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Tetrachloroethylene: Assessment of Human Carcinogenic Hazard

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CONTENTS

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.

3. ANIMAL CARCINOGENICITY

The chronic toxicity of tetrachloroethylene in laboratory animals, in particular its carcinogenic activity, and the implications for human health have been extensively evaluated over the past decade (IPCS, 1984; RIVM, 1984; EPA, 1985; Gezondheidsraad, 1985; IARC, 1987; HSE, 1987; Deutsche Forschungsgemeinschaft, 1988; Werkgroep Deskundigen, 1988; listed in ECETOC, 1989). Other draft regulatory documents exist.

3.1 Oral Studies

The carcinogenicity of tetrachloroethylene administered by gavage has been examined in two experiments in rats and one in mice.

Groups of 50 male and 50 female B6C3F1 mice received time-weighted average doses of 536 and 1,072 mg/kgbw/d (males) and 386 and 772 mg/kgbw/d (females) 5 d/wk for 78 wk, followed by an observation period without treatment of 12 wk. Tetrachloroethylene was administered as a solution in corn oil. Groups of 20 untreated and 20 corn oil treated mice of each sex served as controls. The incidence of hepatocellular carcinoma was approximately 10% in both sexes in untreated and in vehicle control mice and 40% and 65% in low-dose and 40% and 56% in high-dose female and male mice respectively. The mortality rate was high early in the study and toxic nephropathy was observed in nearly all treated mice but not in the controls.

Groups of 50 male and 50 female Osborne-Mendel rats received time-weighted average (TWA) doses of approximately 475 and 950 mg/kgbw/d for 78 wk followed by an observation period without treatment of 32 wk. Tetrachloroethylene was administered as a solution in corn oil. Groups of 20 untreated and 20 corn oil treated rats of each sex served as controls. Toxic nephropathy occurred in more than 85% of the treated male and in 50-80% of the treated female rats. It was not observed in control rats. Tetrachloroethylene did not increase the incidence of

tumours in rats, although a low survival rate prevented definitive conclusions to be drawn by the investigators (NCI, 1977).

Groups of 40 male and 40 female Sprague-Dawley rats received 500 mg tetrachloroethylene/kgbw (15% v/v in olive oil) by gavage once daily, 4 to 5 d/wk for 104 wk. The rats were observed for their lifetime. A group of 50 male and 50 female rats served as controls and were administered similar volumes of olive oil. Renal damage (cytomegaly and/or karyomegaly in renal tubular cells) was observed in 32.5% of the treated male rats, but not in the female rats. The incidence of benign and malignant tumours in the treated groups was not increased when compared with the controls (Maltoni and Cotti, 1986).

3.2 Inhalation Studies

Two groups of 96 male and 96 female Sprague-Dawley rats were exposed to 300 or 600 ppm tetrachloroethylene vapour 6 h/d, 5 d/wk for 12 months. A control group consisted of 192 male and 192 female rats. The surviving rats were killed 31 months after the start of the study. Clinical signs of toxicity were not observed and mean body weights were similar in all groups. Mortality was slightly greater in high-dose males than in the controls. This was thought to be due to earlier onset of spontaneous chronic renal disease. Tetrachloroethylene did not increase the incidence of tumours in the rat under these exposure conditions (Rampy et al, 1978).

Groups of 50 male and 50 female F344 rats or B6C3F1 mice were exposed to tetrachloroethylene by inhalation for 6 h/d, 5 d/wk for 103 wk at atmospheric concentrations of 200 or 400 ppm (rats) or 100 or 200 ppm (mice) respectively. Groups of 50 male and 50 female rats or mice served as controls. There was a statistically increased incidence of hepatocellular carcinoma was observed in treated mice of both sexes accompanied by non-significant increases in the incidence of renal tubular cell adenoma and of adenocarcinoma (a tumour rarely found in control animals) in male rats. The authors reported a statistically

significant increase in mononuclear cell leukemia for both male and female F344 rats. The statistical analysis was based on an evaluation of the stages of development of the leukemia; a method that has not been fully evaluated. The incidence of mononuclear cell leukaemia in the concurrent F344 control rats, in both males and females, was higher than the historical incidences reported by the NTP. In view of the high and spontaneous incidence of this tumour in the F344 rat it is concluded that there is no basis for the association of this increased tumour incidence in the treated groups with exposure to tetrachloroethylene. This conclusion has been reached by other compotent reviewers of the study (HSE, 1987; comments of the NTP Peer Review Committee, 1986).

3.3 Dermal Studies

Two groups of 30 male and 30 female Ha:ICR Swiss mice received either 18 or 54 mg of tetrachloroethylene in 0.2 ml acetone on the shaven dorsal skin, 3 times/wk for the duration of the experiment (imprecisely specified by authors, but at least 440 d). A third group received a single application of 163 mg of tetrachloroethylene followed after 2 wk by 5 µg of phorbol myristate acetate (PMA) in 0.2 ml acetone, 3 times/wk for at least 428 d. There were 3 control groups: PMA alone, acetone alone, and no treatment. Tetrachloroethylene did not initiate nor induce dermal tumours or tumours at other sites (Van Duuren et al, 1979).

3.4 Intraperitoneal Studies

Groups of 20 male A/St mice, 6-8 wk of age, received ip injections of tetrachloroethylene in tricaprylin, 3 times/wk. The groups received 14 injections of 80 mg/kgbw or 24 injections of 200 mg/kgbw or 48 injections of 400 mg/kgbw (concentration and volume not given). The 50 control mice were injected with tricaprylin only. A positive control group received 1 single ip injection of urethane (1,000 mg/kg). The mice were killed 24 wk after the first injection. Special attention was given to the occurrence of lung adenomas. The number of lung tumours was not

increased in the groups injected with tetrachloroethylene whereas an increased incidence was seen in the positive control group (Theiss et al, 1977).

3.5 Tumour Promotion

A rat liver foci bioassay was performed using N,N-diethyl-nitrosamine (DENA) as an initiator and tetrachloroethylene as a promoter (protocol described by Pereira, 1982). DENA was injected ip into male Sprague-Dawley rats 24 h after a partial hepatectomy. Tetrachloroethylene (1,100 mg/kgbw/d) administered by gavage once a day, 5 d/wk for 7 wk, did not increase the number of foci/cm^2 nor the total foci area/cm^2 at 3 d (Lundberg et al, 1987) or 10 d (Lundberg et al, 1987; Holmberg et al, 1986) following the last dose of test compound. The studies thus provided no evidence for tumour promotion in the liver.

A liver foci bioassay was conducted in Osborne-Mendel rats using DENA as initiator. Glutamyl-transpeptidase (GGT) was used as an index of pre-neoplastic change. The incidence of GGT foci was significantly increased by tetrachloroethylene and the extent of the increase was similar whether or not DENA was used (Milman et al, 1988). The significance of the liver foci bioassay and its predictive value for man are uncertain.

In a pulmonary tumour promotion assay in strain A mice, tetrachloroethylene was without promoting activity (Maronpot et al, 1986).

3.6 Metabolites: Trichloroacetic Acid

In a study in mice in which the tumour promoting ability of tetrachloroethylene was compared with that of its metabolite, a group of 22 male B6C3F1 mice received trichloroacetic acid (TCA) in their drinking water at a concentration of 5 g/l for 61 wk. Seven out of the 22 treated animals developed hepatocellular carcinomas, and 8 developed hepato-

cellular adenomas. In a control group of 22 male mice receiving 2 g/l NaCl in their drinking water, 2 developed hepatocellular adenomas although no hepatocellular carcinomas were observed. This study provides some evidence for the direct hepatocarcinogenic effect of a metabolite of tetrachloroethylene in the mouse (Herren-Freund et al, 1987).

3.7 Evaluation

Tetrachloroethylene has been shown to be carcinogenic in male and female B6C3F1 mice, causing hepatocellular carcinomas. The major metabolite, TCA, has been shown to cause hepatocellular carcinomas in male B6C3F1 mice when administered in their drinking water. Tetrachloroethylene also caused an increase in the incidence of renal tubular cell tumours in male F344 rats in one study. Increased incidences of mononuclear cell leukaemia were observed in treated male and female F344 rats but not in Osborne-Mendel or Sprague-Dawley. Mononuclear cell leukemia is known to have a high and variable incidence in F344 rats, this being a strain specific effect.

There is no convincing evidence from a number of assay systems to suggest that tetrachloroethylene acts as a tumour promoter.

4. EPIDEMIOLOGY

Estimates of the number of individuals in the USA potentially exposed to tetrachloroethylene vary from 0.5 million (NIOSH, 1978 [quoted in Santodonato et al, 1985]) to 1.6 million (NIOSH, 1977 [quoted in Brown and Kaplan, 1987]). There are no epidemiological studies of populations exposed exclusively to tetrachloroethylene but studies of some relevance have been performed among workers in the dry-cleaning and laundry industries in the USA.

4.1 Studies in the Dry-cleaning Industry

Tetrachloroethylene was introduced into the dry-cleaning industry in the late 1930s but did not replace other synthetic solvents, such as carbon tetrachloride, trichloroethylene and benzene (used for spot cleaning) until early in the 1950s. Prior to 1960, petroleum derivatives were still the dominant solvents used. By 1977, at least 73% of the dry-cleaning shops in the USA were using tetrachloro- ethylene as the main solvent; a similar figure (75%) has been estimated for 1987 (HSIA, 1989). The use of multiple and mixed solvents for dry-cleaning makes it difficult to draw conclusions from epidemiological studies about a possible association between exposure to any single solvent, including tetrachloroethylene, and the incidence of cancer. In addition, none of the studies reported have given an estimation of the extent of exposure to tetrachloroethylene.

There is some information on the levels of tetrachloroethylene in dry-cleaning establishments. Four recent European studies using personal sampling monitors to determine the exposure of individual workers found that the majority of exposures varied from 50 to 350 mg/m^3 (7 to 50 ppm) (Shipman and Whim, 1980; Lauwerijs et al, 1983; Monster et al, 1983; Buchter et al, 1984). In the USA, Ludwig et al (1983) and Materna (1985) determined atmospheric concentrations in dry-cleaning shops to be 30 to 1,000 mg/m^3 (160-534 ppm) time weighted average (TWA) although during

loading and unloading short-term (peak) concentrations of 1,000 to 3,500 mg/m^3 (146-510 ppm) were recorded. (See Table 20.)

4.1.1 Standardised Mortality Studies

A retrospective cohort mortality study was conducted on 1,597 members of 4 unions in Oakland, Chicago, Detroit and New York, who had been employed in dry-cleaning shops for at least 1 year before 1960 (Kaplan, 1980). Of the cohort, 285 members had died and death certificates were obtained for all but 38; 254 (16%) could not be traced. To calculate Standardised Mortality Ratios (SMRs) the author assumed the same distribution for cause of death in the missing workers as in those for which death certificates were available (an assumption of doubtful validity). For deaths due to malignancy, the incidences and SMRs were compared to the overall USA mortality rates with the following results. Significant excesses of urinary tract and colon cancers were reported:

i. Urinary tract: Observed 5, Expected 2.84 and 2.92 for whites and blacks respectively (SMRs=203 and 198);

ii. Colon: Observed 11, Expected 6.98 and 6.77 (SMRs=182 and 187).

Two of the 5 malignancies of the urinary tract were in the kidney and 3 in the bladder. The original article does not define the expected numbers and SMRs for kidney and bladder separately. Although the number of cases of colon cancer in individual unions was small, the findings of the analysis are compatible with an increased risk of colon cancer occurring in all 4 unions. There were no other statistically significant excesses in tumour incidence.

The study was restricted to union records in which the ethnic origins were not always recorded. The author did not draw definite conclusions because of the small numbers within the cohort.

The study was updated in 1987 at which time the cohort was expanded to 1,690 workers with 42,267 person-years-at-risk (Brown and Kaplan, 1987).

The vital status of 97% of the male workers and 92% of the female workers was known; 493 deaths had occurred. From the original cohort of 1,597 persons (cf Kaplan 1980) a sub-cohort of 615 workers exposed almost exclusively to tetrachloroethylene was defined. In the sub-cohort 137 deaths had occurred. The cause-specific deaths in the cohort and the sub-cohort were compared with the expected incidences. In the main cohort, the SMR for "all causes of death" was lower than expected (SMR=86, confidence limits (Cl) not defined) but the SMR for "all neoplasms" was slightly elevated (SMR=116, 95% Cl 79-136) when compared with the USA national death rates. The urinary tract was the only site that showed a statistically significant excess of cancer deaths (observed 12, expected 4.7, SMR=255, 95% Cl 132-450) primarily due to bladder cancer. Although the incidences of kidney cancer (observed 4, expected 2.0) and bladder cancer (observed 8, expected 2.7) were much higher than expected, only the excess of bladder cancer was statistically significant (SMR=296, 95%Cl 128-586).

In a separate examination using the data for race and group separately, Brown and Kaplan found that the mortality from bladder and kidney cancer was elevated in 3 out of the 4 race-sex groups, although this was based on a small number of deaths. For bladder cancer a statistically significant excess was seen in non-white males (observed 3, expected 0.6, SMR=500). Of the 137 deceased in the sub-cohort no excesses were observed for bladder and kidney cancer. The author concluded that this finding did not preclude an association between tetrachloroethylene and excess cancers but that it weakened the possibility of an association. With respect to other organs, an excess was seen of cancer of the intestinal tract, excluding the rectum: observed 16, expected 11.8, SMR 136 (95% Cl 78-220). No deaths from liver cancer were found whereas 3.5 were expected. In non-white females, the incidence of cervical cancer was higher and that of breast cancer was lower than expected.

A retrospective cohort mortality study was conducted on 11,062 members of a dry-cleaners union employed from 1945-1977 (4,624 white female, 2,231 black female, 795 white male, 1,405 black male and 2,007 unknown). The causes of death of 1,329 workers were compared to those of the general

USA population taking into account the age and year of death. The SMRs for "overall deaths" and "deaths due to all malignancy" were far below those of the general USA population (SMRs=92 and 84 respectively). The incidences of laryngeal cancer, bladder cancer and lymphoma were elevated in workers employed before 1963 (SMRs=256, 156 and 199 respectively). Oesophageal cancer was significantly elevated among black male workers (SMR=280) when the analysis was confined to subjects with medium and high levels of exposure to tetrachloroethylene. The SMR for oesophageal cancer of black male union members with more than 15 years of membership was 463 (Blair et al. 1985).

4.1.2 Proportional Mortality Studies

Blair et al (1979) reported a retrospective study of the mortality among laundry and dry-cleaning workers based on 330 death certificates of former members of 2 trade unions in Missouri during the period 1957-1977. Of these, 279 had worked exclusively in dry-cleaning shops. Within this group, for 146 deceased persons the duration of exposure ranged from less than 1 to 25 years, with a mean of 13 years. The degree, pattern and duration of exposure to tetrachloroethylene and the degree of exposure to other substances were not given. The 330 deaths were predominantly low-waged non-white females. There were 87 deaths from cancer (67.9 expected). Statistically significant excesses of deaths (chi squared test; $P<0.05$) were seen for cancers of the lung, where the Proportional Mortality Ratio (PMR) was 170, cervix uteri (PMR=208) and skin (PMR=429) whilst statistically non-significant excesses were seen for leukaemia (PMR=227) and liver cancer (PMR=235). There was a deficit of breast cancer (PMR=69). The excess of cervical cancer and the deficit of breast cancer (PMR=69) may be related to the low socio-economic status of the cohort. Smoking histories were unknown. The authors commented that smoking could have contributed to the observed excess of lung cancer.

Katz and Jowett (1981) reported a proportional mortality study of 671 death records of white females who had worked of laundries and dry-cleaning shops in Wisconsin (USA) during 1963-1977. The authors compared the cause-specific PMRs for 25 causes of death of laundry and

dry-cleaning workers with that of other female workers from the same area and of similar socio-economic status who had died during the same period. A distinction between workers in dry-cleaning shops and laundries could not be made. There was no elevated risk for "all malignant neoplasms" (PMR=76). The results of the PMR analysis showed a statistically significant elevated risk for cancers of the kidney (observed 7, expected 2.7, PMR=257; $P<0.05$) and genitals (unspecified) (observed 4, expected 0.8, PMR=495; $P<0.01$). For cancers of the skin, bladder and cervix, non-significant excesses were found. The risk of cervical cancer was reduced when the comparison was made with workers in low-wage occupations. The observed number of deaths for leukaemia was lower than expected (observed 4, expected 6.0, PMR=67) as it was for the liver (observed 4, expected 4.5, PMR=89).

Duh and Asal (1984) reported an analysis of the death certificate records of 440 laundry and dry-cleaning workers (no distinction being made) in Oklahoma from 1975-1981. They estimated the Standardised Mortality Odds Ratio's (SMORs) with stratification on sex, race and age of death. There was no excess of deaths from "all cancers" compared with the distribution of causes of death in the USA in 1978 (observed 97, expected 100.5). For cancers of the respiratory tract, lungs and kidneys, the SMORs were statistically significantly increased:

i. Respiratory tract: Observed 29, expected 27.8, SMOR=1.8;
ii. Lungs: Observed 37, expected 22.6, SMOR=1.7;
iii. Kidneys: Observed 7, expected 1.9, SMOR=3.8.

The proportion of deaths from (female) breast, bladder and liver cancer were lower than for the general USA population:

i. Breast: Observed 1, expected 10.5, SMOR=0.1;
ii. Bladder: Observed 1, expected 2.4, SMOR=0.4;
iii. Liver: Observed 1, expected 1.9, SMOR=0.5.

The reduced incidence of deaths due to breast cancer is indicative of a population of low socio-economic standing. It should be noted that over 50% of solvents used by this population were petroleum hydrocarbons.

Nakamura (1985) studied the death certificates of 1,711 members of the "All-Japan Laundry and Dry-cleaning Association" who died during the period 1971-1980. No distinction was made between laundry and dry-cleaning workers. For 294 of the 517 members who died from 1979-1981, the histories of smoking, drinking and exposure were obtained by questionnaire from the families. Significant excesses were seen of the causes of death categorised as "other forms of heart disease", "accidents" and "other diseases of the liver", but not of "malignant neoplasms". The observed number of deaths resulting from malignancies of the small intestine, including duodenum, was significantly increased (SPMR=170 [observed 18; expected 10.6]; $P<0.05$). This excess was due to the excesses in the 15-44 and 65-74 age groups. No data were presented for the colon. In the urinary organs, 8 neoplasms were observed (not significant at $P<0.05$) of which 6 were in the bladder (4.4 expected, SPMR=136) and 2 in the kidney (2.5 expected, SPMR=80). In males, the increase in bladder cancer was only significant for those who died older than 75 (observed 5, expected 1.1, SPMR=455, $P<0.01$). Cancer of the prostate, male genitals and pancreas were all under-represented. The SPMRs recorded for liver and kidney cancer were not significantly increased for any age group. For females, an increase in bone cancer was seen (observed 5; expected 0.5; SPMR=1000, $P<0.05$). The number of female breast cancers was significantly decreased (observed 2, expected 7.1, SPMR=28; $P<0.05$). It was noted that prior to 1985 only 30% of the dry-cleaning shops in Japan used tetrachloroethylene as a solvent.

4.1.3 Case-referent studies

Smith et al (1985) examined the incidence of bladder cancer amongst laundry and dry-cleaning workers in the USA. The data for this case-referent study were drawn from a national bladder cancer study conducted by the National Cancer Institute (NCI) in 1978. Three groups of cases of bladder cancer were compared with controls based on the

population of the State of New Jersey. The groups consisted of individuals who had worked for at least six months in a laundry or dry-cleaning shop (n=103), workers in chemically-related jobs (n=5,776) and non-exposed individuals (n=1,869). Relative risks (RR) and approximate 95% confidence intervals were calculated. After matching and comparing with non-smoking controls, an RR of 1.31 (0.85-2.03) was found for workers in laundries and dry-cleaning shops and an RR of 1.11 (0.99-1.25) in the chemically-related job group.

McLaughlin et al (1987) performed a case-referent study of renal cancers occurring in workers in the Swedish laundry and dry-cleaning industries using data from the Cancer-Environment Registry from 1960-1979. The Standardised Incidence Ratios (SIRs) among 7,405 cases of renal cancer were calculated. In male and female workers, 18 and 25 cases were seen respectively (SIRs=0.99 and 0.86).

No evidence of a relationship between working in laundry and dry-cleaning establishments and the occurrence of bladder or renal cancers was established in these two case-referent studies.

4.2 Limitations of the Studies

Nine studies in the dry-cleaning industry have been reported involving a total of almost 5,000 deceased persons. Important facts are missing from each study reported (Table 21). In general the studies had a low power to detect changes in cancer incidence for specified cancer sites.

In none of the studies were data given with respect to pattern, duration and degree of exposure to tetrachloroethylene. Mixed exposure to solvents is likely to have occurred in most of the populations studied. Furthermore, no distinction could be made between laundry and dry-cleaning workers in 6 out of the 9 studies. This is an important limitation since laundry workers can be assumed not to have been exposed to tetrachloroethylene, thereby reducing the value of these studies in the context of this analysis.

A possible confounding factor has been introduced in that 6 of the studies involved union members only and in some studies selection was based on sex or race where such data were adequately recorded. A further confounding factor was introduced in several studies by comparing mortality figures for people with low socio-economic status with those of the general USA population. This comparison will influence both the SMR and PMR for certain site specific tumours, eg of the cervix uteri and of the breast, when comparing with the general population. Thus their value is limited with respect to establishing a relationship between the exposure to tetrachloroethylene and the occurrence of cancer in man.

4.3 Evaluation

Three standardised mortality and 4 proportional mortality studies have provided information of some relevance to populations which are potentially exposed to tetrachloroethylene. The 4 proportional mortality studies related to workers in laundries and dry-cleaning; the 3 standardised mortality studies (Kaplan, 1980; Blair et al. 1985; Brown and Kaplan, 1987) were concerned with dry-cleaning workers who may be assumed to have been exposed to a mixture of solvents including tetrachloroethylene. No epidemiological studies on groups of individuals exposed solely to tetrachloroethylene were available, although a small sub-cohort of individuals exposed almost exclusively to tetrachloroethylene was defined in one of the studies. Two case-referent studies have also been reported, which concern laundry and dry-cleaning workers.

The SMR studies indicate that the mortality was lower in the cohort groups when compared to the control populations. Statistically significant increases in mortality were found for some site-specific cancers. When comparing the SMR and PMR studies, certain tendencies became apparent.

A higher risk of bladder cancer than expected was observed in one cohort of the SMR study by Brown and Kaplan (1987). However, in this study the higher risk was not present in a small sub-group of workers almost

exclusively exposed to tetrachloroethylene. In the case-referent study of Smith et al (1985) where the possible link between bladder cancer and occupation was examined, no relationship was established between the occurrence of bladder cancer and employment in the dry-cleaning industry. No statistically significant increases in bladder cancer were observed in the 4 PMR studies.

A statistically significant increase in proportional mortality from renal cancer has been observed in 2 out of the 4 PMR studies. This observation was not confirmed in a case-referent study of renal cancer where no increase was observed in the incidence of this cancer among employees of the dry-cleaning industry. The one relevant SMR study showed a non-significant increase in renal cancer when compared with the general USA population (Brown and Kaplan, 1987).

The recurrent finding of elevated cervical cancer incidence and decreased breast cancer in the SMR and PMR studies is most probably due to socio-economic differences between laundry and dry-cleaning workers and the general population with whom they were compared. Differences in the sexual and reproductive history and in the cervical and breast cancer incidences of the different social classes are well recognised (Kinlen, 1988; Leon, 1988).

Other statistically significant observations include an increase of oesophageal cancer (black males only) (Blair, 1985); respiratory tract; skin and "all neoplasms" (Blair, 1979); bones (females only) and small intestine, including duodenum (females only) (Nakamura, 1985). It is to be noted that in none of the studies has a statistically significant excess of liver cancer been reported. A non-significant excess of cancer of the colon observed in one study (Kaplan, 1980) was not reported in the other studies.

Overall, the epidemiological studies of potential relevance are insufficient in both their design and their outcome to demonstrate a relationship between exposure to tetrachloroethylene and the occurrence of cancer in man.

Similar conclusions have been drawn by IARC (1987), the Dutch and German MAC-Committees (Werkgroep Deskundigen, 1987; Deutsche Forschungsgemeinschaft, 1988) the UK Health and Safety Executive (HSE, 1987) the Dutch National Health Council (Gezondheidsraad, 1985) and by various other national agencies in draft documents.

5. METABOLISM AND PHARMACOKINETICS

5.1 Metabolism

Tetrachloroethylene is metabolically transformed via the cytochrome P-450 pathway to trichloroethanol and trichloroacetic acid (TCA), these being the major metabolites in all species, including man (Dekant et al, 1985; Deutsche Forschungsgemeinschaft, 1988; Green et al, 1990).

Urinary metabolites such as oxalic acid have been identified in the rat (Dekant et al, 1986). The presence of oxalic acid is consistent with the formation of the epoxide tetrachloro-oxirane (for which there is no direct evidence) as the first step in a minor metabolic pathway, followed by de-chlorination steps via the vicinal diol (Figure 1). An additional derivative of tetrachloro-oxirane, trichloroacetyl amide, was identified in the urine of rats; this resulted from the reaction of trichloroacetyl chloride with phosphatidyl ethanolamine (Dekant et al, 1986). In a side reaction, oxalylethanolamide is formed by reaction of the intermediate oxalic acid dichloride with phosphatidyl ethanolamine.

Two additional metabolites (1,1,2-trichlorovinyl-cysteine and 1,1,2-trichloro-N-acetyl-cysteine) have been identified. Their formation cannot be explained by the oxidative metabolism of tetrachloroethylene but must result from primary conjugation with GSH. The formation of 1,1,2-trichlorovinyl-cysteine had already been postulated, since this compound produced the same nephrotoxic effects in rats as those produced by the parent compound tetrachloroethylene (Dekant et al, 1986). 1,1,2-tri-trichlorovinyl-cysteine was partially transformed to the corresponding mercapturic acid (1,2,2-trichlorovinyl-N-acetyl-cysteine) which was finally excreted in the urine (Figure 1).

The formation of 1,1,2-trichlorovinyl-cysteine can be explained by conjugation of tetrachloroethylene with GSH in the liver to give 1,1,2-trichlorovinyl-GSH which is excreted in bile, re-absorbed and transported via the blood to the kidney. Following cleavage of the

glutamate and glycine residues, the 1,1,2-trichlorovinyl-cysteine conjugate is metabolised by N-acetylation to the inactive mercapturic acid derivative and excreted in the urine. This conjugate also serves as a substrate for the renal enzyme ß-lyase (Green and Odum, 1985). The reaction of this enzyme is believed to yield trichlorovinylthiol, which could be further transformed to dichlorothioketene (by spontaneous elimination of HCl) or to dichloroethylthionyl chloride (by an intramolecular hydride shift) (Figure 1). From the decay of the reactive trichlorovinylthiol, the formation of dichloroacetic acid would be expected and has been confirmed as an additional urinary metabolite of tetrachloroethylene (Dekant et al, 1986).

5.2 Pharmacokinetics

Tetrachloroethylene is readily absorbed through the lungs and gastrointestinal tract in all species. Tetrachloroethylene, both as liquid and as vapour, can also be absorbed through the skin to some extent. (Jakobson et al, 1987).

Rats exposed to tetrachloroethylene by inhalation (200 ppm 6 h/d, 4 d) showed marked perirenal fat accumulation of the parent chemical (Savolainen et al, 1977).

Using ^{14}C-labelled tetrachloroethylene, it was found that excretion in rats took place predominantly through the lungs (expiration half-life 8 h). Only 2% of a high oral dose was excreted in urine over a period of 18 d; 25% of the radioactivity could be precipitated as chloride and the remainder was TCA (Daniel, 1963).

Following the administration of ^{14}C tetrachloroethylene to rats (1 mg/kgbw oral or 10 ppm by inhalation for 6 h) approximately 70% was excreted within 72 h in the expired air as unchanged tetrachloroethylene. Of the remainder, 26% was excreted as $^{14}CO_2$ or as non-volatile metabolites in the urine and faeces, whilst 3-4% remained in the carcass. Following the administration of tetrachloroethylene orally (500 mg/kgbw)

or by inhalation (600 ppm), 89% was excreted unchanged in the expired air, 9% as $^{14}CO_2$ and urinary and faecal metabolites while 1-2% remained in the body. Pulmonary elimination of tetrachloroethylene was monophasic with a half life of 7 h, independent of the route of administration. The remaining radioactivity was primarily distributed in the kidney, liver and fat tissues (Pegg et al, 1979).

In contrast to the situation in rats, mice exposed to ^{14}C tetrachloroethylene (10 ppm, 6 h) exhaled only 12% of the unchanged compound, whilst 62.5% of of the dose was excreted as urinary metabolites. This finding suggests that the amount of tetrachloroethylene undergoing metabolism is 5-10 times greater in the mouse than in the rat (Schumann et al, 1980).

The pharmacokinetics of tetrachloroethylene in rats and mice are dose-dependent. Rats showed saturable metabolism of tetrachloroethylene at exposure concentrations above 100 ppm (Daniel, 1963; Ikeda et al, 1972; Filser and Bolt, 1980; Bolt, 1987) whilst in mice, saturation was achieved at much higher concentrations (Bolt and Link, 1980; Schumann et al, 1980). Recent studies of inhalation exposure have shown that after exposure to 400 ppm tetrachloroethylene for 6 h the blood level of the principal metabolite TCA was 6 times higher in mice than in rats, indicating that oxidative metabolism was not saturated in mice under these conditions (Odum et al, 1988). Following the administration of tetrachloroethylene to male Swiss-Cox mice by gavage (0-2,000 mg/kgbw in corn oil) Buben and O'Flaherty (1985) found a linear relationship between urinary TCA concentrations and administered doses up to 200 mg/kgbw. Above this dose the amount of TCA found in urine approached a plateau, indicating that the metabolism to TCA is saturable.

Inhalation of tetrachloroethylene by dogs at 700, 1,500 and 2,000 ppm for 1 h showed uptake levels of 47 mg/kgbw at 700 ppm and 103 mg/kgbw at 2,000 ppm (Hobara et al, 1983).

In man, personal monitoring of exposure to airborne tetrachloroethylene accompanied by analysis of urine for total trichlorinated compounds (trichloroethanol and TCA) showed that urinary metabolite levels

increased linearly with external concentrations up to 100 ppm. Plateauing of metabolite excretion was apparent when the external exposure was higher, indicating that, in man, metabolic saturation occurs at approximately 100 ppm (Ikeda et al, 1972; Ohtsuki et al, 1983). Ohtsuki et al (1983) calculated that, at the end of an 8 h shift with exposure to 50 ppm tetrachloroethylene, approximately 38% of the inhaled dose would have been exhaled unchanged and that less than 2% was transformed to urinary metabolites; the remaining 60% would be eliminated later. Following inhalation, the mean half-life of the exhaled tetrachloroethylene was 79 min (Benoit et al, 1985). The half-life of TCA in urine and blood was found to be about 65 to 90 h (Monster et al, 1983).

5.3 Evaluation

The major pathway of tetrachloroethylene metabolism in all species, including man, is cytochrome P-450 mediated oxidation to TCA. Significant species differences have been observed. In both man and the rat, the oxidative pathway appears to be saturated above approximately 100 ppm, since no increase in metabolite (TCA) excretion has been found at higher exposure concentrations. In contrast, the TCA level in the blood of mice continues to increase with the external dose of tetrachloroethylene, indicating that the oxidative metabolism is not saturated following exposure to concentrations up to 400 ppm, the highest concentration tested. After oral administration, saturation of the cytochrome P-450 pathway was observed in mice at doses above 200 mg/kgbw (roughly equivalent to 1,000 ppm/6hr).

A second minor pathway involving conjugation of tetrachloroethylene with GSH has been identified in the rat. The resulting GSH-conjugate is metabolised to a mercapturic acid derivative which is excreted in the urine. This metabolite could subsequently be activated by renal ß-lyase to trichlorovinylthiol and dichlorothioketene.

6. BIOCHEMICAL MECHANISMS OF TUMOUR FORMATION

The lack of proven genotoxicity for tetrachloroethylene in standard assays suggests that the mouse liver tumours seen after exposure to tetrachloroethylene occur by mechanisms that do not involve a direct interaction between tetrachloroethylene and DNA. In the case of rat kidney tumours, three possible mechanisms exist, one of which involves a genotoxic metabolite. Detailed investigations of the metabolism and pharmacokinetics of tetrachloroethylene and its effects on the target tissues of rats and mice have provided evidence on the nature of these mechanisms and also an explanation for the species differences in carcinogenicity. The relevance of these mechanisms and species differences to human cancer hazard assessment is explained below.

6.1 Mouse Liver Tumours

Significant biochemical and morphological changes have been demonstrated in the livers of mice exposed to tetrachloroethylene. Marked peroxisome proliferation has been reported after exposure by gavage (1 g/kg for 10 consecutive days) (Goldsworthy and Popp, 1987) or by inhalation for up to 28 d to 400 ppm, the highest concentration used in the NTP carcinogenicity study (NTP, 1986; Odum et al, 1988). Both studies showed statistically significant increases in liver to body weight ratios up to 130% of control values.

The general lack of genotoxicity of tetrachloroethylene has previously been discussed (Section 2.1.1 and Table 1). In addition, its metabolites from the cytochrome P-450 pathway - trichloroacetylchloride (Reichert et al, 1983) oxalic acid (Sayato et al, 1987) TCA (Andersen et al, 1972; Waskel, 1978) and trichloroethanol (Bignami et al, 1980) - have been shown to be non-mutagenic (see Table 18). It is noted, however, that TCA has been demonstrated to cause chromosome aberrations and spermhead abnormalities in mice following ip administration of 500 mg/kg (Bhunya and Behera, 1987). These observations are consistent with the hypothesis

that the mouse liver tumours arise through a non-genotoxic mechanism. Although the GSH-pathway may produce a genotoxic metabolite in rats (section 6.2), it does so only in the kidneys and it is therefore not relevant to the formation of mouse liver tumours.

A causal relationship has been suggested to exist between peroxisome proliferation produced by a wide variety of chemicals and the occurrence of hepatocellular carcinoma in rodents (Reddy et al, 1980; Reddy and Lalwani, 1983; Rao and Reddy, 1987). There is no evidence that peroxisome proliferators react directly with DNA (Von Daniken et al, 1983, 1984; Goel et al, 1985) and the mechanisms involved in the development of tumours have not been established. One hypothesis is based on the fact that many peroxisome proliferators, including tetrachloroethylene, increase peroxisomal ß-oxidation without increasing catalase activity. The resulting imbalance is believed to lead to increased levels of intracellular hydrogen peroxide causing oxidative damage, cytotoxicity and possibly DNA damage.

TCA, the major metabolite of tetrachloroethylene (Costa and Ivanetich, 1980, 1984; Dekant et al, 1985), has been shown to cause peroxisome proliferation in rodents (Elcombe, 1985; Goldsworthy and Popp, 1987) and also does not interact directly with DNA. TCA has also been shown to cause a marked increase in the incidence of hepatocellular carcinoma in a carcinogenicity study in male B6C3F1 mice (Herren-Freund et al, 1987. Section 3.6). There is strong evidence therefore that the liver tumours seen in mice exposed to tetrachloroethylene are associated with its metabolism to TCA and the resulting peroxisome proliferation.

6.1.1. Species Differences

An increased incidence of liver cancer is not seen in rats exposed to tetrachloroethylene and the absence of significant peroxisome proliferation in the rat exposed to tetrachloroethylene is consistent with this observation. Whereas mice showed a 400-500% increase in peroxisomal ß-oxidation and a 2-3 fold increase in peroxisome volume on exposure to tetrachloroethylene (Goldsworthy and Popp, 1987; Odum et al,

1988) peroxisomal ß-oxidation was increased by only 30-40% in the rat with no measurable increase in peroxisome volume. These differences in peroxisome proliferation and hepatocellular carcinoma formation can be accounted for by pharmacokinetic differences (Odum et al, 1988), the rate of oxidative metabolism of tetrachloroethylene being lower in rats than mice (Schumann et al, 1980; Ikeda and Ohtsuji, 1972). The blood levels of TCA in mice exposed by inhalation to 400 ppm tetrachloroethylene were 7-fold greater, based on the area under the curve, or 13-fold greater, based on the peak blood concentration, than those in rats exposed to the same concentration level (Odum et al, 1988). Although TCA causes peroxisome proliferation in both rats and mice, a threshold concentration must be exceeded before this response occurs to any significant extent (Elcombe, 1985; Prout et al, 1985). Saturation of tetrachloroethylene metabolism in the rat (not the mouse) at atmospheric exposure levels exceeding 100 ppm (Ikeda et al, 1972) prevents this threshold being reached in the rat, with the result that neither a significant level of peroxisome proliferation nor any increase in liver cancer are seen in this species.

TCA production in man is also limited by saturation of the metabolic pathway at inhalation exposures of approximately 100 ppm tetrachloroethylene and above (Ikeda et al, 1972; Ohtsuki et al, 1983). Under these conditions the blood levels of TCA are an order of magnitude lower than those achieved in the rat at saturation (Monster et al, 1979; Green and Odum, 1988). An important difference exists between rodents and man in the response to this metabolite. TCA causes peroxisome proliferation in mice and rats in vivo and in mouse and rat hepatocytes in vitro. Under the same conditions, TCA failed to induce peroxisome proliferation in human hepatocytes (Elcombe, 1985). There are, therefore, three consecutive steps in the mechanism of action of tetrachloroethylene, two of which differ between mice and human beings. Second, tetrachloroethylene itself does not produce proliferative effects in the livers of any species examined. In man, metabolism to TCA is slower and saturable. Blood TCA levels are below those that stimulate peroxisome proliferation in susceptable species even at very high doses. Third, human hepatocytes are refractive to the proliferative effects of TCA. This observation

suggests that the parent chemical, tetrachloroethylene, and its major metabolite are unlikely to be peroxisome proliferators or hepatocarcinogens in human beings (Elcombe, 1985). Species differences in response to peroxisome proliferators have been reported for a variety of other chemicals and drugs (Reddy and Lalwani, 1983; Elcombe and Mitchell, 1986).

6.2 Rat Kidney Tumours

A low incidence of renal tubular cell adenomas and adenocarcinomas was reported in male F344 rats exposed to tetrachloroethylene by inhalation for their lifetime (NTP, 1986). Renal adenoma is rare and adenocarcinoma has not been found in the historical control population of F344 rats used in the NTP studies. Thus, although the incidence was low, the appearance of an uncommon tumour in exposed rats is likely to be of toxicological significance. The mechanism responsible for the development of these tumours could involve (i) cytotoxicity, (ii) hyaline droplet formation or (iii) metabolism to a genotoxic renal metabolite. These mechanisms are considered below.

Some of the life-time carcinogenicity studies in rats (Section 4) were compromised by a low survival rate in the exposed groups. In each case there was evidence that survival was reduced as a result of toxic nephropathy. Although the mortality rate was low in a rat study in which kidney neoplasms were reported, there was evidence of kidney damage characterised by tubular enlargement and hyperplasia (NTP, 1986). Sustained injury to renal tubular cells and the increased cell replication associated with tissue repair is the likely cause of the low incidence of renal tumours; sustained cell damage and repair has been shown to lead to cancer development with a number of non-genotoxic chemicals in several species of laboratory animal (Reitz, 1987).

Other possible mechanisms have also been investigated. Mature male rats given high oral doses of tetrachloroethylene (1-1.5 g/kgbw/d) for up to 42 d accumulate the protein alpha-2μ-globulin (revealed histologically as

hyaline droplets) in renal proximal tubular cells (Goldsworthy et al, 1986, 1988a; Green et al, 1986, 1989). Tubular casts were at the cortico-medullary junction and focal areas of proximal tubular cell regeneration were also reported in these studies. These effects were not seen in female rats nor in mice of either sex. This pattern of response (protein droplet nephropathy) has been demonstrated with several substances which produce renal tumours in male rats only and it is believed that the cycle of necrosis and regeneration results in tumour formation (Bruner, 1984; Charbonneau et al, 1985; Trump et al, 1984; Goldsworthy et al, 1986; Kanerva et al, 1987; Strasser et al, 1988). The mechanism provides an explanation for the kidney tumours seen only in male rats after exposure to tetrachloroethylene. However, protein droplet nephropathy was not observed in male rats exposed to tetrachloroethylene by inhalation at the atmospheric concentration levels used in the NTP study even after exposure to 400 ppm for up to 28 d (Green et al, 1990). Following exposure to 1,000 ppm for 10 d, an increase in the number of protein droplets was seen but this was not accompanied by cast formation nor cellular regeneration. It can be concluded from these studies that there is a threshold dose of tetrachloroethylene which must be exceeded to induce the effect. This dose would appear to be around 1,000 ppm by the inhalation route; precise definition of the dose is precluded by the limited number of doses tested.

Although there is a well established link between protein droplet nephropathy and renal cancer in male rats, the lack of effect at the higher exposure level used in the NTP study (Green et al, 1990) throws doubt on its relevance of protein droplet nephropathy in the development of tumours in that study. However, protein droplet formation was studied over a 28 day period only and the possibility that it may have occurred later during life-time exposure of the animals and contributed to cancer development cannot be ruled out.

Cytotoxicity and protein droplet nephropathy are do not involve a direct interaction between tetrachloroethylene or its metabolites and DNA. The third mechanism which may operate in the rat is formation of genotoxic metabolites in the kidney. Tetrachloroethylene is metabolised by a

second pathway (Figure 2) involving hepatic GSH conjugation (Dekant et al, 1986; Green et al, 1990). The conjugate is further metabolised by the mercapturic acid pathway and excreted in urine as the N-acetyl cysteine derivative. The precursor of this metabolite, S-trichlorovinyl-cysteine (Figure 1), is a substrate for the renal enzyme cysteine conjugate ß-lyase and is mutagenic in the Ames bacterial mutation assay in three strains of Salmonella typhimurium when activated by rat kidney fractions (Green and Odum, 1985; Dekant et al, 1986). The mutagenicity is decreased by aminooxyacetic acid, a ß-lyase inhibitor (Green and Odum, 1985; Dekant et al, 1988; Vamvakas et al, 1988). In vitro investigations in which tetrachloroethylene was incubated with GSH and GSH-S-transferases did not result in mutagenic effects (Deutsche Forschungs-gemeinschaft, 1988). The mutagenic activity of the cysteine conjugate of tetrachloroethylene suggests that there may be a genotoxic component in the development of tumours in the male rat kidney. The lack of response of tetrachloroethylene itself in the Ames assay is most probably due to a failure of the standard assay to replicate the steps involved in the activation of the chemical by the GSH pathway (Figure 1).

6.2.1 Species Differences

Tetrachloroethylene is a nephrotoxin in a number of species (Plaa and Larson, 1965; Klaasen and Plaa, 1966, 1967). Of the species exposed in the lifetime carcinogenicity studies, the male rat was found to be the most sensitive (NCI, 1977; NTP, 1986) and therefore, if the tumours seen are the result of sustained cytotoxicity and tissue repair, would be the most susceptible to the induction of tumours. If so, subtoxic doses would be without carcinogenic risk. The same conclusion can be drawn for all species including man.

Protein droplet nephropathy has been found to occur with a significant number of chemicals that cause cancer in the male rat kidney. The protein, alpha-2µ-globulin, has been found in the male rat but not in female rats, mice of either sex or human beings (Alden et al, 1985; Pitha et al, 1987). If renal tumours resulting from exposure to tetra-

chloroethylene are induced by this mechanism their development would not be relevant to man.

The metabolic rates for the pathway involving GSH conjugation of tetrachloroethylene and activation of the cysteine conjugate by renal ß-lyase have been compared in rats and mice in vivo, and in rat, mouse and human liver cells in vitro (Green et al, 1990). The results of these studies were consistent, the rat being the species most likely to be susceptible to renal cancer by this mechanism.

The first metabolic step in which there are significant species differences in metabolism is conjugation with GSH. In vivo the levels of N-acetyl-S-trichlorovinyl cysteine in rat urine (0.55-2.04 µg/ml) were significantly higher than those in mouse urine (0-0.20 µg/ml) following exposure to 400 ppm tetrachloroethylene by inhalation for up to 14 d. The in vitro studies also found the rat to be the most active species both for the conjugation of tetrachloroethylene with GSH in the liver and for the activation of the cysteine conjugation by ß-lyase in the kidney. The rates for the GSH conjugation of tetrachloroethylene with GSH in rat, mouse and human liver fractions are shown in Figure 3. There was no measurable activity in human liver samples. Based on the limit of detection of the radiochemical assay used in these experiments, the difference between the conjugation rates in rat and human liver was at least 1 order of magnitude (Green et al, 1990).

The second step in which there are significant species differences is the activation of S-trichlorovinyl cysteine by renal ß-lyase. In vitro studies measuring the rate of metabolism of chemically synthesised S-trichlorovinyl cysteine by ß-lyase in rat, mouse and human kidney fractions showed that rat kidney was up to ten times more active than either mouse or human kidney (Figure 4). Although metabolism could be detected in human kidney cells using the cysteine conjugate synthesised in the laboratory it should be noted that hepatic GSH conjugation of tetrachloroethylene, the source of the cysteine conjugate in vivo, could not be detected in human liver samples (Green et al, 1990). Consequently the metabolism observed with the synthetic cysteine conjugate is of no

toxicological significance for man at levels of exposure likely to occur during normal handling and use.

The presence of N-acetyl cysteine conjugate of tetrachloroethylene in female rat and mouse kidney (Green et al, 1990) suggests, however, that the genotoxic glutathione conjugation/ß-lyase mechanism may either not be involved or not be sufficient alone to induce renal tumours. The mechanism occurs in mice and female rats, albeit to a lesser extent than in the male rat, yet kidney tumours were not seen in either.

It appears likely, therefore, that the cell division occurring in the male rat kidney as a result of protein droplet nephropathy or cytotoxicity has a significant role in the development of tetrachloroethylene-induced kidney tumours. The induction of renal cancers only in male rats is most probably due to the fact that these mechanisms either occur only in the male rat or occur to a much greater extent than in female rats or in other species.

In conclusion, the kidney tumours found in male rats exposed to tetrachloroethylene may arise by one or more of the following mechanisms:

i. as a consequence of sustained chronic renal toxicity with prolonged cellular regeneration;
ii. protein droplet nephropathy and cellular regeneration;
iii. GSH conjugation and the formation of a mutagenic cysteine conjugate.

Each of these mechanisms may contribute to the low incidence of renal tumours seen in these animals. Each mechanism is highly dependant upon the dose of the tetrachloroethylene administered. For both chronic toxicity and for protein nephropathy there is a tetrachloroethylene dose threshold below which they are absent. The GSH pathway has been shown to be of little significance at low dose levels such as those experienced during normal handling and use, when tetrachloroethylene is primarily metabolised by oxidation to TCA.

7. ASSESSMENT OF HUMAN CARCINOGENIC HAZARD

Tetrachloroethylene causes tumours in rodents, the most significant for human hazard assessment being hepatocellular carcinomas in male and female mice and renal tumours in male rats. Initial hypotheses on the mechanism of formation of these tumours were based on an assumption that metabolism of tetrachloroethylene was to an epoxide intermediate which is known to be genotoxic (Di Paolo and Doniger, 1982; Kline et al, 1982, Table 18). However, the negative reulsts in mutagenicity assays provided no evidence for the formation of a reactive intermediate of this type. Alternative mechanisms of tumour formation have now been established which are consistent with this lack of genotoxic activity.

Tetrachloroethylene can be metabolised via two different pathways. The major pathway, involving cytochrome P-450 mediated oxidation to TCA, is most likely to be responsible for the mouse liver tumours. The second pathway, involving cytosolic GSH-S-transferase, may play a role in the formation of renal tumours in male rats.

The metabolism of tetrachloroethylene by the two pathways is dose-dependant (Green et al, 1990). At low dose levels, tetrachloroethylene is metabolised almost entirely by the oxidative pathway to TCA. Following saturation of this pathway, increasing exposure to tetrachloroethylene leads to increased metabolism by GSH-conjugation (Figure 2).

Major differences between species in the formation and response to tetrachloroethylene metabolites derived from these pathways provides strong grounds for beleiving that the occurrence of tumours in rodents exposed to tetrachloroethylene is of limited, if any, significance to man.

7.1 Relevance of Mouse Liver Tumours

The oxidative pathway has been shown to be saturated in rats exposed to tetrachloroethylene by inhalation at approximately 100 ppm, whereas in mice this pathway was not saturated up to concentrations of 400 ppm.

This resulted in blood concentrations of TCA in mice being 7-13 times greater than in rats at this exposure level. TCA causes peroxisome proliferation in rats and mice and increases the incidence of hepatocellular carcinomas in mice. However, relatively high levels of TCA have to be achieved before peroxisome proliferation is seen to any significant extent. Saturation of the oxidative metabolism at low concentrations of in the rat result in TCA levels below that required for peroxisome proliferation to occur and no increase in liver cancer has been observed. Since the oxidative pathway reaches saturation at concentrations of approximately 100 ppm in man also, the metabolism of tetrachloroethylene to TCA is limited particularly at high doses.

Another difference appears between rodents and man is that TCA does not cause peroxisome proliferation in human hepatocytes under conditions known to produce this effect in rat and mouse hepatocytes. This suggests that tetrachloroethylene is not a peroxisome proliferator in man and would not be a human liver carcinogen. This is supported by the fact that no increase in the indicence of liver cancer was reported in epidemiological studies of people with potential exposure to tetrachloroethylene. (It is recognised, however, that various shortcomings of these studies limit the strength of the conclusions that may be drawn from them.)

7.2 Relevance of Rat Kidney Tumours

The low incidence of renal tubular cell adenomas and adenocarcinomas observed in the male rat may arise from the interaction of more than one mechanism.

Excessive doses of tetrachloroethylene given to rats for their lifetime cause a reduction in survival rates as a result of toxic nephropathy. Sustained cytotoxicity, cell division and tissue repair in the kidney tubules may contribute to the development of tumours. If the renal tumours seen in male rats are the result of chronic cytotoxicity,

subtoxic doses should be without carcinogenic risk. The same conclusion can be drawn for all species, including man.

The discovery of the GSH-pathway and the mutagenicity of the cysteine conjugate of tetrachloroethylene in the Ames bacterial assay has revealed a genotoxic potential not previously detected by conventional mutagenicity assays. The GSH-pathway has been shown to be very active in the rat, both for the conjugation of tetrachloroethylene with GSH in the liver and for the activation of the resulting cysteine conjugate by ß-lyase in the kidney. Thus, the occurrence of kidney tumours in male rats may involve the formation of genotoxic metabolites. Since tetrachloroethylene does not appear to be metabolised by the GSH-pathway in human liver samples, the development of kidney tumours in man by this pathway seems unlikely.

Toxic nephropathy and the metabolism of tetrachloroethylene by the GSH-pathway occur in both male and female rats and neither of these mechanisms can alone account for the exclusive occurrence of kidney tumours in male rats. However, the protein, alpha-2µ-globulin, accumulates in the proximal tubular cells after exposure to tetrachloroethylene. This response, which occurs with a number of male-rat specific renal carcinogens (eg p-dichlorobenzene and trimethylpentane) appears to be the initial step in a cycle involving cell necrosis and regeneration that results eventually in tumour formation. Accumulation of alpha-2µ-globulin is observed exclusively in male rats. This mechanism, either alone or in combination with one of the other two mechanisms, may explain the sex-specific renal carcinogenic response in rats. The alpha-2µ-globulin mechanism is not relevant to man. Thus the occurence of renal tumours in male rats exposed to tetrachloroethylene is believed to be of no significance for human hazard assessment.

7.3 Relevance of Rat Leukaemias

The observation of increased incidences of mononuclear cell leukaemia in the F344 rat, but not in the Osborne-Mendel nor Sprague-Dawley rat

(sections 3.1 and 3.2) is considered to be of no significance for human hazard assessment because this neoplasm is known to be of a high and variable incidence specifically in the F344 rat, and furthermore this type of leukemia does not occur in man.

7.4 Evaluation

Although tetrachloroethylene causes the development of liver tumours in mice, the mechanism involved is unlikely to occur in man because:

i. the formation of TCA in man is limited by the saturation of the oxidative pathway at concentrations above 100 ppm;
ii. TCA does not induce peroxisome proliferation in human hepatocytes in vitro.

The kidney tumours observed in male rats may arise from one or more of three potential mechanisms:

i. protein droplet nephropathy (sex and species specific for male rats);
ii. sustained chronic toxicity;
iii. hepatic GSH-conjugation and subsequent activation of the resulting cysteine conjugate by renal ß-lyase.

The kidney tumours observed in male rats may be a consequence of the sex specific protein droplet nephropathy developed by the male rats. In addition, (i) sustained chronic toxicity to the kidney tissue and/or (ii) hepatic GSH-conjugation and subsequent activation of cysteine conjugate renal ß-lyase, may have contributed to the development of the renal tumours.

It is unlikely that any of these mechanisms will occur in man exposed to tetrachloroethylene during normal handling and use. GSH-conjugation has not been detected in human liver samples and protein droplet nephropathy is a species and sex specific event exclusively observed in the male rat.

Sustained chronic toxicity will only occur following exposure to high concentrations of tetrachloroethylene over a prolonged period of time.

Thus, interspecies comparisons indicate that the liver tumours in mice and kidney tumours in rats respectively are unlikely to occur in man and therefore these tumour incidences should not be used as the basis for risk assessment in man. Limited support for this view is obtained from the negative results obtained from a number of epidemiological studies.

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TABLE 1

Tetrachloroethylene Studies: Gene Mutation - Prokaryotes

* Stabilized with 0.012 % hydroquinone monomethylether (=HQMME)
** Stabilized with 0.07 % cyclohexeneoxide, 0.05 % ß-ethoxyproprionitrile, 0.011 % HQMME
*** Stabilized with 0.01 % HQMME
**** Stabilized with 0.7 % ß-hydroxypropionitrile, 0.1 % hydroquinone monoethylether, 0.07 % epichlorohydrin, 0.007 % n-methylmorpholine

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Ames/Salmonella TA98, TA100, TA1535, TA1537	Vapour-phase airtight 8h, 37°C	99+ % purity Aldrich	0.025, 0.05, 0.1, 0.5, 1.0 and 1.5 ml added to petriplate at bottom of desicator (9 l)	± Aroclor 1254 induced rat S9 liver (f+m) and mouse S9 liver (f+m)	-ve	1.5 ml toxicity	Milman et al, 1988; SRI International, 1983
Ames/Salmonella TA100	Tedlar vaporization desiccator technique	Unknown	Unknown	± induced hamster S9 liver	-ve	Toxic levels achieved	Warner et al, 1988
Ames/Salmonella TA98, TA100, TA1535, TA1537, TA1538	Vapour-phase airtight 18h, 37°C	Perchlor 200 * 99.93 % purity PPG Industries	1 % (v/v) TA98, TA1538 and TA1537 0.1, 1.0, 2.5, 5.0, 7.5 and 10 % (v/v) TA100 and TA1535	± Aroclor 1254 induced rat S9 liver	+ve (2.5 %) TA100 TA1535	2.5 % : (> 97 % toxic) 3-6 fold response ± activation, dose-response not established	Williams and Shimada 1983; Shimada et al, 1985
Ames/Salmonella TA98, TA100, TA1535, TA1537, TA1538	Vapour-phase airtight 18h, 37°C	Perchlor 230 ** 99.80 % purity PPG Industries	1 % (v/v) TA98, TA1538 and TA1537 0.1, 1.0, 2.5, 5.0, 7.5 and 10 % (v/v) TA100 and TA1535	± Aroclor 1254 induced rat S9 liver	+ve (2.5 %) TA100 TA1535	2.5 % : (> 98 % toxic) 3-10 fold response ± activation, dose-response not established	Williams and Shimada 1983; Shimada et al, 1985
Ames/Salmonella TA100, TA1535	Vapour-phase airtight 18h, 37°C	High purity Perchlor *** 99.98 % purity PPG Industries	0.1, 1.0 and 2.5 % (v/v)	± Aroclor 1254 induced rat S9 liver	-ve	2.5 % : toxic	Williams and Shimada 1983; Shimada et al, 1985

TABLE 1 (continued)

Tetrachloroethylene Studies: Gene Mutation - Prokaryotes

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Ames/Salmonella TA98, TA100, TA1535, TA1537	Preincubation 20 min 37°C plate-test	Technical grade Fisher 772783	3, 10, 33, 100 and 333 µg/plate in DMSO	± Aroclor 1254 induced rat S9 liver and hamster S9 liver	-ve	333 µg/plate toxic	Haworth et al, 1983
Ames/Salmonella TA97a, TA98, TA100, TA102	Preincubation plate-test	Unknown	Unknown	+ S9	-ve	Doubtful response in TA97A, insufficient details for proper evaluation	Calandra et al, 1987
Ames/Salmonella TA100	Plate-test	99.7 % purity Merck-Darmstadt	0-1791 µg/plate in DMSO (4 mM)	Phenobarbital induced mouse S9 liver, with and without NAPDH, G6P	-ve	> 224 µg/plate toxic (= 0.5 mM)	Bartsch et al, 1979
Ames/Salmonella TA1535	Plate-test	99.0 % E. Merck	100 µg/plate in ether	Absent	-ve		Kringstad et al, 1981
Ames/Salmonella TA98, TA100, UTH8414, UTH8413	Plate-test	Purity unknown Eastman Kodak	50, 100, 500, 1000 and 2000 µg/plate in DMSO	± Aroclor induced male rat S9 liver	-ve		Connor et al, 1985
Ames/Salmonella TA98, TA100, TA1535, TA1537, TA1538	Plate-test airtight	Non stabilized high purity Detrex Chemical Industries	0.01, 0.05 and 0.1 ml/plate (16-160 mg/plate)	± Aroclor 1254 induced rat S9 liver	-ve		Margard, 1978
Ames/Salmonella TA98, TA100, TA1535, TA1537, TA1538	Plate-test airtight	Stabilized **** 99.84 % purity Detrex Chemical Industries	0.01, 0.05 and 0.1 ml/plate (16-160 mg/plate)	± Aroclor 1254 induced rat S9 liver	(+) -S9 + +S9 (0.1 ml) TA100 (?)	0.1 ml : (> 90 %) toxic. 10-17 fold increase with activation, 2 fold without activation	Margard, 1978
Ames/Salmonella TA98, TA100, TA1535, TA1538 TA1952, TA1951 TA1950	Spot-test	Unknown	1 and 10 % in 0.05 ml DMSO or undiluted (= 0.01, 0.1, 1 mg/ml)	Absent	+ve	Mutagenic activity of undiluted compound in all strains, dose dependance in TA100 only. Insufficient details for proper evaluation.	Cerna and Kypenova, 1977
E. Coli K 12 arg + nad + gal + MIR	Liquid 2 h at 37°C	Analytical grade Merck-Darmstadt	0.6 mM (= 150 µg/ml)	+ Phenobarbital induced mouse S9 liver ± NADPH cofactors	-ve	99 % survival	Greim et al, 1975

TABLE 2

Tetrachloroethylene Studies: Gene Mutation and Recombination in Non-Mammalian Eukaryotic Systems

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Saccharomyces cerevisiae D7 ilv-1 (reverse mutation) trp-5 (mitotic gene conversion) ade-2 (mitotic recombination)	2 h, 37°C suspension	99.5 % purity 0.01 % thymol Carlo Erba	5, 10, 20, 60, 85 mM (0-14.1 mg/ml) in DMSO	Stationary phase; ± phenobarbital and ß-naphthoflavone induced mouse S9 liver	-ve	Significant decreased survival from 10 mM	Bronzetti et al, 1983
Saccharomyces cerevisiae D7 ilv-1 (reverse mutation) trp-5 (mitotic gene conversion) ade-2 (mitotic recombination)	1 h	Not specified purity, 0.01 % thymol Eastman Kodak	813 µg/ml (4.9 mM) ilv-1 813 and 1094 µg/ml (4.9-6.6 mM) trp-5, ade-2	log phase	-ve ilv +ve trp ade	Positive at 1094 µg/ml 42 % toxicity (1459 µg/ml 100 %). No positive control	Callen et al, 1980
Saccharomyces cerevisiae D7 liv 1-92 (reverse mutation) trp 5 (mitotic gene conversion)	2 h, 30°C	Analytical grade EGA Chemie	9.8, 14.7 mM in phosphate buffer	Late log phase or stationary phase; +/- aroclor 1254 induced mouse liver S9	Unknown	No evaluation possible due to high toxicity (70% at 9.8 mM)	Koch et al 1988
Drosophila melanogaster SLRL	7 h inhalation	91.43 % purity North Strong IBI No 2537	100, 500 ppm	Absent	-ve	Only small sample size examined	Beliles et al, 1980
Drosophila melanogaster Canton-S SLRL	3 d feeding or injection (0.3 µl)	Technical grade Fisher 772783	4000 ppm (feeding) 1000 ppm (injection) in 10 % ethanol	Absent	-ve		NTP, 1986; Valencia et al, 1985

TABLE 3

Tetrachloroethylene Studies: Gene Mutation - Host Mediated Assays

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Host-mediated Salmonella TA1950, TA1951, TA1952	Unknown	Unknown	0.5 LD50, LD50	Female ICR mice	+ve	No dose dependence, insufficient details for proper evaluation	Cerna and Kypenova, 1977
Host-mediated Salmonella TA98	Ip after last exposure (inhalation, 5 d, 7 h/d) 3 h incubation	91.43 % purity North Strong LBI No 2537	100, 500 ppm	Male and female Swiss CD-1 mice	+ve female 500 ppm + male 100 ppm	+ve control (0.8 mg/kg 2-amino-anthracene, IM) ineffective. -ve in S9 activated Ames plate incorporation assay (TA98). Low purity.	Beliles et al, 1980
Host-mediated Saccharomyces cerevisiae D7 stationary phase trp-5 (mitotic gene conversion) ilv-1 (reverse mutation)	Retro-orbital sinus injection direct before (final) oral exposure, 4 h incubation (liver, lungs, kidneys)	99,5 % purity 0,01 % Thymol Carlo Erba	11,000 mg/kg or 26,000 mg/kg (total of 12 adm over 3 w)	Male swiss CD-1 mice	-ve	No +ve control, unusual protocol	Bronzetti et al, 1983

TABLE 4

Tetrachloroethylene Studies: Gene Mutation - Mammalian Systems in Vitro or in Vivo

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Mouse lymphoma L5178Y/TK +/-	4 h, 37°C	Unknown	12.5, 25, 50 nl/ml (6.25, 100 nl/ml for + S9) (75, 150 nl/ml for - S9)	+ Aroclor 1254 induced rat liver S9	-	Growth was inhibitor at top dose level	NTP, 1986

TABLE 5

Tetrachloroethylene Studies; Chromosomal Aberrations - in Vitro Mammalian Systems

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Chinese hamster ovary cells	2 h (+ S9) 8-10 h (- S9) 37°C	Unknown	17, 34, 68 µg/ml in DMSO (+ 136 µg/ml, - S9)	± Aroclor 1254 induced rat liver S9	-ve		NTP, 1986; Galloway et al, 1987

TABLE 6

Tetrachloroethylene Studies; Chromosomal Aberrations - in Vivo Mammalian Systems

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Result	Comments	Reference
Rat Bone marrow	Inhalation, 6 h/d, 5 d/w, 12 mnth	99.9 % purity 3 ppm IRI 2 ppm C CL4 44 ppm 4-methyl morpholine	300, 600 ppm	-ve	Very low number of metaphases scored in females	Rampy et al, 1978
Rat Bone marrow	Inhalation 7 h BM cells 6, 24, 48 h	91.4 % purity North Strong LBI No 2537	100, 500 ppm	-ve	Weak clastogenic effects (breaks, fragments, deletions, aneuploid cells) in 500 ppm males at 24 h	Belles et al, 1980
Rat Bone marrow	Inhalation 7 h/d, 5 d BM cells 6 h	91.4 % purity North Strong LBI No 2537	100, 500 ppm	-ve	Slight increase of aberrations in females at 100 ppm	Belles et al, 1980
Mouse Bone marrow	I.p.injection single dose	Unknown	0.5 LD50	-ve		Cerna and Kypenova, 1977
Mouse Bone marrow	I.p.injection 1 x/d, 5 d	Unknown	0.16 LD50	-ve		Cerna and Kypenova, 1977
Human lymphocytes	Workers of a degreasing workshop	Technical grade	92 (30-220) and 10-40 ppm	-ve		Ikeda et al, 1980

TABLE 7

Tetrachloroethylene Studies: SCE in Vitro Mammalian Systems

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Chinese hamster ovary cells	2 h, 37°C	Unknown	16.4, 54.5, 164.0 µg/ml DMSO (- S9) 80.4, 109.9, 124.6 µg/ml DMSO (+ S9)	± Aroclor 1254 induced rat liver S9	-ve		NTP, 1986; Galloway et al, 1987

TABLE 8

Tetrachloroethylene Studies: Chromosome Loss - Non Mammalian Systems

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Result	Comments	Reference
Drosophila melanogaster	Inhalation, 7 h	91.4 % purity North Strong LBI No 2537	100, 500 ppm	-ve		Belliles et al, 1980

TABLE 9

Tetrachloroethylene Studies: SCE and Mitotic Index - in Vivo Mammalian Systems

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Result	Comments	Reference
Human lymphocytes	Workers from degreasing workshop	Technical grade	92 (30-220) and 10-40 ppm for 3 m to 10 y	-ve		Ikeda et al, 1980

TABLE 10

Tetrachloroethylene Studies: Dominant Lethal

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Result	Comments	Reference
Rat males	Inhalation 7 h/d, 5 d	91.4 % purity North Strong LBI No 2537	100, 500 ppm	-ve		Belliles et al, 1980

TABLE II

Tetrachloroethylene Studies: DNA Damage - in Vitro

* Stabilized with 0.012 % HQMME
** Stabilized with 0.011 % HQMME, 0.07 % cyclohexeneoxide, 0.05 % ß-ethoxyproprionitrile

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
UDS Human fibroblast (diploid WI-38)	Liquid phase	91.4 % purity North Strong LBI No 62537	0.1, 0.5, 5, 1, 5 µl/ml DMSO	± Aroclor induced rat liver S9	-ve (e)	Toxic at 5 µl/ml +ve controls gave weak responses	Beliles et al, 1980
UDS Rat hepatocytes	3 h, 18 h, liquid phase	Perchlor 200 * 99.93 % purity PPG Industries	0.0001, 0.001, 0.1, 1.0 % (v/v)	Absent	-ve		Williams and Shimada, 1983
UDS Rat hepatocytes	3 h, 18 h, liquid phase	Perchlor 230 ** 99.80 % purity PPG Industries	0.001, 0.01, 0.1, 1.0 % (v/v)	Absent	-ve		Williams and Shimada, 1983
UDS Rat hepatocytes	3 h, 18 h, vapour phase	Perchlor 230 ** 99.80 % purity PPG Industries	0.1, 1.0, 2.5 % (v/v) - target	Absent	(+ve) 0.1 %	Toxicity (25-50 %) at 0.1 %; toxicity (ca 100 %) at 1.0 and 2.5 %	Williams and Shimada, 1983; Shimada et al, 1985
UDS Rat hepatocytes	3 h, 18 h, vapour phase	Perchlor 200 ** 99.93 % purity PPG Industries	0.1, 1.0, 2.5 % (v/v) - target	Absent	(+ve) 0.1 % (3 h)	Toxicity (ca 75 %) at 0.1 %; toxicity (ca 100 %) at 1.0 and 2.5 %	Williams and Shimada, 1983; Shimada et al, 1985
UDS Rat, mouse hepatocytes	Liquid phase 18 h	99+ % purity Aldrich Chemical Company	0.00001, 0.0001, 0.001, 0.01, 0.1 % (v/v)	Absent	-ve	Toxic at 0.01 %	Williams, 1983; Milman et al, 1988
UDS Rat hepatocytes	Liquid phase 2.5 h, 37°C airtight	Merck-Darmstadt	2.5 mmol in ethanol	Phenobarbital	-ve	Viability of hepatocytes affected at > 2.5 mmol	Costa and Ivanetich, 1984

TABLE 12

Tetrachloroethylene Studies: DNA Damage - in Vivo/in Vitro

Test system	Protocol	Purity/ ce (% w/w)	Concentrations tested	Result	Comments	Reference
UDS male rats (kidney)	Oral 12,24 h incubation	Unknown	1000 mg/kg	-ve		Goldsworthy et al, 1988b

TABLE 13

Tetrachloroethylene Studies: DNA Damage - in Vivo

Test system	Protocol (% w/w)	Purity/Source tested	Concentrations activation	Result	Comments	Reference
Mouse, male single strand breaks (liver kidney, lungs)	Ip 1 h and 24 h incubation detection limit 1SSB/ $5x10^6$ nucleotides	99.8 % purity Merck-Schuchardt	4-8 mmol/kg in Tween-80	+ve (liver, kidney 1 h)	No damage in lung DNA after 1 h All damage repaired at 24 h	Walles, 1986

TABLE 14

Tetrachloroethylene Studies: DNA Binding - in Vivo

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Result	Comments	Reference
Mouse (liver)	Inhalation (6 h) or oral single exposure	Dowper, 99+ % purity 56 ppm 4-methyl-morpholine	600 ppm inhalation 500 mg/kg oral	-ve	Binding less than 10-14.5 alkylations per 10^6 nucleotides	Schumann et al, 1980
Rat, mouse (liver, lung, kidney, stomach)	Ip + 22 h incubation detection limit 0.13-0.94/10^6 nucleotides	^{14}C-labeled, 97 % purity (hexachloro-ethane) spec. act. 14.6 mCi/mmol	8.70 µmol/kg	+ve (mouse liver)		Mazzullo et al, 1987

TABLE 15

Tetrachloroethylene Studies: DNA Binding - in Vitro

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Calf (thymus)	90 min, 37°C	^{14}C-label, 97% purity (hexa-chloroethane) spec. act. 14.6 mCi/mmol	2.5 µCi	± Phenobarbi-tone induced rat, mouse liver S9 or cytosol	+ve	Cytosol enzymes more effective than S9	Mazzullo et al, 1987

TABLE 16

Tetrachloroethylene Studies: Cell Transformation

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Metabolic activation	Result	Comments	Reference
Rat embryo cells F 1706 p 108	48 h incubation	99.9 % Eastman Kodak 755428	97 - 970 µM (= 16.1-161 µg/ml)	Absent	+ve	Cytotoxic at 97µM; positive control: 3-methylcholanthrene	Price et al, 1987
BALB/C-3T3 mouse		97-99 % purity Aldrich	0 - 250 µg/ml	Absent	-ve	Cytotoxic at 250 µg/ml (4 % survival)	Tu et al, 1985; Milman et al, 1988
Hamster BHK 21/C13	Vapour phase	Purity unknown ICI MD 516	Unknown	+ Aroclor induced rat liver S9	-ve		Longstaff and Ashby, 1978

TABLE 17

Tetrachloroethylene Studies: Germ Cell Effects - in Vivo Mammalian Systems

Test system	Protocol	Purity/Source (% w/w)	Concentrations tested	Result	Comments	Reference
Sperm head abnormality Rat Mouse	Inhalation (7 h/d, 5 d) + 1,4 and 10 weeks	91.4 % purity North strong LBI No 2537	100, 500 ppm	-ve (rat) +ve (mouse)	500 sperm/animal examined damage to spermatocytes, spermatogenia	Beliles et al, 1980

TABLE 18

Tetrachloroethylene Studies: Metabolites of Tetrachloroethylene

Test compound	Test system	Protocol	Metabolic activation	Result	Reference
Tetrachloroethylene oxide	Ames/*Salmonella* TA1535	Preincubation 20 min, 37°C	Absent	+ve	Kline *et al*, 1982
Trichloroacetyl chloride	Ames/*Salmonella* TA98, TA100	Preincubation 90 min, 37°C	± Aroclor 1254 induced rat liver S9	-ve	Reichert *et al*, 1983
Trichloroacetic acid (TCA)	Ames/*Salmonella* TA98, TA100, TA1535	Plate incorporation	± Aroclor 1254 and phenobarbital induced rat liver S9	-ve	Waskell, 1978; Andersen *et al*, 1972
Trichloroethanol	Ames/*Salmonella* TA100, TA1535	Spot- and plate-incorporation	± Aroclor 1254 induced rat liver S9	-ve	Bignami *et al*, 1980
Oxalic acid	Ames/*Salmonella* TA97, TA98, TA100, TA102, TA104	Plate incorporation, preincubation 20 min, 37°C	± Phenobarbital and 5,6-benzoflavone induced rat liver S9	-ve	Sayato *et al*, 1987
1,1,2-Trichloro-vinylcysteine	Ames/*Salmonella* TA100	Plate-incorporation	± Aroclor 1254 induced rat kidney S9	+ve	Green and Odum, 1985
	Ames/*Salmonella* TA98, TA100, TA2638	Preincubation 120 min, 37°C	± Rat liver and kidney S9 or cytosol	+ve except TA2638	Dekant *et al*, 1986
1,1,2-Trichloro-vinyl-N-acetyl cysteine	Ames/*Salmonella* TA100	Unknown	± Rat kidney cytosol	+ve	Vamvakas *et al*, 1987
Trichloroacetic acid	Mouse *in vivo*	500 mg/kg ip	Absent	Sperm head abnormalities chromosome aberrations	Bhunya and Behera, 1987

TABLE 19

Exposure-Related Increases in Incidences of Tumours in Rats and Mice Exposed to Tetrachloroethylene by Inhalation (after NTP, 1986)

* = P<0.005
** = P<0.001
NS = Not significant

Species - dose	Hepatocellular Carcinoma	Renal Tubular Cell Adenomas and Adeno-carcinomas (combined)	Mononuclear Cell Leukaemia
Mouse	Male Female		
- control	7/49 1/48		
- 100 ppm	25/49** 13/50**		
- 200 ppm	26/50** 36/50**		
Historical incidence	20.3% 4.5%		
range	16-32% 0-14.6%		
Rat		Male	Male Female
- control		1/49	28/50 18/50
- 200 ppm		3/49 NS	37/50* 30/50*
- 400 ppm		4/50 NS	37/50* 29/50*
Historical incidence		0.2%	29.5% 18.6%
range		0-2%	10-60% 6-38%

TABLE 20

Air Monitoring at the Working Place in Dry-Cleaning Industries

* PS = Personal Sampling
** STEL = Short Term Exposure Limit (15 min TWA) during loading and unloading
*** TWA = Time Weighted Average concentration during 8 h

Number of shops	Measuring method (*)	STEL (**) (mg/m^3)	TWA (***) (mg/m^3)	Reference
2 persons	PS	680 - 1000 at 1 m distance	100 - 300	Buchter et al, 1984
44	Unknown	300	30 - 1000 machine operator 150 presser 25	Ludwig et al, 1983
6	PS		60 - 250	Lauwerijs et al, 1983
3	PS		50 - 350	Monster et al, 1983
67	Unknown	75 - 3500	200	Materna, 1985
408	Unknown		almost all <335	CEFIC, 1981
90	PS		<70 - <3,500 (97%<700)	Shipman and Whim, 1980
160 factories	PS		<70 - <4,200 (93%<700)	Shipman and Whim, 1980

TABLE 21

Limitations of the Epidemiological Studies for the Assessment of Carcinogenicity in Man

SMR = Standardised Mortality Ratio
PMR = Proportional Mortality Ratio
CR = Case-Referent

X = Apparent limitation because ...
1) = For 16% of the cohort
2) = For 18% of the cohort

	SMR			PMR				CR	
Reference:	Kaplan 1980	Blair 1985	Brown 1987	Blair 1979	Katz 1981	Duh 1984	Nakamura 1985	Smith 1985	McLaughlin 1987
Country	USA	USA	USA	USA	USA	USA	Japan	USA	Sweden
Confined to union members	X	X	X	X			X		
Dry-cleaning and laundry workers together				X	X	X	X	X	X
Sex and/or race restrictions		X		X	X				
Mixed exposure to tetrachloroethylene - plus other solvents - plus other chemicals	X?	X	(X)	X?	X?	X?	X		
Exposure histories unknown	X	X	X		X	X	X	X	X
Confounding factors unknown	X	X	X	X		X	X		X
Low socio-economic status				X	X				
Small numbers in - the cohort - for specific causes of death	X		X	X		X			
Substantial data on deceased missing	x[1]	x[2]							

TABLE 22

Comparison of Cause-Specific Risks Among

Mortality Studies of Dry-Cleaning and Laundry Workers

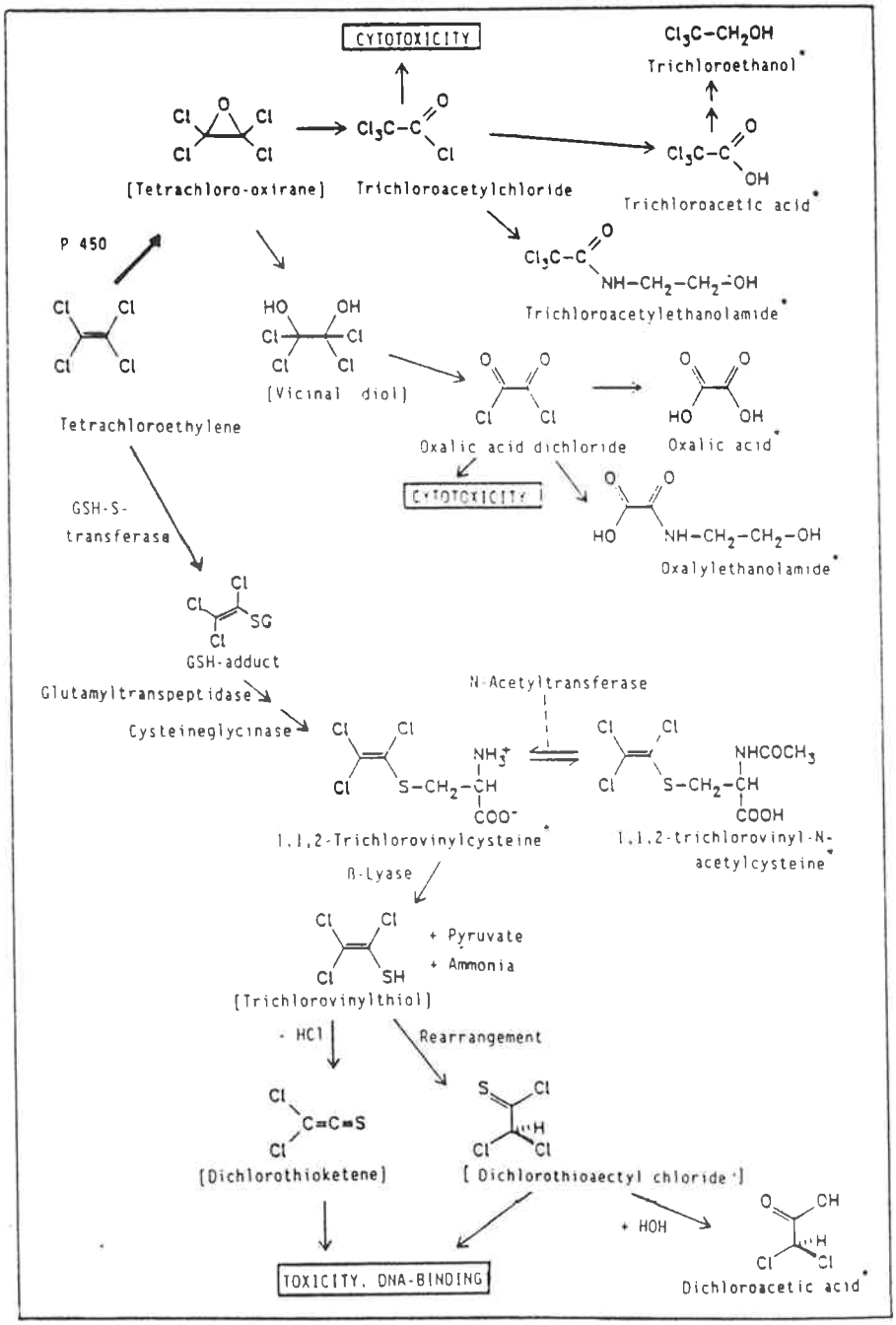
Codes	Authors	SMR - COHORT MORTALITY				PMR - COHORT MORTALITY				
		Kaplan 1980		Brown and Kaplan 1987	Blair et al 1985	Blair et al 1979	Katz and Jowett 1981	Duh and Asal 1984	Nakamura 1985	
ICD-9 **	Number of Deceased in Cohort	N=285		N=493	N=1329	N=330	N=671	N=440	N=1711	
	All causes			86	92	(100%)	(100%)	(100%)	(100%)	
	All neoplasms			116	84	128	96	90	100	
		Whites	Blacks						Females	Males
50	Oesophagus				280*				154	76
53	Colon	182	187	136			103			
55	Liver					235	89	50	67	104
57	Pancreas	167	127	172		129	117	50	48	
165	Tractus respiratorius	148	133	114		170*		180*	138	85
61	Larynx				278				333	103
62	Lung						98	170*		
70	Bone								1000*	
73	Skin					429*	207	150		143
74	Female breast			87		69	72	10	28*	
80	Cervix uteri			196		208*	195*	130		
88	Bladder	} 203	198	296	146	83	189	40		136
89	Kidney			200		200	257*	380*	250	80
93	Thyroid									
200	Lymfoma						179	80		
208	Leukaemia					227	67			
	Hodgkin								154	91

PMR = Proportional Mortality Ratio SMR = Standardised Mortality Ratio * = Statistically Significant

** = International Code of Disease, 9th edition

FIGURE 1

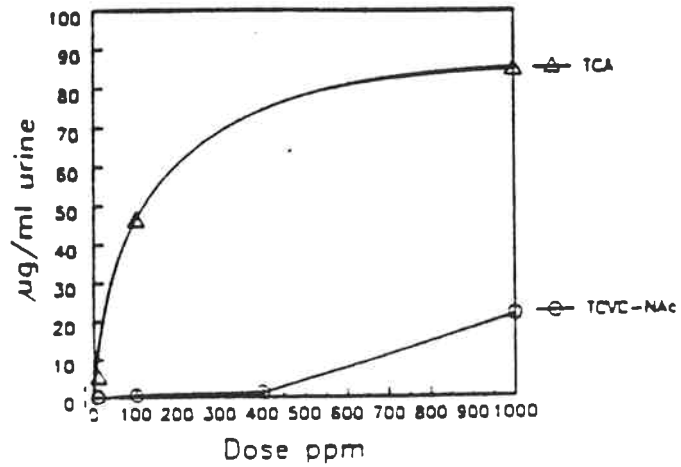
Review of the oxidative and reductive biotransformation of tetrachlorethylene (after Deutsche Forschungsgemeinschaft, 1988)



[ ] = Theoretical intermediates
* = Identified urinary metabolites
GSH = Glutathion

FIGURE 2

Effect of Dose on Tetrachloroethylene Metabolism in Rats Exposed by Inhalation (6 h) (Green et al, 1990)



TCA = Trichloroacetic acid
TCVC-NAc = N-acetyl trichlorovinyl cysteine

FIGURE 3

Hepatic metabolism of tetrachloroethylene (10 mmol) by the Glutathion-S-transferase pathway (Green et al, 1990)

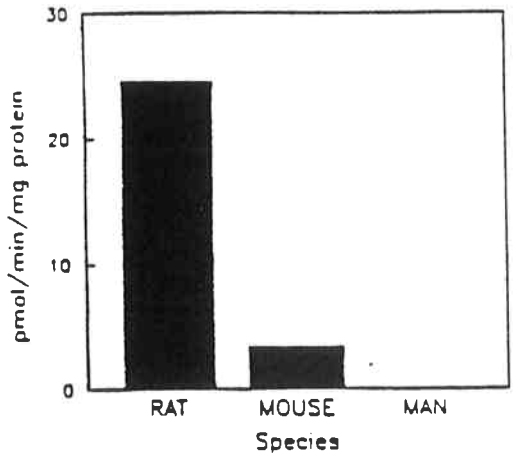
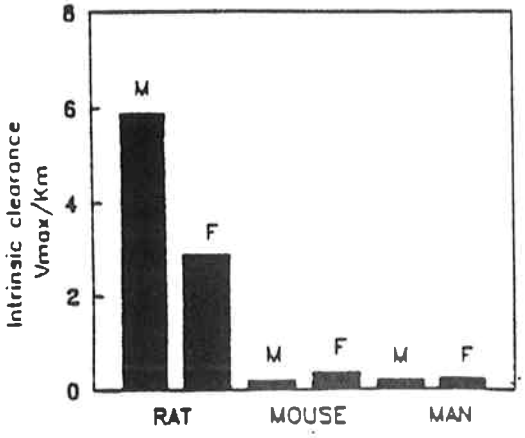


FIGURE 4

Metabolism of S-Trichlorovinyl-cysteine by Renal ß-Lyase in Vitro (Green et al, 1990)



APPENDIX 1

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