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Tetrachloroethylene: Assessment of Human Carcinogenic Hazard

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SUMMARY

Evidence for the carcinogenicity of tetrachloroethylene from animal toxicity, mutagenicity, pharmacokinetic, metabolism and epidemiological studies has been reviewed and critically appraised.

The mutagenicity of tetrachloroethylene has been investigated in a wide variety of tests. No mutagenic effects were observed in several well conducted studies using validated test systems. Weakly positive or equivocal results were obtained in a number of tests using technical or commercial grade material (containing mutagenic stabilisers) and in non-validated test systems. An overall assessment, taking into account the quality of conduct and reporting and the results of the *in vivo* and *in vitro* assays, has concluded that pure tetrachloroethylene is itself non-mutagenic.

The carcinogenic activity of tetrachloroethylene has been studied in a number of long-term oral, inhalation and dermal assays in rats and mice and in studies of its initiator and promoting ability. Tetrachloroethylene has been shown to cause hepatocellular carcinomas in mice and renal tubular cell tumours in male rats. An increased incidence of mononuclear cell leukaemia was seen in male and female F344 rats but not Osborne-Mendel nor Sprague-Dawley rats exposed to tetrachloroethylene.

The observation of mononuclear cell leukemia in the F344 rat is considered to be of no significance for human hazard assessment because of its high and variable incidence and the fact that this type of leukemia does not occur in man.

No epidemiological studies are available on groups of individuals exposed soley to tetrachloroethylene. Seven studies of cancer mortality among employees in the laundry and dry-cleaning industry, who may have been exposed to tetrachloroethylene and other solvents, have revealed increased incidences of cancers (eg renal cancer, bladder cancer,

cervical cancer). An association between tetrachloroethylene and bladder or renal cancer was not confirmed in case-referent studies in laundry and dry-cleaning workers. It is concluded that the increased incidences were either due to social factors or were isolated findings. None of the studies reported an increased incidence of liver cancer in the exposed groups. It is concluded that, overall, the design and outcome of epidemiological studies failed to demonstrate a relationship between exposure to tetrachloroethylene and the occurrence of cancer in man.

Evidence from metabolic studies in various species, including man, suggests that the major metabolic pathway in all species is a saturable cytochrome P-450 mediated oxidation to (principally) trichloroacetic acid (TCA). Marked species differences have been observed between man and rodent species in the utilisation of this pathway. A second minor pathway involving conjugation with glutathione-S-transferase (GSH) has been identified which could be of major significance in relation to the carcinogenicity of tetrachloroethylene to rats.

A considerable body of evidence is available on the mechanisms of tumour formation by tetrachloroethylene in rodent species. Liver tumours in mice are most probably due to its metabolism to TCA, a known peroxisome proliferator and a non-genotoxic liver carcinogen in rodents. The kidney tumours found in male rats could be caused by three possible mechanisms, none of which is relevant to man under normal conditions of exposure.

Knowledge that significant differences exist in the utilisation of the two metabolic pathways by mice, rats and man strongly suggests that the mechanisms linked to induction of cancers in animals are unlikely to occur in man. This hypothesis is supported by the existing epidemiological studies which, although not adequate in design to show a conclusive relationship between exposure to tetrachloroethylene and cancer in man, have failed to show any consistent excess of tumours at sites highlighted by the animal studies, ie the liver and the kidney. The observed tumour incidences in mice and rats cannot be taken to indicate a human hazard and should not be used as the basis for human carcinogenic risk assessment.

1. INTRODUCTION

Tetrachloroethylene is a widely used, non-flammable, volatile solvent. Its principle uses are in the dry cleaning of clothing and in metal cleaning. It is also used in textile processing and as a chemical intermediate (CEFIC, 1984). Commercial grade tetrachloroethylene is 99.9% pure, although up to 0.2% of stabilisers are added to commercial formulations. A large number of people may be exposed to tetrachloroethylene and hence its possible effects on human health have been extensively studied.

Tetrachloroethylene has been shown to be carcinogenic in animal studies. For example, hepatocellular tumours were induced in male and female mice, mononuclear cell leukaemia and renal cell carcinoma in male rats and mononuclear cell leukaemia in male and female rats, following long-term inhalation exposure to tetrachloroethylene (NTP, 1986).

Tetrachloroethylene has generally been found to be non-mutagenic in vivo and in vitro, so the liver tumours observed in animal studies may not be a direct consequence of a genotoxic effect of the chemical. If an alternative, non-genotoxic mechanism is operative, its relevance to man must be fully understood if a valid assessment of the carcinogenic hazard of tetrachloroethylene is to be made.

Following publication of the NTP data, experiments were conducted into the mechanism of the carcinogenic activity of tetrachloroethylene in rodents. This report reviews information on mechanisms relevant to the assessment of the human carcinogenic hazard following exposure to tetrachloroethylene; mutagenicity and animal carcinogenicity data, and recent biochemical and mechanistic studies have been considered and experience with man, as reflected by epidemiological studies, has been reviewed.

2. MUTAGENICITY

The details of mutagenicity assays are summarised in Tables 1-18.

2.1 Gene Mutation

2.1.1 Bacterial Assays

Studies using bacterial assays are summarised in Table 1.

The ability of tetrachloroethylene to cause gene mutations in bacteria has been investigated in Salmonella typhimurium and Escherichia coli. In several plate-incorporation assays negative results were obtained, either with or without pre-incubation, using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 and two additional strains which are not DNA repair deficient (UTH 8413 and 8414). Toxicity was observed at 224-333 µg/plate. Metabolic activation was achieved with liver S9 fractions obtained from rats, mice or hamsters induced by phenobarbital or Aroclor (Margard, 1978; Bartsch et al, 1979; Kringstad et al, 1981; Haworth et al, 1983; Connor et al, 1985).

Tetrachloroethylene in the vapour phase has been tested on different Salmonella typhimurium strains (see Table 1). Tetrachloroethylene of high purity and containing low concentrations of stabilisers gave negative results in these assays up to levels which were toxic to the organisms in the absence or presence of Aroclor induced rat, mouse or hamster liver S9 fractions (SRI International, 1983; Williams and Shimada, 1983; Milman et al, 1988; Warner et al, 1988).

Positive responses in both plate incorporation and vapour phase Ames assays have been obtained with certain commercial and technical preparations of tetrachloroethylene but only at toxic concentrations. No dose-response relationship was established in these tests. Furthermore, non-stabilised, highly pure tetrachloroethylene gave a negative response

in these studies (Margard, 1978; Williams and Shimada, 1983). The positive findings may be due to the presence of mutagenic contaminants and/or added stabilisers such as cyclohexene oxide and epichlorohydrin; both contaminants gave positive results with Salmonella typhimurium TA100 at concentrations greater than 0.25 ppm (vapour), and Salmonella typhimurium TA1535 at 0.2 µg/ml agar (Bridges, 1978; Koorn, 1987).

Tetrachloroethylene of undefined purity was tested in a spot test, using Salmonella typhimurium strains TA98, TA100, TA1535, TA1950, TA1951 and TA1952. Mutagenic activity was shown in all strains. A dose-dependent response was observed only in strain TA100 (Cerna and Kypenova, 1977). Insufficient details were provided for an assessment of this study.

A screening test on the ability of tetrachloroethylene to induce survival repair mechanisms (SOS chromotest) in Escherichia coli PQ37 was negative (Von der Hude et al, 1988).

Tetrachloroethylene caused no increase in the frequency of forward or reverse mutations in a bacterial assay with Escherichia coli K12, in the presence or the absence of mouse liver S9 fractions (Greim et al, 1975).

2.1.2 Yeast Assays (Table 2)

Tetrachloroethylene did not induce mutagenic activity in a yeast culture of Saccharomyces cerevisiae D7 in stationary phase in the absence or the presence of an exogenous activation system (Bronzetti et al, 1983).

Tetrachloroethylene has also been studied in Saccharomyces cerevisiae D7 and D4 strains using log-phase cultures (Callen et al, 1980; Koch et al, 1988). The results were positive but were not reliable because of poor survival. The purity of the tetrachloroethylene used was incompletely described.

2.1.3 Host Mediated Assays (Table 3)

Oral administration of tetrachloroethylene of high purity (99.5%) to CD-1 mice for 5 days gave negative results in a host-mediated assay using stationary Saccharomyces cerevisiae D7 as the indicator organism (Bronzetti et al, 1983). The protocol, which involved intravenous injection, was unusual and as no positive control was used, interpretation of this study is impossible.

Using Salmonella typhimurium strains TA1950, TA1951 and TA1952 as indicators, an increase in mutagenicity was observed in a host-mediated assay with tetrachloroethylene of unknown purity administered to female ICR mice. No dose dependence was found (Cerna and Kypenova, 1977). In another host-mediated assay using Salmonella typhimurium strain TA98, tetrachloroethylene administered by inhalation at 100 and 500 ppm to female mice yielded a clear (four-fold) increase in mutations at a dose of 500 ppm (Beliles et al, 1980). The material used was of low purity (91.43%) but it was negative when tested with TA98 in the presence of an S9 fraction.

The results obtained from the host-mediated assays are of uninterpretable because of the absence of suitable controls or the use of an unconventional route of administration.

2.1.4 Drosophila Assays (Table 2)

Sex-linked recessive lethal tests in Drosophila melanogaster in which tetrachloroethylene was administered by inhalation, feeding or injection showed no mutagenic effect (Beliles et al, 1980; Valencia et al, 1985).

2.1.5 Mammalian Systems (Table 4)

A study of gene mutation in vitro in a mouse lymphoma cell line (L5178Y/TK+/-) in the presence of an induced rat liver S9 fraction gave negative results up to dose levels which were toxic to the cells (NTP, 1986).

2.2 Chromosomal Effects

2.2.1 In Vitro Mammalian Systems (Tables 5 and 7)

Tetrachloroethylene did not induce chromosomal aberrations or sister chromatid exchanges (SCEs) in an *in vitro* study on Chinese hamster ovary cells in the presence or absence of rat liver S9 metabolic activation (NTP, 1986; Galloway *et al*, 1987).

2.2.2 In Vivo Mammalian Systems (Table 6)

Administration of tetrachloroethylene to rats at concentrations up to 600 ppm for 12 months to rats and by single or repeated ip injection to mice did not reveal exposure related chromosome aberrations in bone marrow (Cerna and Kypenova, 1977; Rampy *et al*, 1978; Beliles *et al*, 1980).

A dominant lethal study in male rats exposed to tetrachloroethylene by inhalation (100-500 ppm, 7 h/d for 5 d) showed no mutagenic effects (Beliles *et al*, 1980).

2.2.3 Non Mammalian Systems (Tables 8 and 10)

An inhalation study of the effects on chromosomes, including sex chromosome loss, in *Drosophila melanogaster* (100-500 ppm for 7 h) failed to demonstrate mutagenic activity (Beliles *et al*, 1980).

2.2.4 In Vivo Human Systems (Tables 6 and 9)

Studies on lymphocytes from 10 factory workers occupationally exposed to tetrachloroethylene for 3 months to 18 years showed no significant dose-related differences from controls in numerical or structural chromosomal aberrations, SCE rate, the proportion of M_2 and M_3 metaphases and mitotic index (Ikeda *et al*, 1980). The study is of limited value because the workers studied were not matched to the control group with regard to age, sex, race or social-economic status. No indication was available of the medical histories of the subjects.

2.3 DNA Damage (Tables 11 to 15)

Unscheduled DNA synthesis (UDS) is a measure of reparative (rather than replicative) synthesis resulting from damage to DNA. Tetrachloroethylene of varying purity has been evaluated *in vitro* by means of UDS test systems using human fibroblasts and rat or mouse hepatocytes. No effects indicative of DNA damage were observed (Beliles *et al*, 1980; Williams, 1983; Williams and Shimada, 1983; Costa and Ivanevitch, 1984; Milman *et al*, 1988). In assays conducted in the vapour phase, weak positive responses were observed at levels which killed more than 25% of cells (Williams and Shimada, 1983). The tetrachloroethylene used in this study was stabilised (see Table 11) and gave a positive response in a *Salmonella* assay (see Table 1). In an *in vivo*/*in vitro* rat kidney cell assay, in which tetrachloroethylene was administered orally (1,000 mg/kg), reparative DNA synthesis was not induced (Goldsworthy *et al*, 1988b).

Single-strand breaks were found in cells of the liver and kidney but not of the lungs of mice 1 h after ip administration of tetrachloroethylene; the sensitivity of detection was 1 single-strand break per 5×10^6 nucleotides. All damage was repaired by 24 h (Walles, 1986). The origin of the single-strand breaks induced by tetrachloroethylene as is not clear. No studies are available from which to evaluate the effects of prolonged administration of tetrachloroethylene on the persistence of these single-strand breaks.

The ability of tetrachloroethylene to bind covalently to DNA was studied *in vivo* in mice following inhalation (600 ppm for 6 h) and oral administration (500 mg/kg) (Schumann *et al*, 1980). No evidence of alkylation was found; the study had a power to detect 1 alkylation in 10^5 nucleotides. DNA binding was reported to occur in mouse liver following ip injection of tetrachloroethylene and in calf thymus DNA under certain metabolic conditions in an *in vitro* study (Mazzullo *et al*, 1987). An unusual pattern of binding in the *in vivo* study was reported, the level bound to RNA being significantly higher than that bound to protein or DNA. No distinction was drawn between covalent binding and the

incorporation of radioactivity through the C-1 pool, making interpretation of the results impossible.

In conclusion, although tetrachloroethylene produced a low incidence of single-strand breaks, the limited studies of DNA damage failed to provide evidence of DNA alkylation. Tetrachloroethylene did not induce UDS either in vitro or in vivo.

2.4 Miscellaneous Test Systems (Table 16)

Tetrachloroethylene has been tested for its ability to induce transformation in various cell systems. No effects were observed in BHK21/C113 and BALB/C-3T3 mouse cells (Longstaff and Ashby, 1978; Tu et al, 1985; Milman et al, 1988). Transformation were induced in an unusual test system using Rauscher leukaemia virus-infected Fischer rat embryo cells (Price et al, 1979). Conflicting results from different cell transformation test systems is common and makes their relevance to and reliability in predicting carcinogenic activity uncertain.

2.4.1 Germ Cell Effects (Table 17)

Effects on germ cells were studied in a sperm morphology test in mice and rats (Beliles et al, 1980). Tetrachloroethylene of low purity, which also produced weakly positive responses in other test systems, induced an increase in the proportion of sperm with aberrant morphology in mice but not in rats. As sperm morphology can be affected by non-genetic mechanisms, no conclusions regarding germ cell mutagenicity can be drawn from these findings.

2.5 Metabolites (Table 18)

The mutagenicity of key metabolites derived from tetrachloroethylene is considered in Section 6.1 and Table 18.

2.6 Evaluation

The mutagenic activity of tetrachloroethylene has been investigated in a wide variety of tests.

No mutagenic effects were observed in the following studies (the number performed indicated in brackets):

A) In Vitro

1) Gene mutation	Prokaryotes	Salmonella typhimurium (9)
		Escherichia coli (2)
	Fungi	Saccharomyces cerevisiae (1)
		Saccharomyces cerevisiae with mouse as host (1)
	Mammals	Mouse cell line (1)
2) Chromosome damage		
Chromosome aberration	Mammals	Hamster cell line (1)
	Man	Lymphocytes (1)
Sister chromatid exchange	Mammals	Hamster ovary cells (1)
	Man	Lymphocytes (1)
3) DNA damage (UDS)	Mammals	Rat/mouse (in vitro) (4)
	Man	Fibroblasts (1)

B) In Vivo

1) Gene mutation	Insects	Drosophila melanogaster (2) 2)
Chromosome damage		
Chromosome aberration	Mammals	Mouse (in vivo) (2)
		Rat (in vivo) (3)
Chromosome damage	Insects	Drosophila melanogaster (1)
3) Germ cell	Mammals	Rat (dominant-lethal) (1)
4) DNA damage (UDS)	Mammals	Rat (in vitro/in vivo) (1)
5) Sperm morphology	Mammals	Rat (1)

Weakly positive or equivocal results were obtained from a number of tests on technical and commercial grade material or in non-validated test systems. The presence of mutagenic stabilisers in the samples of tetrachloroethylene tested is the most likely explanation for the weakly positive findings and therefore confounds the interpretation of these results. The significance of some of the studies could not be judged because of inadequate reporting, the lack of appropriate controls or the unconventional test-system used. Positive results were obtained only at concentrations of tetrachloroethylene which were toxic to the organisms or cells and no dose dependence was established. Tetrachloroethylene exposure did not produce significant DNA damage or binding. The conflicting results in different cell transformation systems and the different response between mouse and rat in a sperm morphology test are more likely to be due to the inherent properties of the test system than to an expression of the genotoxicity of tetrachloroethylene. Negative results were obtained when pure tetrachloroethylene was tested in a range of more reliable and better validated studies.

It is concluded from an overall assessment of the available data from a range of in vivo and in vitro assays, taking into account the quality of conduct and reporting of the studies, that tetrachloroethylene is non-mutagenic.