Jorgensen *et al*., 1985).

Dog: Groups of 8–16 male and 8–16 female pure-bred beagle dogs, 18–24 weeks of age, were given chloroform [purity not specified] in a toothpaste base orally by gelatin capsule at concentrations of 0, 15 or 30 mg/kg bw per day, on seven days per week for at least seven years. One group of eight males and eight females was untreated, and another control group received an alternative toothpaste. Survival was excellent, with 84/96 animals still alive after seven years. Exposure to chloroform was not associated with any increase in tumour incidence (Heywood *et al*., 1979).

3.2 Inhalation

Mouse: Groups of 50 male and 50 female BDF_1 mice, six weeks of age, were given chloroform (purity, > 99%) in air by inhalation for 6 h per day on five days per week for 104 weeks at concentrations of 0, 5, 30 or 90 ppm. The 30- and 90-ppm doses were acutely lethal to the mice; thus, mice were first exposed to 5 ppm for two weeks, then to 10 ppm for two weeks (and, in the 90 ppm group, then 30 ppm for a further two weeks) before the 30 and 90 ppm concentrations were maintained. Under these conditions, chloroform produced renal tubular tumours (adenomas and carcinomas) in male mice at

the two highest concentrations: control, 0/50; low-dose, 1/50; mid-dose, 7/50; high-dose, 12/48 (Nagano *et al*., 1998).

Rat: Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were given chloroform (purity, 99%) in air by inhalation for 6 h per day on five days per week for 104 weeks at concentrations of 0, 10, 30 or 90 ppm. Chloroform did not increase the incidence of tumours (Nagano *et al*., 1998).

3.3 Administration with known carcinogens

Mouse: Groups of 23–39 male and 25–45 female CD-1 Swiss mice, 15 days of age, were given a single intraperitoneal injection of 0, 5 or 20 mg/kg bw *N*-ethyl-*N*-nitrosourea (ENU) in 1 mol/L sodium acetate. At five weeks of age, the mice either received no further treatment or were given chloroform (purity, > 99%) in the drinking-water at a concentration of 1800 mg/L (ppm) for the next 46 weeks (i.e. until 51 weeks of age). All mice were killed at 52 weeks of age. Chloroform did not alter the incidence of ENU-initiated lung tumours in mice of either sex nor of liver tumours in female mice. Chloroform decreased the incidence of ENU-initiated liver tumours in male mice by approximately one-half (Pereira *et al.*, 1985).

Rat: In a two-stage model of liver carcinogenesis, groups of 12 male Sprague-Dawley rats weighing 225–275 g received a two-thirds partial hepatectomy followed 20–22 h later by a single oral gavage dose of 1.5 mmol/kg bw chloroform [purity not specified] in tricaprylin, or 0.5 mmol/kg bw *N*-nitrosodiethylamine (NDEA) in distilled water as a positive control. Three days later, sodium phenobarbital was added at a concentration of 500 mg/L (ppm) to the drinking-water for 47 days, and the animals were killed six days later. Chloroform did not increase the incidence of γ-glutamyltranspeptidase-positive foci of hepatocellular alteration in either the partially hepatectomized or the intact rats (Pereira *et al*., 1982).

In the promotion part of the above two-stage model of liver carcinogenesis, groups of 15 or 16 male Sprague-Dawley rats weighing 225–275 g were given by gavage an initiating dose of NDEA in distilled water, and three days later either an oral gavage dose of 1.5 mmol/kg bw chloroform [purity not specified] in tricaprylin twice weekly for 53 days, 2 mL/kg bw tricaprylin alone or 500 mg/L (ppm) of sodium barbital in the drinking-water as a positive control. Rats were killed four to five days after the 53 days of promotion regimen. Chloroform did not statistically significantly increase the incidence of γ-glutamyltranspeptidase-positive foci of hepatocellular alteration (Pereira *et al*., 1982).

In a two-stage initiation–promotion model of liver carcinogenesis, groups of four to six female Sprague-Dawley rats, three weeks of age, received a single oral gavage dose of 8 mg/kg bw NDEA followed one week later by an oral gavage dose of 25, 100, 200 or 400 mg/kg bw chloroform (purity, 99%) twice weekly for 11 consecutive weeks, at which time the surviving animals were killed. These doses of chloroform were also administered to groups that had not been initiated with NDEA. Chloroform increased the incidence of adenosine-5′-triphosphatase-deficient, γ-glutamyltranspeptidase-positive and

glycogen-positive foci of hepatocellular alteration in the NDEA-initiated groups, but not in the uninitiated groups (Deml & Oesterle, 1985, 1987).

In a rat model of gastrointestinal carcinogenesis, groups of 40 male Fischer 344 rats, seven weeks of age, received a subcutaneous injection of 200 mg/kg bw 1,2-dimethylhydrazine, followed seven days later by administration of chloroform (preservative-free grade) at concentrations of 900 or 1800 mg/L in drinking-water for 39 weeks, at which time all the surviving animals were killed. Survival was 98–100%. Chloroform produced a statistically significant ($p < 0.001$) reduction in the incidence of gastrointestinal tract tumours (including those of the stomach, duodenum, jejunum, caecum and colon); when the incidence of colon tumours was analysed independently, it was significantly reduced ($p < 0.001$) at both doses (Daniel *et al*., 1989).

In a two-stage initiation–promotion model of liver carcinogenesis, groups of 11 or 12 male Fischer 344 rats weighing 150–160 g were subjected to a partial (67%) hepatectomy followed 18 h later by a single oral gavage dose of either 0.5 mmol/kg bw NDEA in saline or 2 mL/kg bw sterile saline. Two weeks after the hepatectomy, the groups were given water containing 500 mg/L (ppm) phenobarbital or 1800 mg/L chloroform (preservative-free high-performance liquid chromatography-grade), drinking-water containing both phenobarbital at concentrations of 650, 700, 800 or 950 mg/L and chloroform at concentrations of 200, 400, 900 or 1800 mg/L, or distilled water alone, for the next 12 weeks, at which time all the surviving animals were killed. Chloroform reduced the number of γ-glutamyltranspeptidase-positive and glutathione-*S*-transferase (placental form)-positive foci of hepatocellular alteration in a dose-related manner (Reddy *et al*., 1992).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

After inhalation of approximately 5 mg [^{38}Cl]chloroform, ~80% of the chloroform was found to have been absorbed (Morgan *et al*., 1970). Eight volunteers expired 18–67% of an oral dose of 500 mg [^{13}C]chloroform (in capsules of olive oil) unchanged; in two subjects, about half of the dose was eliminated in the expired air as $^{13}CO_2$. The decline in the concentration of chloroform in blood was described by a two-compartment model, with initial and second-phase half-lives of 14 and 90 min, respectively, averaged over four subjects (Fry *et al.*, 1972).

The uptake and elimination of inhaled chloroform (2–2.5% v/v in air) by eight patients during general anaesthesia was rapid, with an average blood concentration of about 175 mg/L (Poobalasingham & Payne, 1978). Smith *et al*. (1973) reported that concentrations of 70–165 mg of chloroform per litre of blood produced adequate surgical anaesthesia; at a concentration of 50 mg/L, patients became responsive again. One hour after anaesthesia was terminated, the arterial chloroform concentration was 20–40 mg/L.

Chloroform has been detected *post mortem* in tissue samples from various organs at concentrations of 1–68 μg/kg wet tissue, the concentration in body fat being the highest (McConnell *et al*., 1975). When subjects inhaled a mixture of oxygen/nitrogen (20/80) containing less than 0.025 μg/L chloroform, traces up to 11 μg/h per subject were found in expired air (Conkle *et al*., 1975).

After chloroform was applied to the forearm of male volunteers aged 23–36, and exhaled air and urine were collected and analysed, absorption of chloroform was ~7.8% after application of 50 μg in water and ~1.6% after application of 250 μg in ethanol. More than 94% of the absorbed dose was excreted via the lungs between 15 min and 2 h after dosing, 69 and 88% of which was CO_2 after application in ethanol and water, respectively (Dick *et al.*, 1995).

Cytochrome P450 (CYP) 2E1 was shown to be a major enzyme in the oxidation of chloroform in human liver microsomes (Guengerich *et al*., 1991).

4.1.2 *Experimental systems*

Chloroform is rapidly absorbed and distributed to all organs, with relatively high concentrations in nervous tissue (Von Oettingen, 1964). After intraduodenal injection of [^{14}C]chloroform to rats, 70% of the radiolabel was associated with unchanged chloroform in the expired air and 4% with CO_2 after 18 h. In an experiment with tissue slices *in vitro*, the liver and, to a much lesser extent, the kidney were the main organs in which CO_2 was formed (Paul & Rubinstein, 1963). Mink *et al.* (1986) administered [^{14}C]chloroform by intragastric intubation to fasted male Sprague-Dawley rats (100 mg/kg bw) and male B6C3F_1 mice (150 mg/kg bw) and found that the majority of the compound was eliminated in expired air through the lungs of both species within 8 h. The mice eliminated 40–81% of the total dose as $^{14}CO_2$ and 5–26% as the parent compound, whereas the rats exhaled 4–18% as $^{14}CO_2$ and 41–67% as unmetabolized chloroform.

The blood concentrations in male Fischer 344 rats during 24-h dermal exposure to pure chloroform peaked after 4–8 h at 52 μg/mL and remained constant for the duration of the exposure (Morgan *et al*., 1991).

[^{14}C]Chloroform in olive oil given periodically to mice, rats and monkeys (60 mg/kg bw) was completely absorbed, since 93–98% of the radiolabel was recovered from exhaled air, urine, faeces and the carcass. Most of the dose was excreted unchanged by monkeys, as $^{14}CO_2$ by mice and as a mixture of the two by rats. Three metabolites were detected in the urine of rats and mice, one of which was identified as urea (Brown, D.M. *et al*., 1974; Taylor *et al*., 1974).

A study of the tissue distribution of chloroform in three strains of mice (CF/LP, CBA and C57) by whole-body autoradiography after oral dosing with 60 mg/kg bw [^{14}C]chloroform showed the greatest amounts of radiolabel in liver and kidneys, with significant amounts in the renal cortex but not the medulla of male mice. In female mice, the highest levels of radiolabel were found in the liver, intestine and bladder with much less in kidney (Taylor *et al*., 1974). No sex differences in renal binding were found in rats or monkeys (Brown, D.M. *et al*., 1974).

In male Wistar rats, the integrated area under the blood concentration–time curve after oral administration of chloroform at 75 mg/kg bw in water was 8.7 times greater than after administration in vegetable oil. In both cases, chloroform was well absorbed, peak blood concentrations being reached approximately 6 min after administration; uptake from aqueous solution gave rise to higher peak blood concentrations (Withey *et al*., 1983). In male Fischer 344 rats, administration of chloroform in a low-dose volume (2 mL/kg bw) of either corn oil or 2% emulphor:water did not appear to affect the absorption or tissue dosimetry of chloroform; however, in $B6C3F_1$ mice treated with chloroform in a high-dose volume (10 mL/kg bw) of the vehicle, these parameters were affected by the vehicle used (Dix *et al*., 1997).

The fraction of the dose exhaled as unchanged chloroform did not increase proportionately to that administered in either male Osborne-Mendel rats or male $B6C3F_1$ mice exposed to 90, 360 or 1040 ppm [440, 1800 or 5100 mg/m^3] or 10, 90 or 360 ppm [49, 440 or 1800 mg/m^3] [^{14}C]chloroform for 6 h, respectively. $^{14}CO_2$ was the major metabolite exhaled. The data indicate metabolic saturation at the higher doses (Corley *et al*., 1990).

The metabolism of chloroform has been extensively reviewed (Hathway, 1974; Charlesworth, 1976; Pohl, 1979; Davidson *et al*., 1982). There is considerable evidence for the metabolism of chloroform to phosgene (Mansuy *et al*., 1977; Pohl *et al*., 1977, 1979, 1980). The finding of 2-oxo-thiazolidine-4-carboxylic acid (4-carboxy-thiazolidine-2-one) in incubates is strong evidence for the formation of phosgene, as the reactive metabolite phosgene is formed by mixed-function oxidation of chloroform to trichloromethanol after dehydrochlorination (Mansuy *et al*., 1977; Pohl *et al*., 1977, 1980). Phosgene reacts with water to give CO_2 and HCl, which explains the presence of CO_2 as a metabolite *in vivo* in a number of studies. Phosgene also reacts with tissue nucleophiles to form covalently bound products (Mansuy *et al*., 1977; Pohl *et al*., 1980). In phenobarbital-treated rats, liver necrosis was observed only with doses of chloroform high enough to decrease the concentration of reduced glutathione in liver (Docks & Krishna, 1976). Chloroform depletes liver glutathione by reacting with it (Pohl *et al*., 1980, 1981). Covalent binding of [^{14}C]chloroform-derived radiolabel to microsomal proteins *in vitro* was inhibited by cysteine (Pohl *et al*., 1977).

Chloroform is metabolized to a greater extent in mice than in rats (Mink *et al*., 1986). More covalent binding of [^{14}C]chloroform metabolites to liver and kidney proteins *in vivo* was seen in $B6C3F_1$ mice than in Osborne-Mendel rats. The uptake of chloroform by male $B6C3F_1$ mice in a closed chamber was more rapid than that by male Fischer 344 rats after exposure to initial concentrations of chloroform ranging from 1000 to 5000 ppm [4900–25 000 mg/m^3] and 103–2581 ppm [510–13 000 mg/m^3], respectively (Corley *et al*., 1990). This difference was attributed to the higher rate of chloroform metabolism in mice.

The metabolism of chloroform to phosgene in mouse kidney has been implicated as the reactive pathway, as in the liver (Branchflower *et al*., 1984). Strain differences in sensitivity to chloroform-induced nephrotoxicity correlate with the ability of the kidney to metabolize chloroform. Chloroform metabolism in renal cortical slices from male, but

not female, ICR mice was found to be responsible for the sensitivity of male mice to chloroform-induced nephrotoxicity (Smith & Hook, 1983, 1984). Homogenates of kidney from male DBA/2J mice metabolized chloroform twice as fast as those from C57BL/6J mice (Pohl *et al*., 1984), and covalent binding of chloroform to renal microsomes was much greater in male DBA mice than in male C57BL/6 mice, consistent with the sensitivity of the DBA strain to chloroform-induced nephrotoxicity (Clemens *et al*., 1979).

Purified CYP 2El in the presence of liver microsomes prepared from acetone-induced rats metabolized chloroform (Brady *et al.* 1989; Guengerich *et al*., 1991).

Testai and Vittozzi (1986) demonstrated that reductive biotransformation of chloroform takes place in rat microsomes *in vitro* and is dependent on inducible P450 enzymes. Reductive metabolism was negligible with microsomes from uninduced animals. The results of several investigations suggest that reductive metabolism of chloroform *in vivo* would be significant only at high concentrations of chloroform (5 mmol/L) under very low pO_2 (0–1%) and would be totally inhibited by the presence of higher concentrations of oxygen (Luke *et al*., 1988; De Curtis *et al*., 1994; Gemma *et al*., 1996).

4.1.3 *Comparison of humans and rodents*

The metabolism of chloroform in human and rat (male Sprague-Dawley) liver microsomes *in vitro* is similar. The kinetics in the two species were quite similar, but less total irreversible binding was seen in human samples. Cysteine inhibited covalent binding of chloroform metabolites to macromolecules in both human and rat microsomes (Cresteil *et al.*, 1979).

The metabolism of ^{14}C[chloroform] in liver and kidney microsomes prepared from male Fischer 344 rats, Osborne-Mendel rats, B6C3F_1 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 the kidneys of the various species were less active than liver microsomes. The metabolism of [^{14}C]chloroform in kidney microsomes was greatest in mice (B6C3F_1) 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.

Mice were exposed on day 11, 14 or 17 of gestation to [^{14}C]chloroform for 10-min in an all-glass chamber in which 100 μCi were mixed with maize oil and gently heated. The radiolabel was observed to cross the placenta. In mid-gestation, the amniotic fluid accumulated radiolabel from non-volatile metabolites, but chloroform itself did not accumulate in fetal brain or other tissue. Radiolabel attached to non-volatile compounds peaked in the fetus around 1 h after inhalation by the dam (Danielsson *et al*., 1986).

4.2 Toxic effects

4.2.1 *Humans*

Extensive exposure to chloroform is fatal to humans, rapid death being attributed to cardiac arrest and delayed death to liver and kidney damage (Challen *et al.*, 1958;

Matsuki & Zsigmond, 1974). The symptoms of exposure to chloroform include respiratory depression, coma, renal damage and liver damage as measured by elevated serum enzyme levels (Storms, 1973). According to Royston (1925), chloroform was not toxic to the fetus or newborn when administered to women during prolonged labour. Chloroform anaesthesia, under normal conditions, was found to induce minimal or no toxic side-effects, even in young children (Whitaker & Jones, 1965).

4.2.2 *Experimental systems*

The acute toxicity of chloroform is species-, strain-, sex- and age-dependent. Thus, the acute oral LD_{50} in young and older adult male Sprague-Dawley rats was 1300 and 1200 mg/kg bw, respectively whereas it was 440 mg/kg bw in 14-day-old animals (Kimura *et al.*, 1971). The LD_{50} of a single oral dose in male mice varied from 120 mg/kg bw in DBA/2J mice to 490 mg/kg bw in C57BL/6J mice (Hill *et al.*, 1975). The dose that caused a 50% incidence of acute neurological effects (ataxia, loss of coordination and anaesthesia) was 480 mg/kg bw (Balster & Borzelleca, 1982).

Males of many mouse strains are susceptible to renal tubular necrosis, whereas females are not similarly affected. The response to chloroform increased with the age of the mice. Strains C3H, $C3H_f$, A and HR were susceptible, and strains C57BL, C57L, C57BR/cd and ST were resistant to exposure (Deringer *et al.*, 1953).

Renal tubular injury has been observed in mice of a number of strains, including $B6C3F_1$ (Larson *et al*., 1994a,b, 1996), DBA (Hill *et al*., 1975), ICR (Smith *et al*., 1983), C57BL (Hill *et al*., 1975) and BDF_1 (Templin *et al*., 1996a, 1998), after oral or inhalational exposure to chloroform. A number of studies indicate that male but not female mice are susceptible to chloroform-induced nephrotoxicity (Eschenbrenner & Miller, 1945; Larson *et al*., 1996; Templin *et al*., 1996a, 1998).

Male Sprague-Dawley rats were given 0, 1.2, 2.5, 3.1, 3.7 or 4.4 mmol/kg [0, 140, 300, 370, 440 or 530 mg/kg bw] chloroform in either corn oil or EL 620 emulphor (10 mL/kg) and killed 48 h later. Toxicity to the kidney was evaluated by measuring *para*-aminohippuric acid incorporation into renal cortical slices prepared from treated rats. The nephrotoxicity appeared to be more severe in rats given chloroform in corn oil (Raymond & Plaa, 1997). [The Working Group noted that the large volume of the vehicle used may have influenced these results.]

Liver damage was the cause of death of rats and mice after accidental long-term exposure to and acute administration of chloroform (Doyle *et al.*, 1967; Brown, B.R. *et al.*, 1974). Early dilatation of granular endoplasmic reticulum, with detachment of the ribosomes, was observed in the livers of treated rats (Scholler, 1968). In rats, rabbits and guinea-pigs exposed to 125, 250 or 425 mg/m^3 chloroform in air and in dogs exposed to 125 mg/m^3 for 7 h a day on five days a week for six months, higher mortality rates, changes in organ weights and histopathological alterations in the liver and kidney were observed; centrilobular granular degeneration was seen in rat liver. These effects appeared to be reversible in rats exposed to 125 mg/m^3 (Torkelson *et al.*, 1976).

Chloroform increases ornithine decarboxylase activity in Fischer 344 rats (Savage *et al*., 1982, 1987) and male B6C3F_1 mice (Pereira *et al*., 1984). Ornithine decarboxylase has been proposed to be a molecular marker for tumour promotion.

Various treatments that affect hepatic drug-metabolizing enzymes alter the hepatotoxicity of chloroform, indicating that a metabolite of chloroform may be responsible for the liver necrosis (McLean, 1970; Scholler, 1970). After administration of [^{14}C]-chloroform, there was extensive, presumably covalent, binding of ^{14}C to liver and kidney proteins in male C57BL/6 mice. The livers of female mice showed similar amounts of covalent binding and a similar degree of hepatic necrosis; the kidneys of female mice appeared to be resistant to chloroform-induced necrosis and showed much less protein binding (Ilett *et al*., 1973). In similar experiments, binding in the kidneys of mice of two strains was related to their susceptibility to kidney lesions (Vesell *et al*., 1976). In mice, covalent binding of chloroform to renal proteins correlated with the degree of renal tubular necrosis (Ilett *et al*., 1973; Smith & Hook, 1983). Other factors, such as the availability of glutathione (Brown, B.R. *et al*., 1974), the concentration of cytochrome P450 and oxygen tension (Uehleke & Werner, 1975; Sipes *et al*., 1977), affected the extent of covalent binding and of hepatic centrilobular damage.

Chloroform induced concentration-dependent cytotoxicity in male B6C3F_1 mouse and Fischer 344 rat hepatocytes at concentrations greater than 1 mmol/L, which is the threshold concentration for glutathione depletion. Cytochrome P450-dependent metabolism and glutathione depletion, but not reductive metabolism, were found to be involved in the toxicity (Ammann *et al*., 1998).

Chloroform caused a sustained cytotoxic and regenerative cell proliferative response in the livers of female B6C3F_1 mice under conditions of treatment by gavage similar to those that produce cancer (corn oil, 10 mL/kg bw) (Larson *et al*., 1993, 1994a). Similar total daily doses of chloroform administered in drinking-water at concentrations as high as 1800 ppm [260 mg/kg bw] did not induce hepatocellular damage, cell proliferation or liver tumours (Jorgenson *et al*., 1985; Larson *et al*., 1994a). A similar pattern of hepatocellular damage and regeneration was observed in B6C3F_1 mice given chloroform by gavage or in the drinking-water (Pereira, 1994).

Male Fischer 344 rats were given oral doses of 0, 10, 34, 90 or 180 mg/kg bw per day chloroform in corn oil for four days, or for five days a week for three weeks. A second group of rats were given chloroform *ad libitum* in the drinking-water at concentrations of 0, 60, 200, 400, 900 or 1800 ppm [0, 6, 17–19, 32–33, 62–68, 57–110 mg/kg bw per day] for four days or three weeks. Bromodeoxyuridine was administered via an osmotic pump 3.5 days before necropsy, and the labelling index was evaluated immunohistochemically. Administration of chloroform by gavage caused more severe hepatic and renal toxicity than exposure in the drinking-water, and regenerative proliferation in the kidney and liver depended on the route of administration (Larson *et al*., 1995a). Male and female Fischer 344 rats appear to be equally susceptible to chloroform-induced hepatotoxic effects, but the nephrotoxic effects, including the proliferative response, were more severe in females than in males (Larson *et al*., 1995a,b). Templin *et al*.

(1996b) showed that male Fischer 344 and Osborne-Mendel rats have similar susceptibility to chloroform-induced renal injury.

Female and male $B6C3F_1$ mice were exposed by inhalation to 0, 0.3, 2, 10, 30 or 90 ppm chloroform [0, 1.5, 9.8, 49, 150 or 440 mg/m^3] for 6 h a day on seven days a week for four days or for 3, 6 or 13 consecutive weeks. Additional groups were exposed for five days a week for 13 weeks or exposed for six weeks and then examined at 13 weeks. Bromodeoxyuridine was administered via osmotic pumps implanted 3.5 days before necropsy, and the labelling index was evaluated immunohistochemically. Treatment-induced dose- and time-dependent histological lesions and increased labelling indices were found only in the livers and nasal passages of female and male mice and in the kidneys of male mice. Significant increases in the labelling index (cells in S-phase, a measure of cell proliferation) were sustained in the group exposed to 90 ppm, and no adverse effects were observed in mice exposed to 10 ppm (Larson *et al*., 1996).

Male and female Fischer 344 rats were exposed to 0, 2, 10, 30, 90 or 300 ppm [0, 9.8, 49, 150, 440 or 1500 mg/m^3] chloroform for 6 h a day on seven days a week for four days or for 3, 6 or 13 weeks. Additional groups were exposed for five days per week for 13 weeks. The primary target in Fischer 344 rats was the kidney, which showed a significantly increased labelling index in the epithelial cells of the proximal tubules of the cortex at concentrations of 30 ppm and above (seven days per week) and 90 ppm and above (five days per week). A concentration-dependent response in the labelling index in the kidney was found in both male and female rats. Hepatocyte alterations were seen mainly in rats exposed to 300 ppm at all times and in those exposed to 90 ppm at later times (Templin *et al.*, 1996c).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The effects of chloroform on the development of rats, mice and rabbits have been reviewed (Smith *et al*., 1986). Adverse clinical effects on the dams of each species and some evidence of embryotoxic and fetotoxic effects (predominantly reduced fetal size and weight and retarded skeletal ossification) were reported at the highest doses tested: 300 ppm for 7 h per day by inhalation and up to 400 mg/kg orally on days 6–15 of gestation. Teratogenic effects were reported in rats and mice exposed by inhalation but not in rats or rabbits treated by oral gavage. In one study, abnormal sperm were reported in mice exposed by inhalation to 400 or 800 ppm for 4 h per day for five days (Land *et al*., 1981).

Sprague-Dawley rats were exposed by inhalation to 0, 30, 100 or 300 ppm chloroform for 7 h per day on days 6–15 of gestation, and their fetuses were examined on day 20 of gestation for viability, growth and morphological appearance. All three doses resulted in significantly reduced maternal body-weight gain, and this effect was marked

in females at 300 ppm. Only 3/20 of females at this dose had viable fetuses at term, compared with 88% of the controls. No effects on viability or body weight were noted in the fetuses of dams at the two lower doses, but both viability and body weight were considerably reduced at the high dose, as was crown–rump length. The crown–rump length of fetuses of dams at the low dose was also reduced, and delayed skeletal ossification and an increased incidence of fetuses with wavy ribs were seen. Fetuses of dams at 100 ppm had increased incidences of absent or short tails, imperforate anus, subcutaneous oedema and anomalies of the skull and sternum, with delayed ossification of the sternebrae. The authors concluded that chloroform is not strongly teratogenic but highly embryotoxic (Schwetz *et al*., 1974).

Groups of 15 Sprague-Dawley rats were exposed to 0, 100, 200 or 400 mg/kg bw chloroform by gavage on days 6–15 of gestation, and their fetuses were examined on day 22. Maternal body-weight gain was decreased in all treated groups, and three females at the high dose died. The relative liver weights were increased at all doses, while the kidney weights were increased only in dams at the high dose. The only fetal effects were a decrease in weight and increased incidences of runts, aberrations of the sternebrae and interparietal malformations at the high dose. The last two effects were considered to be indicative of fetotoxicity (Ruddick *et al*., 1983).

Male and female mice received 0 or 31 mg/kg bw per day chloroform in an Emulphor-saline vehicle by oral gavage for 21 days before mating, throughout mating (21 days or until a vaginal plug was detected) and throughout gestation and lactation. The offspring in five control and five treated litters received the same doses daily beginning on postnatal day 7 and continuing to the end of the study. No effects were reported on offspring body weights or on a variety of neurobehavioural measures (righting reflex, forelimb placing response, forepaw grasp, rooting reflex, cliff-drop aversion, auditory startle response, bar-holding ability, eye opening, motor activity and passive avoidance learning) (Burkhalter & Balster, 1979).

CF-1 mice were exposed to 0 or 100 ppm chloroform by inhalation for 7 h per day on days 1–7, 6–15 or 8–15 of gestation, and their fetuses were examined on day 18 of gestation for viability, growth and morphological appearance. Reduced body-weight gain during the first few days of exposure was seen in all treated dams. Exposure on days 1–7 or 6–15 significantly reduced the percentage of females that maintained pregnancy. Fetal body weights and crown–rump lengths were reduced in groups treated on days 1–7 or 8–15, and there was a significant increase in the incidence of cleft palate in the latter group (Murray *et al*., 1979).

The reproductive effects of chloroform were evaluated in CD-1 mice in the continuous breeding protocol of the United States National Toxicology Program (Anon., 1997). As reported in summary form, the mice were exposed to 0, 8, 20 or 50 mg/kg bw per day by gavage, and males and females were maintained in breeding pairs throughout the exposure. There were no reported treatment-related effects on the general health of the parental generation (e.g. body and organ weights, clinical signs) or on their reproductive function (e.g. numbers of litters produced, litter size or pup weight). Mating of

control and high-dose offspring to produce an F_2 generation resulted in a higher fertility index and larger litter sizes in the treated groups, while the relative liver weights were increased in treated female offspring (F_1), and the relative weight of the epididymides was increased in treated F_1 males. All treated females showed some degree of hepatocellular degeneration, while individual cases of hepatitis and hepatocellular degeneration were observed in males.

Sprague-Dawley rat embryos (12–15 somites) at day 10.5 of gestation were exposed in whole-embryo culture to chloroform at concentrations of 0.53–3.7 μmol/mL for 40 h. Concentrations of 2 μmol/mL and higher retarded embryonic development. At the highest concentration, yolk sac vascularity was reduced within 4 h of exposure; extensive cell death was seen in the neuroepithelium after 16 h of culture (Brown-Woodman *et al*., 1998).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 4 for references)

Chloroform did not induce prophage or SOS DNA repair in *Escherichia coli* in two studies carried out in the presence and absence of exogenous metabolic activation. Weak differential toxicity was observed in *E. coli* in one study with exogenous metabolic activation, but another study gave negative results. A similar assay with *Bacillus subtilis* showed a positive result in the absence of activation, but a second study again gave negative results.

Chloroform did not cause forward mutations in *Salmonella typhimurium* and did not cause reverse mutation in several studies in *S. typhimurium* strains TA100, TA135, TA1537, TA1538 and TA98 and *E. coli* WP2 and WP2 *uvr*A in the presence or absence of exogenous metabolic activation; two exceptions were positive responses in *S. typhimurium* TA1535 transfected with rat glutathione *S*-transferase and in strain TA98 in the presence of metabolic activation from mouse liver.

In lower eukaryotes, chloroform had mixed effects. In one study, it induced mitotic gene conversion, mitotic crossing-over and reversion in the D7 strain of *Saccharomyces cerevisiae* containing cytochrome P450-dependent monooxygenases and, in another study, it induced deletions via intrachromosomal recombination. In contrast, no DNA damage, mitotic gene conversion, mitotic crossing-over, reverse mutation or increase in mitotic aneuploidy were observed in *Saccharomyces cerevisiae* in the presence or absence of exogenous metabolic activation. In *Aspergillus nidulans*, chloroform did not induce mitotic crossing-over, somatic segregation or gene mutation in the absence of metabolic activation; aneuploidy was observed in one of two studies.

In two studies, chloroform did not induce sex-linked recessive lethal mutation in *Drosophila melanogaster*.

Chloroform did not elicit DNA fragmentation in primary rat hepatocytes or DNA repair in either mouse or rat hepatocytes, and it did not induce gene mutation at the *hprt*

Table 4. Genetic and related effects of chloroform

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage induction, SOS repair, DNA strand breaks, cross-links or related damage	–	–	0.05 µL/mL	Thomson (1981)
Escherichia coli PQ37, SOS repair	–	–	3000	Le Curieux *et al*. (1995)
Escherichia coli pol A/W3110-P3478, differential toxicity (liquid suspension)	–	(+)	250 µg/plate	Rosenkranz *et al.* (1981)
Escherichia coli rec strains, differential toxicity	–	–	NR	Green (1981)
Bacillus subtilis rec strains, differential toxicity	+	NT	NR	San Agustin & Lim-Sylianco (1978)
Bacillus subtilis rec strains, differential toxicity	–	–	20 µL/plate	Kada (1981)
Salmonella typhimurium, forward mutation	–	–	300	Skopek *et al*. (1981)
Salmonella typhimurium Ara forward mutation	–	–	9.6 µmol/plate	Roldán-Arjona *et al.* (1991); Roldán-Arjona & Pueyo (1993)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	5 mg/plate	Simmon *et al.* (1977)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	NT	–	15 mg/plate	Nestmann *et al*. (1980)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	3600 µg/plate	Gocke *et al*. (1981)
Salmonella typhimurium TA100, TA98, TA1537, reverse mutation	–	–	5000 µg/plate	MacDonald (1981)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	1000 µg/plate	Van Abbé *et al*. (1982)
Salmonella typhimurium TA100, reverse mutation (fluctuation test)	–	–	10 000	Le Curieux *et al*. (1995)
Salmonella typhimurium TA1535, reverse mutation	–	NT	0.3% v/v	San Agustin & Lim-Sylianco (1978)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA98, TA1535, TA1537, reverse mutation	–	–	10	Gatehouse (1981)
Salmonella typhimurium TA1535, TA1537, TA1538, reverse mutation	–	–	10 000 µg/plate	Richold & Jones (1981)
Salmonella typhimurium TA1535 transfected with human *GST* gene, reverse mutation	(+)	NT	226	Pegram *et al*. (1997)
Salmonella typhimurium TA1537, reverse mutation	–	NT	0.05% v/v	San Agustin & Lim-Sylianco (1978)
Salmonella typhimurium TA98, reverse mutation	–	NT	0.02% v/v	San Agustin & Lim-Sylianco (1978)
Salmonella typhimurium TA98, reverse mutation	NT	+	0.2 mL/2000 cm^3	Norpoth *et al.* (1980)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	1000	Gatehouse (1981)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	10 000 µg/plate	Kirkland *et al*. (1981)
Escherichia coli WP2, reverse mutation	–	–	10 000 µg/plate	Kirkland *et al*. (1981)
Saccharomyces cerevisiae, gene conversion	+	NT	6400	Callen *et al*., (1980)
Saccharomyces cerevisiae, gene conversion	–	–	333 µg/plate	Jagannath *et al.* (1981)
Saccharomyces cerevisiae, gene conversion	–	–	1000	Sharp & Parry (1981)
Saccharomyces cerevisiae, gene conversion	NT	–	3000	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae, homozygosis by mitotic recombination or gene conversion	+	NT	6400	Callen *et al*. (1980)
Saccharomyces cerevisiae, homozygosis by mitotic recombination or gene conversion	–	–	1000	Kassinova *et al*. (1981)
Aspergillus nidulans, genetic crossing-over	–	NT	5 mL/20 L	Crebelli *et al*. (1984)
Aspergillus nidulans, genetic crossing-over	–	NT	0.5%	Gualandi (1984)
Saccharomyces cerevisiae, reverse mutation	+	NT	6400	Callen *et al*. (1980)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae, reverse mutation	–	–	1111	Mehta & von Borstel (1981)
Aspergillus nidulans, forward mutation	–	NT	0.5%	Gualandi (1984)
Saccharomyces cerevisiae, aneuploidy	–	–	100	Parry & Sharp (1981)
Aspergillus nidulans, aneuploidy	–	NT	5 mL/20 L	Crebelli *et al*. (1984)
Aspergillus nidulans, aneuploidy	+	NT	0.16% v/v	Crebelli *et al*. (1988)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		2975	Gocke *et al*. (1981)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		0.2% (adult feeding)	Vogel *et al.* (1981)
DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	–	NT	357	Sina *et al*. (1983)
Unscheduled DNA synthesis, rat hepatocytes *in vitro*	–	NT	1000	Althaus *et al.* (1982)
Unscheduled DNA synthesis, mouse hepatocytes *in vitro*	–	NT	1190	Larson *et al*. (1994c)
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	2.5% gas × 24 h	Sturrock (1977)
Sister cromatid exchange, Chinese hamster cells *in vitro*	–	–	0.71% gas v/v	White *et al*. (1979)
Sister chromatid exchange, Chinese hamster cells *in vitro*	NT	–	10 µg	Perry & Thomson (1981)
Sister chromatid exchange, rat leukemia cells *in vitro*	–	+	119	Fujie *et al*. (1993)
Cell tranformation, SA7/Syrian hamster embryo cells	+	NT	0.25 mL/chamber	Hatch *et al*. (1983)
Unscheduled DNA synthesis, human lymphocytes *in vitro*	–	–	15000	Perocco & Prodi (1981)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	119	Butterworth *et al.* (1989)
Sister chromatid exchange, human lymphocytes *in vitro*	NT	–	400	Kirkland *et al*. (1981)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	1190	Morimoto & Koizumi (1983)
Chromosomal aberrations, human lymphocytes *in vitro*	NT	–	400	Kirkland *et al*. (1981)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
S. typhimurium TA1537, reverse mutation in urine from mice	+		700 × 1	San Agustin & Lim-Sylianco (1978)
Host-mediated assay, *S. typhimurium* TA1535 in mouse peritoneal cavity	–		NG	San Agustin & Lim-Sylianco (1978)
Host-mediated assay, *S. typhimurium* TA1537 in mouse peritoneal cavity	+		NG	San Agustin & Lim-Sylianco (1978)
Host-mediated assay, microbial cells in animal hosts	–		800 ip × 1	Hellmér & Bolcsfoldi (1992)
DNA strand breaks, cross-links or related damage, animal cells *in vivo*	–		400 po	Petzold & Swenberg (1978)
DNA strand breaks, cross-links or related damage, animal cells *in vivo*	–		480 po × 1	Kitchin & Brown (1989)
Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		400 po	Mirsalis *et al.* (1982)
Unscheduled DNA synthesis, mouse cells *in vivo*	–		477 po × 1	Larson *et al*. (1994c)
Gene mutation, B6C3F1 *lac*I transgenic mice *in vivo*	–		90 ppm 6 h/d × 180 d	Butterworth *et al.* (1998)
Sister chromatid exchange, animal cells *in vivo*	+		50 po × 4	Morimoto & Koizumi (1983)
Micronucleus formation, mice *in vivo*	(+)		700 × 1	San Agustin & Lim-Sylianco (1978)
Micronucleus formation, mice *in vivo*	–		952 ip × 2	Gocke *et al*. (1981)
Micronucleus formation, mice *in vivo*	?		132 ip	Salamone *et al.* (1981)
Micronucleus formation, mice *in vivo*	–		0.06 ml/kg ip × 2	Tsuchimoto & Matter (1981)
Micronucleus formation, rats (kidney) *in vivo*	+		4 mmol/kg po × 1	Robbiano *et al*. (1998)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, other animals (newt) *in vivo*	–		50 µg/mL	Le Curieux *et al*. (1995)
Chromosomal aberrations, animal bone-marrow cells *in vivo*	+		10 µmol/kg ip × 1	Fujie *et al*. (1990)
Chromosomal aberrations, animal bone-marrow cells *in vivo*	+		1 mmol/kg po × 5	Fujie *et al*. (1990)
Binding (covalent) to DNA *in vitro*	NT	–	0.8	Diaz Gomez & Castro (1980a)
Binding (covalent) to DNA *in vitro*	–	+	95	Di Renzo *et al.* (1982)
Binding (covalent) to rat liver cell protein *in vitro*	+	NT	0.0008	Diaz Gomez & Castro (1980b)
Binding (covalent) to DNA and RNA, mouse liver cells *in vivo*	–		750 ip × 4	Diaz Gomez & Castro (1980a)
Binding (covalent) to RNA or protein, rat liver cells *in vivo*	+		5 ip × 1	Diaz Gomez & Castro (1980a)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose unless otherwise stated; in-vitro test, µg/mL; in-vivo test, mg/kg bw per day; NR, not reported; ip, intraperitoneal; po, oral

locus in Chinese hamster V79 cells in the absence of metabolic activation. No increase in the frequency of sister chromatid exchange was detected, in the presence of a metabolic system from rat liver, in two studies in Chinese hamster ovary cells. A modest but significant increase in sister chromatid exchange frequency was induced by chloroform in cultured rat erythroblastic leukaemia cells, but only in the presence of metabolic activation, and it enhanced transformation of Syrian hamster embryo cells by SA7 adenovirus.

In single studies in cultured human lymphocytes, chloroform increased the frequency of sister chromatid exchange in the absence of metabolic activation, but did not elicit DNA repair with or without activation; it also did not induce chromosomal breakage or sister chromatid exchange in the presence of activation. In cultures of primary human hepatocytes, no DNA repair synthesis was observed after exposure to chloroform.

In a host-mediated assay in mice, chloroform was mutagenic in males but not in females, and the response was weakly positive in *S. typhimurium* strain TA1537 but not in strain TA1535; chloroform was inactive in another host-mediated assay in mice in which the relative survival of *E. coli* k-12 *uvrB/recA* DNA repair-deficient strain was measured in blood, liver, lungs, kidney and testes.

DNA fragmentation was not observed in the livers of rats given a single oral dose of chloroform, and negative responses were obtained in in-vivo–in-vitro assays for DNA repair in rat and mouse hepatocytes. No increase in *lacI* mutant frequency was seen in the livers of female transgenic $B6C3F_1$ mice exposed to chloroform by inhalation for 180 days. Daily oral dosing of mice with chloroform for four successive days caused a significant increase in sister chromatid exchange frequency in bone-marrow cells. Weak induction of micronuclei in polychromatic erythrocytes was observed in chloroform-treated mice in one study, whereas another study of micronucleus formation in mouse bone marrow gave equivocal results and two studies gave negative results. A dose-dependent increase in the frequency of chromosomal aberrations was detected in the bone marrow of rats given a single intraperitoneal dose of chloroform or five successive daily oral doses. A statistically significant increase in the induction of micronucleated cells was observed in the proximal tubules of the kidneys of rats given a single oral dose of chloroform. Micronuclei were not, however, induced in newts.

No covalent binding of reactive metabolites of chloroform to DNA or RNA was observed in the livers of mice and rats injected intraperitoneally with [^{14}C]chloroform or in DNA exposed *in vitro* to [^{14}C]chloroform in the presence of a microsomal suspension containing an NADPH-generating system or liver tissue slices. In contrast, [^{14}C]chloroform bound covalently to calf thymus DNA after activation by hepatic microsomes from phenobarbital-induced rats.

4.5 Mechanistic considerations

There is strong evidence that cytotoxicity is a critical component of the induction of tumours in rodents by chloroform, since it has been shown that oxidative metabolism is necessary for activation of this compound and correlates with cytotoxicity, regenerative

cell proliferation and tumour response in the target cell population. The concordance between these responses is strongest for hepatic and renal tumours in mice.

Chloroform-induced liver tumours have been observed in mice only after prolonged exposure to cytotoxic bolus doses given by oral gavage and not after administration of the same total daily dose in drinking-water. The incidence and severity of toxicity in the liver and kidney have been related to the degree of covalent binding of oxidative metabolites of chloroform to tissue proteins. The relationship between metabolism and toxicity is exemplified by localization of covalent binding to protein in both liver and kidney and the increases and decreases in toxic responses that result from pretreatment with inducers and inhibitors of cytochrome P450 activity, respectively. There is a consistent association between oxidative metabolism of chloroform, the pattern of covalent tissue binding and toxic injury (Davidson *et al*., 1982). A strong dose-related correlation is also seen between induction of hepatic cytotoxicity, a sustained increase in regenerative cell proliferation and induction of liver tumours.

In the kidney of male mice, a strong correlation is also seen between the degree of oxidative metabolism, renal cytotoxicity, compensatory cell regeneration and renal tubular tumours. This correlation includes a strong concordance of these end-points with the sex of the mice and genetic variability. For example, male DBA mice, a parental strain of BDF_1 mice in which chloroform induces renal tumours, developed much more severe renal injury than C57BL mice, in which chloroform does not induce renal tumours.

The evidence for a correlation between cytotoxicity, a sustained increase in regenerative cell proliferation and tumours is weakest in rat kidney and in the tumour-susceptible Osborne-Mendel strain, which has not been investigated in depth; however, the observation of sustained renal cell injury in a two-year bioassay with Osborne-Mendel rats that correlated with tumour-inducing doses is consistent with induction of renal tumours via cytotoxicity induced by high doses and compensatory cell proliferation.

Thus, with the high-dose regimens used in cancer bioassays, chloroform has been shown to induce cytolethality and regenerative cell proliferation in the target organs for cancer.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

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

5.2 Human carcinogenicity data

Two cohort studies of cancer and drinking-water quality were carried out in the United States. One conducted in Maryland showed excess mortality from cancers of the liver and breast in association with water chlorination, while that conducted in Iowa

showed increased risks for cancers of the colon and lung and skin melanoma associated with chloroform concentrations in drinking-water.

Eight case–control studies have been reported on bladder cancer in relation to chlorinated drinking-water in the United States. Significant results were obtained in five studies, but there was little consistency in the risk pattern in subgroups defined by sex or surrogate measures of chloroform intake. Significant increasing trends in the risk for bladder cancer were seen in two studies. The study in Colorado showed increasing risk with years of exposure to chlorinated water; the study in Iowa showed increasing risk with lifetime intake of trihalomethanes (from drinking-water), but only in men and not in women.

Seven case–control studies addressed the risk for cancers of the large bowel in association with consumption of chlorinated water. In two of these studies, lifetime exposure to trihalomethanes was assessed. Two studies showed significant associations with rectal cancer. Overall, however, the results were inconsistent with regard to the subsite of the large bowel and sex, and the quality of the studies varied widely.

Exposure to chloroform in the workplace was addressed in two case–control studies, both of which had limited statistical power. The study on brain cancer gave negative results. The other included a number of sites (but not the brain) and showed associations with cancers of the prostate and lung, but no association was seen with bladder cancer.

The presence of various water chlorination by-products, including trihalomethanes, is likely to be highly correlated. Although chloroform is the most ubiquitous, the other by-products therefore may act as confounders in studies of water-mediated exposure. In addition, important sources of chloroform other than drinking-water were ignored in the majority of the studies.

Although the epidemiological evidence for an association between consumption of chlorinated drinking-water and the risk for some cancers, particularly those of the urinary bladder and rectum and possibly of the colon, seems to favour an interpretation of mild excess, a causal inference cannot be made with regard to chloroform because of incomplete control for confounding by other water impurities and other factors and lack of concordance in the results for men and women. Use of surrogate indicators for exposure to chloroform adds to the uncertainty.

5.3 Animal carcinogenicity data

Chloroform was tested for carcinogenicity in several experiments in mice, rats and dogs. In three studies by oral administration and in one study by inhalation exposure in mice, it produced renal tubular tumours and, in one study, hepatocellular tumours. In three studies by oral administration in Osborne-Mendel rats, chloroform produced renal tubule tumours. No increased incidence of tumours was observed in one study in dogs.

5.4 Other relevant data

Chloroform is metabolized by oxidative and reductive pathways. Under normal conditions, oxidative metabolism is the major pathway, and reductive metabolism does not play a significant role. Oxidative metabolism of chloroform results in the generation

of phosgene, which either reacts with water to give carbon dioxide and hydrogen chloride or binds covalently to tissue macromolecules. The formation of carbon dioxide as a metabolite of chloroform has been shown in a number of studies in both rodents and humans *in vivo*.

The metabolism of chloroform is more rapid in mice than in rats, and human tissues (liver and kidney) have the lowest activity. CYP2E1 is the predominant enzyme involved in the metabolism of chloroform in both rodent and human tissues.

There is a consistent, tissue-, species-, strain- and sex-specific pattern in the rate of metabolism, cytotoxicity and cell proliferation produced by chloroform in rodent liver and kidney. Under the conditions of the high-dose regimens used in cancer bioassays in which tumours are produced, chloroform induced cytotoxicity and regenerative cell proliferation in the target organs for cancer. These findings are consistent with a mode of action for tumorigenesis in the liver and kidney of rodents that involves cytotoxicity.

Chloroform has been tested for developmental toxicity in mice and rats by gavage and inhalation. Fetal toxicity in the form of growth retardation has been observed in several studies, concurrent with evidence of maternal toxicity. Malformations were observed in one study in rats exposed by inhalation. In a continuous breeding study, no reproductive effects were noted.

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.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of chloroform.

There is *sufficient evidence* in experimental animals for the carcinogenicity of chloroform.

Overall evaluation

Chloroform is *possibly carcinogenic to humans (Group 2B)*.

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