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CARCINOGENIC AND MUTAGENIC PROPERTIES OF CHEMICALS IN DRINKING WATER

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ABSTRACT

Isolated cases of careless handling of industrial and domestic waste has lead to a wide variety of dangerous chemicals being inadvertently introduced into drinking water. However, chemicals with established carcinogenic and mutagenic properties that occur with a high frequency and in multiple locations are limited in number. To date, the chief offenders have been chemicals of relatively low carcinogenic potency. Some of the more common chemicals are formed as by-products of disinfection. The latter process is generally regarded as essential to the production of a "microbiologically safe" drinking water. Consequently, any reductions in what may be a relatively small carcinogenic risk must be balanced against a potential for a higher frequency of waterborne infectious disease.

The results of recent toxicological investigations will be reviewed to place the potential carcinogenic and mutagenic hazards frequently associated with drinking water into perspective. First, evidence for the carcinogenicity of certain volatile organic compounds such as trichloroethylene, tetrachloroethylene and carbon tetrachloride is considered. Second, the carcinogenic activity that can be ascribed to various by-products of chlorination is reviewed in some detail. Finally, recent evidence that other chemicals derived from the treatment and distribution of drinking water is highlighted as an area requiring move systematic attention.

INTRODUCTION

A wide variety of chemicals have been found in drinking waters around the world. Modern analytical techniques have identified such a diversity of chemicals that it renders comprehensive review of toxic and potentially carcinogenic properties of each substance virtually impossible. However the sources of toxic chemicals in finished drinking water are derived from three general sources:

1. Contamination of source water.
2. By-products of treatment processes.
3. Leaching from the distribution system.

Examples of contaminants from each source will be discussed in turn.

CONTAMINANTS OF SOURCE

A great variety of chemicals can contaminate sources of drinking water. By far the most frequent contaminants in this category are trichloroethylene

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and tetrachloroethylene because of their large production volumes and widespread use in commerce. The carcinogenicity of trichloroethylene, tetrachloroethylene and to a lesser degree carbon tetrachloride are somewhat more controversial. Consequently, this section will provide an updated review of the data bearing on the carcinogenic properties of these chemicals. Consideration of other contaminants will be for comparative purposes only. A summary of pertinent carcinogenicity and mutagenicity data associated with contaminants of source waters is provided in Tables 1 and 2.

Carbon Tetrachloride

Studies of carbon tetrachloride (Cameron and Karunaratne, 1936; Reuber and Glover 1967a; Reuber and Glover 1967b) have documented the ability of carbon tetrachloride to induce preneoplastic changes in the liver of rats (e.g., hyperplastic nodules and cholangiofibrosis). Subsequent studies (Reuber and Glover, 1970) demonstrated that subcutaneous administration of carbon tetrachloride in corn oil at a dose of 2080 mg/kg twice weekly gave rise to hepatocellular carcinomas in male Japanese rats, Osborne-Mendel rats, and Wistar rats. Black rats and Sprague-Dawley rats failed to survive long enough to develop carcinomas following the same treatment. The authors' called attention to the fact that carcinoma development appeared to be inversely related to the degree of liver cirrhosis across the strains tested. However, this relationship may be fortuitous since those strains developing severe cirrhosis failed to survive. The single level of treatment utilized in this study precludes a clear resolution of this issue. A more recent study (NCI, 1976) of the carcinogenicity of carbon tetrachloride in Osborne-Mendel rats failed to confirm its carcinogenic effects at doses of up to 160 mg CCl_4/kg in corn oil administered 5 times weekly for 78 weeks (47 and 94 mg/kg for males, 80 and 159 mg/kg for females). However, it should be noted that the doses utilized were much lower than used in previous studies.

Eschenbrenner and Miller (1943) demonstrated that carbon tetrachloride was able to induce hepatomas in Strain A/J mice. Tumor yield was generally dose-related, but the effect was enhanced as the spacing between doses was increased from 1 to 5 day intervals to the same total dose of carbon tetrachloride. In subsequent experiments Eschenbrenner & Miller (1946) examined the relationship between liver necrosis and tumor development and found that the liver became resistant to necrotizing doses of carbon tetrachloride with chronic treatment. This observation was most probably related to the inactivation of cytochrome P-450 by carbon tetrachloride observed in modern metabolic studies (Glende et al., 1975). Overall, there

appeared to be a relationship between obvious necrosis and the development of hepatomas. However, hepatomas could be induced by doses of carbon tetrachloride that did not induce obvious necrosis in the liver. The authors pointed out that they could not rule out the possibility of a slightly greater rate of liver cell replacement at these lower doses.

They did find evidence of cirrhosis at doses not producing obvious necrosis, indicating some degree of liver injury. Kiplinger and Kensler (1963) also observed the induction of hepatomas following oral administration of carbon tetrachloride in C3H mice relative to historical controls, however, this study did not include concurrent controls. The National Cancer Institute (NCI, 1976) documented a high incidence of hepatocellular carcinomas in the B6C3F1 mice administered at doses of 1250 and 2500 mg/kg by gavage in a corn oil vehicle.

Despite the fact of its recognized carcinogenic effects in rodents there is little convincing evidence that carbon tetrachloride possesses genotoxic properties (IARC, 1979b; USEPA, 1984). On the other hand, the ability of hepatonecrotic doses of carbon tetrachloride to promote the yield of liver tumors in mice is well documented (Pound and McGuire, 1975). Therefore, the bulk of the evidence indicates that the cancer producing activity of carbon tetrachloride are secondary to its hepatoxic effects and carbon tetrachloride might be considered as a carcinogen that does not act by a "genotoxic" mechanism.

Trichloroethylene

Trichloroethylene (TCE) was first shown to be carcinogenic in a study sponsored by the National Cancer Institute (NCI, 1976). In this study, male B6C3F1 mice were exposed to time-weighted average doses of 1169 and 2339 mg/kg body weight, whereas, females received 869 and 1739 mg/kg of TCE dissolved in corn oil by gavage for a period of 78 weeks. In the same study both male and female Osborne-Mendel rats were administered doses of 549 and 1097 mg/kg TCE by the same means and for the same duration of exposure. Dose-related increases in the incidences of hepatocellular carcinomas were observed in both male and female mice, although the response was much more marked in the males. There were no statistically significant increases in tumor yield in either male or female Osborne-Mendel rats.

Inhalation studies conducted by Henschler et al. (1980) at concentrations of TCE in air of 100 and 500 ppm for 6 h/day, 5 days/week for a period of 18 months demonstrated an increased incidence of malignant lymphoma in female NMRI mice but was without apparent effect in male mice, Wistar rats or Syrian

TABLE 1. Evidence for Carcinogenicity of Carbon Tetrachloride, Trichloroethylene and Tetrachoroethylene

Chemical	Species & Strain (Sex)	Highest Dose	Route	Vehicle	Result	Reference
Carbon Tetrachloride	C3H Mice (M&F)	0.04 ml 2 to 3 times weekly for 8-16 weeks	Oral	Olive Oil	Hepatoma	Edwards, et al., 1941
	Strain L	0.04 ml 46x over a 4 month period (no	"	"	Hepatoma	Edwards, et al., 1941
"	Strain A Mice (M&F)	2.5g/kg to a total of 30 doses	Oral	Olive Oil	Hepatoma	Eschenbrenner & Miller, 1943
"	B4C3F1 Mice (M&F)	2500/mg/kg 5 X weekly for 78 wks	Oral	2-5% solution in corn oil	Hepatocellular carcinoma	NCI, 1976
"	C3H Mice	1.6 g/kg 3 X weekly for 10 wks	Oral	Corn Oil	Hepatoma	Kiplinger & Kensler, 1963
"	C3H Mice	0.04 ml 2 times weekly for 20-26 weeks	Rectal	Olive Oil	Liver Tumors	Confer & Stenger, 1965
"	Osborne-Mendel Rat (M&F)	160 mg/kg 5 X weekly 78 weeks	Oral	Corn Oil	Negative	NCI, 1976
"	Syrian golden hamsters	12.5 ul/hamster weekly no controls	Oral	Corn Oil	Liver cell carcinomas	Della Porta et.al., 1976
"	Japanese rat (M)	2g/kg bw 2 X weekly	s.c.	Corn Oil	Hepatocellular carcinoma	Reuber & Glover, 1970
"	Osborne-Mendel (M)	" " " "	s.c.	" "	Hepatocellular carcinoma	Reuber & Glover, 1970
"	Wistar - (M)	" " " "	s.c.	" "	Hepatocellular Carcinoma	Reuber & Glover, 1970

TABLE 1. (Continued)

Chemical	Species & Strain (Sex)	Highest Dose	Route	Vehicle	Result	Reference
Carbon Tetrachloride	Black rat (M)	2g/kg bw 2 X weekly	s.c.	Corn Oil	(Died early)	Rebuer & Glover, 1970
"	Sprague-Dawley (M)	" " " "	"	" "	"	Reuber & Glover, 1970
Trichloroethylene	B6C3F1 Mice (M&F)	2339 mg/kg TWA for 78 weeks	Oral	Corn Oil	Hepatocellular carcinoma	NCI, (1976)
"	Osborne-Mendel (M&F)	1097 mg/kg TWA for 78 weeks	Oral	Corn Oil	Negative	NCI, (1976)
"	ICR Mice (F)	0, 150 & 450 ppm in air 7 h	Inh.	Air	Pulmonary adenocarcinoma	Fukuda, et al., 1983
"	Sprague-Dawley rats (F)	0, 150 & 450 ppm in air 7 h per day, 5 days a week	Inh.	Air	Negative	" "
"	Mice (M&F)	500 ppm for 18 months	Inh.	Air	Malignant lymphoma	Henschler et al., 1980
"	Rats (M&F)	500 ppm for 18 months	Inh.	Air	Negative	" "
"	Hamsters (M&F)	500 ppm for 18 months	Inh.	Air	Negative	" "
Tetrachloroethylene	B6C3F1 Mice (M&F)	1072 mg/kg TWA for 78 weeks	Oral	Corn Oil	Hepatocellular carcinoma	NCI, (1977)
"	Osborne-Mendel (M&F)		Oral	Corn Oil	Negative	NCI, (1977)
"	Sprague-Dawley (M&F)	600 ppm in air for 12 months	Inh.	-	Negative	Rampy et al., 1978

TABLE 2. (Continued)

Chemical	Test System	Special Conditions	Highest Dose Tested	Result	Reference
Trichloroethylene	Covalent binding to protein in rat liver microsomes	In vitro	Unlike vinyl chloride binding is not specific to SH containing protein.	pos.	Bolt and Filser 1977
"	S. Typhimurium TA100	+/- Aroclor 1254 induced rat liver S-9 fraction	100 ug/ml in top agar	neg. with and without S-9	Henschler et al., 1977
"	Saccharomyces cerevisiae XV185-14C	+/- mouse liver S-9 fraction	20 ul/ml	Pos. with S-9 only	Shahin & Von Borstel 1977
"	Fischer Rat F1706 embryo cell system		1.1×10^{4} M	Pos.	Price et al., 1977
"	Covalent binding to hepatic proteins in the S.D. Rat	In vivo and In vitro		Pos.	Allemand, et al., 1978
"	Saccharomyces cerevisiae D7	Suspension test, +/- mouse liver 10,000 x g supernatant	40 mM	Pos. point mut. and give conversion required S-10 to be active	Bronzetti, et al., 1978

TABLE 2. (Continued)

Chemical	Test System		Special Conditions	Highest Dose Tested	Result	Reference
Trichloroethylene	Saccharomyces cerevisiae D7		Host mediated	400 mg/kg	pos. ilv reversion + trp conversion	Bronzetti, et al., 1978
"	" D4		" "	400 mg/kg	Pos. at ade and trp loc.	" "
"	" D4		Host mediated	3700 mg/kg (22 repeated doses of 150 mg/kg + 400 mg/kg day of sacrifice).	pos. trp and ade loci	" "
	Schizosaccharomyces pombe	P1	+/- mouse and rat liver S-9 fraction pretreated phenobarbital or B-naphthoflavone	22 mM	neg. at ade loci 1,3,4, 5 & 9	Rossi, et al. 1983
"	Schizosaccharomyces pombe	P1	Host mediated	2 g/kg	neg.	Rossi, et al. 1983
"	DNA binding in in vitro & in vivo		Calf thymus, in vitro mouse, in vivo		pos. in vitro neg. in vivo	Bergman, 1983
Tetrachloroethylene	E. Coli K12	gal + arg + nad + MTR	+/- Mouse microsomes, phenobarbital pre-treated	0.9 mM in media	neg.	Greim et al. 1975

TABLE 2. (Continued)

Chemical	Test System	Special Conditions		Highest Dose Tested	Result	Reference
Tetrachloroethylene	S. typhimurium	TA1950 TA1951 TA1952 TA1535 TA1538 TA100 TA98	Spot test-no S-9 used in vitro		neg. neg. neg. neg. neg. pos. neg.	Cerna & Kypenova, 1977
"	S. typhimurium	TA1950 TA1951 TA1952	Host mediated Female ICR mice	LD_{50} and 1/2 LD_{50}	Pos. no dose response evident	Cerna & Kypenova, 1977
"	Macromolecular binding in rat and liver tested in vivo			500 mg/kg (oral dose)	Binding to macro-molecules but no binding detected in purified DNA	Schumann et al. 1980
"	Saccharomyces cerevisiae	D7	Suspension assay +/- mouse liver S-9 Host mediated assay	85 mM 11 g/kg (single dose) 2g/kg x 12 + 4 g/kg	Neg. Neg. Neg.	Bronzetti et al. 1983

hamsters. More recently, Fukuda et al. (1983) were able to show a small increase in the incidence of pulmonary adenocarcinomas in female ICR Swiss mice exposed to 150 and 450 ppm TCE (16 and 15%, respectively, versus a control incidence of 2%) in air 7 h per day, 5 days a week for 104 weeks. No evidence of carcinogenic activity was observed in female Sprague-Dawley rats subjected to the same treatment. Consequently, evidence that TCE is carcinogenic is limited to mice, although three different tumors have been identified in each of the mouse strains that have been studied.

As a result of the initial observation that TCE was carcinogenic in mice and because it comes from the same chemical class as vinyl chloride (chloroethylene), an established human carcinogen, there have been many studies of the compound's genotoxic properties. Simmon et al. (1977) reported that TCE increased the reversion rate of S. typhimurium strain TA100 only in the presence of a 9000 X g supernatant of homogenates made of mouse or rat liver. The experiments in this study were conducted in a desiccator and avoided the volatilization of TCE that is encountered in the standard plate assay of the Ames test when such precautions are not taken. Cerna and Kypenova (1977) were unable to demonstrate mutagenic activity of TCE in the spot test utilizing a variety of strains of S. typhimurium in the absence of a metabolic activation system, but were able to demonstrate positive activity in host-mediated assays in female ICR mice using strains TA1950, TA1951 and TA1952. Greim et al. (1975) demonstrated that TCE was capable of more than doubling the spontaneous mutation rate at the arg+ locus of E. coli strain K12 after incubation with a liver microsomal preparation taken from mice that had been pretreated with phenobarbital. No activity was attributable to TCE in the absence of metabolic activation. Henschler et al. (1977) failed to confirm the mutagenic activity of TCE in S. typhimurium strain TA100 both in the presence and absence of a metabolic activation system derived from Aroclor 1254 pretreated rats.

In addition to studies in bacterial systems, TCE has been shown to induce mutations in one yeast test system by two different research groups (Shahin & von Borstel, 1977; Bronzetti et al., 1978) either in the presence of a metabolic activation system or in host-mediated assays, but negative under similar circumstances in another (Rossi et al., 1983). Price et al. (1978) were able to demonstrate that TCE was capable of transforming Fischer rat embryo cells. Transformed cells were capable of growth in semisolid agar and produced undifferentiated fibrosacromas when inoculated into newborn Fischer rats. Henschler et al., (1977) suggested that the mutagenic activity and the carcinogenic activity of TCE might be accounted for by the presence of

epichlorohydrin and 1,2-epoxibutane. However, these chemicals produced a mutagenic effect in the absence of a metabolic activation system, an observation that was not observed in prior positive tests of TCE by other authors.

It has been clearly shown that TCE is metabolized to a form capable of covalently interacting with tissue proteins at a level comparable to that observed with vinyl chloride (Van Duuren & Banerjee, 1976; Bolt & Filser, 1977; Allemand et al. 1978). Only very low levels of interaction of TCE with DNA in vivo have been demonstrated (Stott et al., 1982; Bergman, 1983). Under in vitro conditions and in the presence of a metabolic activation system, a higher degree of binding of a TCE metabolite to calf thymus DNA has been observed (Bergman, 1983).

The evidence that TCE is carcinogenic was considered to be limited by IARC (1979d). At that time the evidence that TCE has genotoxic properties was obscured by reports of impurities present in the technical grade solvent (Henschler et al., 1977). However, the mutagenic activity associated with impurities have always shown activity in the absence of metabolic activation, whereas samples of TCE have been routinely negative in the absence of metabolic activation. As indicated above, positive results have been specifically observed under circumstances where metabolic activation was possible in E. coli, S. typhimurium, and Saccharomyces cerevisiae. Moreover demonstration of transformation in Fischer rat embryo cells supports the notion that TCE is able to act as a carcinogen. Negative results using bacterial and yeast systems in vitro appear to involve failure to consider the volatility of TCE or employed lower concentrations than utilized in positive studies. The reasons for the discrepancy in results using two yeast strains in host-mediated assays is not as clear.

The lack of strong evidence of TCE interaction with DNA in vivo (Stott et al., 1982; Bergman, 1983) presents a problem in invoking a genotoxic event as being responsible for the carcinogenic response. The difficulty in demonstrating such an interaction is in sharp contrast to that observed with vinyl chloride, a closely related chemical (Green and Hathway, 1978). On the other hand, it is difficult to completely rule out the possibility that the minor interaction observed (Stott et al., 1982) could be responsible for some of the apparent genotoxic effects of TCE.

The observations of pulmonary adenocarcinomas (Fukuda et al., 1983) and malignant lymphoma (Henschler et al., 1980) in addition to hepatocellular carcinomas previously reported (NCI, 1976), indicate that TCE has some carcinogenic activity in mice. It should be pointed out, however, that these

tumors have an appreciable background incidence in the strains of mice utilized. In the liver of mice, doses that produced liver tumors produced definite histological evidence of hepatocellular damage and a dose-related hypertrophic response to trichloroethylene (Stott et al., 1982). These data suggest that liver tumors in long-term studies may have resulted from repeated tissue damage and hypertrophy.

Tetrachloroethylene

To date, the evidence that tetrachloroethylene (perchloroethylere, PCE) is carcinogenic is limited to a study conducted by the National Cancer Institute (1977). The results of this study indicated that time weighted average doses of 536 and 1072 mg/kg to male and 386 and 772 mg/kg given to female B6C3F1 mice by gavage using a corn oil vehicle for 78 weeks resulted in the development of hepatocellular carcinomas. No evidence of increased tumor incidence was observed in Osborne-Mendel rats given time weighted average doses of 471 and 941 mg/kg in males and 474 and 949 mg/kg in females administered under the similar conditions in the same study. Rampy et al. (1978) also failed to observe increased tumor incidences in Sprague-Dawley rats exposed to 600 ppm TCE in air for a period of 12 months.

Evidence that PCE has genotoxic properties is also limited. PCE failed to increase the mutation frequency of E. coli K12 strain (Greim et al. 1975) in the presence or absence of mouse liver microsomes. It was reported (in abstract form) to produce an increase in the reversion rate of S. typhimurium strain TA100 in the absence of metabolic activation in the spot test, but was inactive in all other strains employed (Cerna & Kypenova, 1977). On the other hand, these same authors indicated that PCE was positive in a host-mediated assay utilizing S. typhimurium strains TA1950, TA1951 and TA1952 in female ICR mice at the LD50 and 1/2 of the LD50 in the same abstract. There was no evidence of dose-response. Bronzetti et at. (1983) were unable to demonstrate an effect of PCE using Saccharomyces cerevisiae strain D7 in a suspension assay in the presence or absence of mouse liver S-9 fraction or in a host-mediated assay. It is notable that these same authors had previously demonstrated positive results with TCE under both of these conditions (Bronzetti et al. 1978).

The IARC (1979e) evaluated the evidence that PCE was carcinogenic and found only limited evidence that it was active in mice. There is no substantive basis on which to modify that opinion at the present time.

BY-PRODUCTS OF TREATMENT PROCESSES

The trihalomethanes were the first chemicals that were recognized to be by-products of the chlorination of drinking water (Bellar et al., 1974; Rook, 1974). Trichloromethane (chloroform), dichlorobromomethane, dibromochloromethane, and tribromomethane (bromoform) are the most commonly encountered trihalomethanes found in drinking water. Iodinated derivatives are less commonly observed. A recent, comprehensive review of its carcinogenic, mutagenic, and teratogenic effects has been published by Davidson et al., (1982). The present section will primarily concern itself with data that has become available since this earlier review.

More recently, it has become quite apparent that the trihalomethanes (THM) are only one class of by-products produced in the chlorination of drinking water. Many of the non-THM by-products have been shown to be mutagenic in bacterial systems, but of these only the haloacetonitriles and chlorinated phenols have been shown carcinogenic in experimental animals. Thus, only these two additional classes will be covered in the present review. However, chlorine is known to increase mutagenic activity in drinking water (e.g. Zoeteman et al. 1982; Kool et al. 1982; Meier and Bull, 1985) and in reactions with humic acid, (Meier et al. 1983; Coleman et al., 1984; Meier et al., 1985). On the other hand, it should be remembered that chlorination is the only practice extensively studied. Many by-products remain to be identified with chlorine as well as other alternative modes of disinfection and even those which have been identified have been poorly characterized toxicologically.

In addition to by-products of drinking water disinfection, other treatment processes have the potential for the introduction of carcinogens into drinking water. In this light the carcinogenicity of acrylamide is discussed for illustrative purposes.

Chloroform

A study sponsored by the National Cancer Institute (NCI, 1976) demonstrated that B6C3F1 mice of both sexes developed hepatocellular carcinomas while male Osborne-Mendel rats had increased yields of renal tumors following chronic exposure to chloroform (Table 3). Chloroform was administered to both mice and rats in a corn oil vehicle by stomach tube.

Because of the primary source of chloroform in drinking water is side reactions of chlorination and the necessity for producing microbiologically safe drinking water a more thorough examination of the dose response relationships involved in chloroform-induced cancer was undertaken (Jorgenson et al. 1985). The doses used in this study was extended to a lower range and

the group sizes were substantially expanded. Chloroform was administered in the drinking water. This design was to avoid problems that may have been associated with gavage dosing and the corn oil vehicle. The expanded group size at low doses would provide a better basis for extrapolation of the results to estimate human risks. Serum and tissue biochemical measurements were made in separate groups of animals on parallel treatments. Additional control groups were utilized for both species in which were restricted in their water consumption to the amount consumed by the high dose groups for each species. This provided a check on the effects that might be secondary to decreased water consumption. Only female B6C3F1 mice and male Osborne-Mendel rats were included in the experiment as these were the sexes which gave the greatest response in the NCI (1976) study.

In the male Osborne-Mendel rat very similar results were observed in the Jorgenson et al. study (1985) as had been obtained in the prior NCI study (1976). In particular, there was a dose-related increase in renal tubular adenomas and adenocarcinomas. The results obtained with the female B6C3F1 mice were, however, substantially different than observed in the original NCI study. There was a complete absence of any dose-related increases in the incidence of hepatocellular carcinomas in mice. This result was particularly significant, since the daily doses of chloroform in the two high exposure groups bracket the low dose which gave rise to an 80% incidence of hepatocellular carcinomas in the earlier NCI study.

These results are interesting in the context of evidence of chloroform carcinogenicity that was obtained prior to the NCI study (Table 3). Eschenbrenner and Miller (1945) noted that hepatocarcinogenic effects of chloroform were confined to doses which produced frank liver necrosis. Studies quoted in Table 3 that failed to produce increases in tumor incidence involved dose levels lower than those used in either the NCI (1976) or Jorgenson et al. (1985) studies. Roe et al. (1979), however, did find increased incidence of renal tumors in ICI mice when chloroform was administered in a toothpaste base at a dose of 60 mg/kg per day. Renal tumors were not produced in the C57BL, CBA, and CF/1 strains at the same dose in the same study. No liver tumors were not observed in any of these mouse strains.

It has been argued that the carcinogenicity was secondary to tissue necrosis followed by regenerative hyperplasia largely because of the early observations of Eschenbrenner and Miller (1945). Chloroform at doses found capable of inducing tumors produces substantial increases of tritiated thymidine incorporation into DNA of both the liver and kidney of the B6C3F1 mouse (Reitz et al., 1982). This result provides evidence of a regenerative

TABLE 3. Evidence for Carcinogenicity of Chloroform in Experimental Animals

Species and Strain	Vehicle	Highest Dose Tested	Route	Tumor	References
A Mice	Olive Oil	2350 mg/kg	oral	Liver	Eschenbrenner & Miller, 1945
C57BL Mice	Toothpaste	60 mg/kg/d	oral	none	Roe et al., 1979
CBA Mice	Toothpaste	60 mg/kg/d	oral	none	"
C1/1	Toothpaste	60 mg/kg/d	oral	none	"
ICI Mice	Toothpaste	60 mg/kg/d	oral	Kidney	"
Sprague-Dawley Rat	Toothpaste	60 mg/kg/d	oral	none	"
Beagle Dog	Toothpaste	20 mg/kg/d	oral	none	"
"	"	"	"	Total Neoplasms	Reuber, 1979
Osborne-Mendel Rat	Corn Oil	180 mg/kg/d	oral	Kidney	NCI, 1976
"	"	"	"	Thyroid	Reuber, 1979
"	"	"	"	Cholangiofibromas	" "
"	"	"	"	Cholangiocarcinomas	" "
B6C3F1 Mice	Corn Oil	477 mg/kg/d	oral	Liver	NCI, 1976
"	"	"	"	Lymphoma	Reuber, 1979
Osborne-Mendel Rat	Drinking Water	130 mg/kg/d	oral	Kidney	EPA-NCI Study.
B6C3F1 Mice	Drinking Water	400 mg/kg/d	oral	None	EPA-NCI Study.

response and is consistent with this hypothesis. Along the same line, Moore et al. (1982) provided evidence that doses of 60 mg/kg of chloroform in corn oil resulted in damage to the kidney and evidence of tubular regeneration as measured by tritiated thymidine uptake. These same doses administered in a toothpaste base were without effect. At 240 mg/kg similar effects were seen with both vehicles. This type of response was not strongly apparent in the liver or kidney of Osborne-Mendel rats.

In animals receiving parallel treatments to those used in the Jorgenson et al. study (1985) there was a dose-related increase in the liver fat content apparent in chloroform-treated mice (Jorgenson et al., 1982). This effect was observed only at much higher doses in the rat. Before the changes in liver fat occurred in the rat they were preceded by substantial a depression of serum triglycerides. Data on serum triglycerides of the mice under these conditions are not presently available. Based on the rat data one can speculate that the use of corn oil may have contributed to the development of hepatocellular carcinomas in the first NCI study through some type of interaction with the hepatoxic effects of chloroform.

The difficulty that has been encountered trying to demonstrate that chloroform possesses genotoxic properties (Table 4) indirectly supports the thesis that chloroform induced tumors are the result of overt tissue necrosis. A number of independent workers have failed to show a direct interaction of chloroform or a metabolite with DNA in the mouse or rat liver or kidney (Diaz-Gomez and Castro, 1980; Reitz, et al. 1982; Pereira et al. 1982). On the other hand, chloroform has been shown capable of inducing chromosome breakage and increased sister chromatid exhange frequencies in human lymphocytes in vitro and increased sister chromatid exchange in the bone marrow of mice treated with chloroform in vivo (Morimoto and Koizumi, 1983). It has also been found to enhance the transformation of Syrian Hamster Embryo (SHE) cells by S7 adenovirus (Hatch et al., 1983). It is notable that the concentrations of chloroform tested in these positive experiments tend to be higher than those utilized in similar experiments that had negative results (Kirkland et at., 1981). It must be said, however, that the exact mechanism by which chloroform induces cancer remains elusive. Attempts to directly demonstrate an alternative mechanism for chloroform-induced cancer have produced equivocal data. For example, Pereira et al. (1982) failed to clearly demonstrate either tumor initiating or tumor promoting activity in the rat liver.

In summary, the extent to which chloroform in drinking water represents a carcinogenic hazard to man is still uncertain. There is no doubt that

chloroform is capable of producing renal tumors in at least one strain of mice and rats. Chloroform can also induce liver tumors in mice, but this effect seems dependent on the vehicle used. In view of the apparent dependence of the carcinogenic effects of chloroform in the mouse liver on the vehicle in which it was administered, it is suggested that chloroform's carcinogenic activity in the rat may be more appropriate for estimating carcinogenic risk in humans.

Haloacetonitriles

The dihalogenated chlorine and bromine derivatives of acetonitrile have been the principal species identified in chlorinated drinking water. (Trehy & Bieber, 1981; Oliver, 1983). Investigations into the carcinogenicity of these chemicals have been confined to the three members of the chlorinated series, bromochloroacetonitrile and dibromoacetonitrile. Simmon et al. (1977) first documented that dichloroacetonitrile (DCAN) was mutagenic in Salmonella typhimurium. Bull et al. (1985) confirmed the mutagenic properties of DCAN in Salmonella and demonstrated that bromochloroacetonitrile (BCAN) is mutagenic under similar circumstances. Chloroacetonitrile (CAN), trichloroacetonitrile (TCAN) and dibromoacetonitrile (DBAN) were found to be inactive in the presence or absence of a 9000 X g supernatant fraction (S-9) of a liver homogenate taken from rats previously treated with Arochlor 1254. All five haloacetonitriles induced sister chromatid exchange (SCE) in Chinese hamster ovary cells, in vitro, in the absence of rat liver S-9 fraction. None of these same five haloacetonitriles, however, were found capable of inducing mutagenic activity in the mouse micronucleus assay in vivo (5 male and 5 female CD-1 mice in each group) at doses of 0, 12.5, 25 and 50 mg/kg bw for 5 consecutive days in this same study.

Direct evidence of the carcinogenicity of the haloacetonitriles is limited to initiation/promotion experiments in the mouse skin. Experiments employing the oral route of administration (a total dose of 150 and 300 mg/kg bw split between 6 individual doses) followed by a 20 week promotion schedule with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) failed to demonstate a significantly elevated incidence of either benign or malignant skin tumors within a 1 year observation period (Bull et al., 1984). On the other hand, higher doses applied topically (1200, 2400 and 4800 mg/kg, also split between 6 applications), yielded significantly higher tumor incidences with CAN, BCAN and DBAN. DCAN and TCAN produced small increases in cumulative tumor yields, but the changes were not significantly different from controls.

TABLE 4. Evidence for Genotoxic Activity of Chloroform

Test System		Special Conditions	Highest Dose Tested	Result	Reference
S. Typimurium	TA1535	+/- Mouse & Rat Liver and kidney S-9	10 mg/plate	neg.	Van Abbe et al. 1982
"	TA1537	"	"	"	"
"	TA1538	"	"	"	"
"	TA98	"	"	"	"
"	TA100	"	"	"	"
Syrian Hamster Embryo Cells- Enhanced viral transformation		Applied in sealed chamber	0.5 ml/chamber	pos.	Hatch et al., 1983
V79 cells - 8-azaguanine		Applied in flow through system, then sealed	3% in air	neg.	Sturrock, 1977
Human Lymphocyte - Sister Chromatid Exchange Induction		+/ - Rat Liver S-9	400 ug/plate	neg.	Kirkland et al., 1981
Human Lymphocyte Chromosome Damage		+/ - Rat Liver S-9	400 ug/plate	neg.	"
E. Coli WP2p and WP2uvr A⁻p		+/ - Rat Liver S-9	10 mg/plate	neg.	"
Human Lymphocyte - Sister Chromatid Exchange, in vitro			6 mg/ml	pos.	Morimoto & Koisumi, 1983
Mouse Bone Marrow - Sister Chromatid Exchange, in vivo			4 x 200 mg/kg/1	pos.	"
Rat Liver, DNA - binding in vivo				neg.	Diaz-Gomez & Castro, 1980

TABLE 4. (Continued)

Test System	Special Conditions	Highest Dose Tested	Result	Reference
Sprague Dawley Rat, DNA binding *in vivo*	Liver	N.A.	neg.	Pereira et al., 1982
	Kidney	N.A.	neg.	"
B6C3F1 mice, DNA binding,	Liver	N.A.	neg.	"
B6C3F1 mice, DNA binding *in vivo*	Liver	N.A.	neg.	Reitz et al., 1982

A preliminary investigation of the haloacetonitriles ability to increase the incidence of lung tumors in strain A/J mice was reported by Bull and Robinson (1985). Doses of 10 mg/kg administered three times weekly for 8 weeks significantly increased lung tumor yields at 9 months of age with CAN, TCAN, and BCAN (Bull and Robinson, 1985). However, the tumor incidence was increased to only 32, 28 and 31% respectively. Control incidence of this spontaneous tumor was 10%. Although statistically significant, this small increase in lung tumors is difficult to interpret because of the variable background rate of this tumor in A/J mice.

These data indicate that certain members of the haloacetonitrile class do possess weak carcinogenic properties. This evidence has been confined to the topical route of administration and coupled to the subsequent application of a potent tumor promoting agent, TPA. There is no evidence available concerning the ability of these compounds to induce cancer by a systemic route of administration. Because of their relatively common occurrence as a result of the chlorination of drinking water it is critical that studies examining this issue be initiated.

Chlorinated Phenols

Chlorination of drinking water often gives rise to small quantities of chlorinated phenol derivatives. The principle products are 2-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol. There is substantive evidence of carcinogenic properties for only 2,4,6-trichlorophenol. The National Cancer Institute (NCI, 1979) conducted a feeding study of the effects of 5,000 and 10,000 ppm of 2,4,6-trichlorophenol in the diet of F344 rats and 5,214 and 10428 ppm in the diet of B6C3F1 mice. The incidence of lymphomas or leukemias was increased in male rats in a dose-related manner. Hepatocellular carcinomas were increased in both male and female mice and the incidences were dose-dependent.

Chlorinated Aldehyde and Ketone Derivatives

A number of chlorinated aldehydes and ketones have been identified in drinking water or related matrices following chlorination. A number of these by-products have been shown to be mutagenic (Bull and Robinson, 1985). Preliminary results from our studies, indicate that at least two of these chemicals, 2-chloropropenal and 1,3-dichloroacetone are capable of initiating tumors in mouse skin (unpublished results).

Other Treatment Chemicals

A wide variety of chemicals are necessary in the production of a safe and wholesome drinking water. For the most part these chemicals are generally regarded as safe and pose no substantive hazard to the health of the consuming public. However, if the synthesis of some products is improperly controlled they can contain contaminants that are of potential concern. An example of such products is the group of polymeric chemicals that are commonly used clarifying water, the coagulant aids.

The coagulant aids are high molecular weight polymers that are essentially non-toxic and are removed from the finished water by precipitation and filtration. However, the monomeric components used in the synthesis of these polymers are often reactive chemicals that are highly toxic and if sufficiently stable in water could be expected to be in the finished drinking water. One example of such a monomer is acrylamide.

Acrylamide has been recently shown capable of initiating papillomas and carcinomas in the skin of mice whether administered by the oral, intraperitoneal or topical routes of administration (Bull et al., 1984a). It was also shown to be capable of increasing the yield of lung adenomas in strain A/J mice in the same study. More recently, very similar results have been reported with ICR-Swiss mice (Bull 1984b).

Although acrylamide is inactive in the standard plate assay of the Ames' test (Bull et al., 1984a) it does increase the frequency of sister chromatid exchange in Chinese hamster ovary cells, in vitro (Bull et al., 1983). Shiraishi and Yamamoto (1978) and Shiraishi (1978) reported that acrylamide produces aneuploid and polyploid cells in both bone marrow cells and spermatogonia of mice treated with acrylamide by oral or intraperitoneal administration. Bull et al. (1983) observed dose-related increases in spermhead abnormalities in mice with acrylamide. More recently, Vanhorick and Moens (1983) found that acrylamide was a weak inducer of SV40 DNA amplification in SV40-transformed Chinese hamster cells as measured by in situ hybridization techniques. However, acrylamide synergistically enhanced the induction of SV40 DNA synthesis by a number of other carcinogens.

DISTRIBUTION SYSTEM

Contribution of toxic chemicals from the distribution system to finished drinking water can be considerable under certain circumstances. It is beyond the scope of this paper to deal comprehensively with this subject. However, it should be pointed out that certain products used in drinking water distribution are made of monomeric components that are recognized carcinogens. The most obvious example is polyvinyl chloride pipe which is a polymer of the

well established human carcinogen vinyl chloride (IARC, 1979). Modern manufacturing techniques have reduced the monomer content of these products to negligible concentrations, but users of such products should carefully avoid substandard materials.

Another product that was widely used in lining of certain water mains, storage tanks and pipes are coal tar and asphaltic-based paints. The high polyaromatic hydrocarbon content of coal tar paints is partially, but not completely responsible for the high carcinogenic activity of these products in the mouse skin (Robinson et al., 1984). Although still containing carcinogenic components, the asphaltic based paints used in contact with potable water are approximately 3 orders of magnitude less potent than their coal tar analogs. Because of the extremely low water solubility of the polyaromatic hydrocarbons that are partially responsible for the carcinogenic activity of coal tar these chemicals would not be expected to be rapidly leached from coatings. However, the extent to which these coatings provide a relatively constant reservoir for the leaching of carcinogen from the surface of the distribution system has not been well studied. However, some information exists to suggest that as these coatings age they can begin to shed fine particulates containing high concentrations of polyaromatic hydrocarbons into the finished drinking water.

DISCUSSION

It is clear from the previous discussion that chemical carcinogens find their way into drinking water through a variety of means. Obviously, industrial contamination is only one and in most cases a minor source of chemicals in drinking water. Evidence that the chlorination by-products 2,4,6-trichlorophenol and the haloacetonitriles have carcinogenic properties indicate that we must look beyond the trihalomethanes as the only potential carcinogenic hazards associated with disinfection. However, whatever the carcinogenic risk these products might represent they must be balanced against the obvious reduction of disease from waterborne infectious disease that results from chlorination. Finally there is a clear need to carefully scrutinize products that are utilized in the treatment and distribution of drinking water since these products can be a major source of harmful chemicals presented at the tap for human consumption.

Despite the need to accept the general notion that carcinogenicity in experimental animals signals cause for concern about human exposures to the same chemical, it must be recognized that considerable uncertainty underlies the extrapolation of that information to man. These uncertainties are both

qualitative and quantitative and have been discussed in detail elsewhere (IARC, 1982c). For example, if a chemical induces malignant tumors in more than one species, particularly if it produces tumors that are rare in the particular strain or species its carcinogenic properties are widely accepted (IARC, 1982c). On the other hand, a chemical that increases the incidence of a tumor that has a high spontaneous incidence in the test animal employed are felt to provide only limited evidence of carcinogenicity. Certain of the low molecular weight chlorinated hydrocarbons fall into this category. Other chemicals from the same class, such as vinyl chloride, cause little controversy because they produce malignant tumors in multiple sites and different species. It should be recognized that this view does not suggest that increased incidence of spontaneous tumors is of less concern than induction of rare tumors. Rather it is a statement related to the wide variety of factors that can modify the yield of a spontaneous tumor, whereas induction of a rare tumor probably requires the properties of a complete carcinogen.

It is ironic that most of the chemicals that occur frequently in drinking water fall into a class of carcinogens whose carcinogenic effects are controversial. In general, the controversy involves those chemicals which induce liver tumors, in particular those whose activity is limited to the induction of liver tumors in mice. Part of this concern is illustrated by the fact that the yield of hepatocellular carcinomas in B6C3F1 mice can be increased substantially by simple performance of a partial hepatectomy (Newberne et al., 1982). The chemicals most directly involved in this controversy are trichloroethylene and tetrachloroethylene, but also involve considerations of the carcinogenicity of carbon tetrachloride and chloroform.

Carbon tetrachloride should in many ways be considered the prototype for this group, although its carcinogenicity seems somewhat more generally accepted than the other three compounds. As pointed out above, carbon tetrachloride is capable of producing liver tumors in a variety of species. Despite many attempts (many of which have not been published), it has been virtually impossible to demonstrate any genotoxic activity with carbon tetrachloride (IARC, 1979b; USEPA, 1984). However, the studies that have demonstrated carbon tetrachloride induced liver cancer has involved very high doses, from 1000 to more than 2000 mg/kg administered in an vegetable oil vehicle 2 to 5 times weekly for extended periods of time.

The experimental results with trichloroethylene in many ways contrast sharply with that obtained with carbon tetrachloride. Trichloroethylene has been shown to only induce hepatocellular carcinomas in mice (NCI, 1976).

Although of a rather low potency, the genotoxic properties of trichloroethylene have been demonstrated by a number of investigators. In this respect, evidence of its carcinogenic activity would have to be considered stronger than that of carbon tetrachloride. However, the fact remains that trichloroethylene has only been shown capable of increasing the yield of neoplasms only in strains of mice which have high background incidence of these same neoplasms (Henschler et al., 1980; Fukuda et al. 1983; NCI, 1976b).

Examination of the livers of mice receiving chloroform in drinking water demonstrated a dose-related increase in liver fat content that extend to levels much lower than those utilized for the original NCI corcinogenesis study (Jorgenson et al., 1982). Similar changes were not observed in the livers of Osborne-Mendel rats until much higher levels of chloroform were administered in drinking water. These results suggest that corn oil was in some way involved in the liver pathology that develops in mice treated with chloroform. If so, this would imply that tumors induced by other hepatoxic agents may depend heavily on the corn oil vehicle commonly used in testing of non-polar chemicals. Such an interpretation would suggest that results that have been obtained with chemicals such as trichloroethylene and tetrachloroethylene may not be extrapolated to other species without appropriate qualification. In fact, these data also call into similar question the carcinogenicity of carbon tetrachloride since it has only been shown capable of producing liver tumors when administered in a vegetable oil vehicle.

Tetrachloroethylene increases hepatic DNA synthesis in B6C3F1 mice but not in rats following doses of 1000 mg/kg per day for 11 days (Schumann, et al., 1980). Similarly, Reitz et al. (1982) found substantial increases in DNA synthesis of the liver and kidney of B6C3F1 mice treated with single doses of 60 and 240 mg/kg of chloroform and much smaller increases in the liver and kidney of male Osborne-Mendel rats. These data indicate that the toxic effects of these chemicals in the mouse liver results in substantial tissue regeneration of dose levels utilized in the carcinogenesis bioassays of these chemicals. Consequently, the possibility exists that these chemicals are capable of increasing liver tumor development from spontaneously initiated cells in the same way that carbon tetrachloride has been shown to increase hepatomas in the liver of diethylnitrosamines initiated mice (Pound and McGuire, 1978).

These questions become extremely important when attempting to estimate the risk to human beings consuming drinking water. Presumably the damage that

is produced by initiators of cancer (i.e. those that act through damage to DNA) produce a certain degree of irreversible or non-repairable damage that is directly related to the dose of carcinogen. Theoretically, this would lend itself to a linear relationship to dose at low response rates and risks at low concentrations can be reliably estimated from experiments conducted at high doses. Increased tumor incidence due to non-genotoxic effects of chemicals or interactions with nutritional state presumably involve reversible processes. Consequently, the assumption of a linear relationship with dose at low response rates may not be justified. This may substantially affect any estimate of the health hazard associated with the low concentrations of these chemicals that occur in drinking water that is based upon the high doses used in animal experiments.

The data available at present is not suited for projecting the risk to humans from the use of materials in the treatment and distribution of drinking water. Identification of carcinogenic properties of by-products of disinfection other than the trihalomethanes, acryamide and coal tar paints, however, does indicate that the use of such material in contact with potable water requires closer scrutiny. The industry should examine critically time-honored practices in the treatment and distribution of drinking water to gradually replace these materials with safer alternatives.

In summary, evidence that a chemical induces cancer in experimental animals does suggest that there is a potential risk to man regardless of the mechanism involved. Although it is certainly possible that a chemical that produces cancer in experimental animals would not do so in man, it is difficult to develop a prudent course of action on this assumption because the evidence which presently exists would suggest that the reverse is the general case. If tumorigenesis is dependent upon tissue damage, it should be remembered that these agents are also capable of inducing liver damage in humans. Consequently, it should be recognized that the argument presented regarding relationship of tissue damage to tumorigenicity is more of a quantitative consideration in the judgement of risks in man and less of a question of across-species extrapolation than discussions of the issue would ordinarily imply. Therefore, the appearance of all the above chemicals in drinking water is cause for concern and to be avoided wherever possible and practical.

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