# CARBON TETRACHLORIDE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987a).

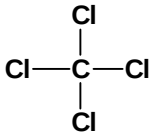
## 1. Exposure Data

### 1.1 Chemical and physical data

1.1.1 *Nomenclature*

*Chem. Abstr. Serv. Reg. No.*: 56-23-5
*Chem. Abstr. Name*: Tetrachloromethane
*IUPAC Systematic Name*: Carbon tetrachloride
*Synonyms*: Benzinoform; carbona

1.1.2 *Structural and molecular formulae and relative molecular mass*




$CCl_4$ Relative molecular mass: 153.82

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, clear, nonflammable, liquid with a characteristic odour (Budavari, 1996)
(*b*) *Boiling-point*: 76.8°C (Lide, 1997)
(*c*) *Melting-point*: –23°C (Lide, 1997)
(*d*) *Solubility*: Very slightly soluble in water (0.05% by volume); miscible with benzene, chloroform, diethyl ether, carbon disulfide and ethanol (Budavari, 1996)
(*e*) *Vapour pressure*: 12 kPa at 20°C; relative vapour density (air = 1), 5.3 at the boiling-point (American Conference of Governmental Industrial Hygienists, 1991)
(*f*) *Conversion factor*: $mg/m^3 = 6.3 \times ppm$

### 1.2 Production and use

Production in the United States in 1991 was reported to be approximately 143 thousand tonnes (United States International Trade Commission, 1993). Information

available in 1995 indicated that carbon tetrachloride was produced in 24 countries (Chemical Information Services, 1995).

Carbon tetrachloride is used in the synthesis of chlorinated organic compounds, including chlorofluorocarbon refrigerants. It is also used as an agricultural fumigant and as a solvent in the production of semiconductors, in the processing of fats, oils and rubber and in laboratory applications (Lewis, 1993; Kauppinen *et al*., 1998).

### 1.3 Occurrence

#### 1.3.1 *Occupational exposure*

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), approximately 70 000 workers in Europe and as many as 100 000 workers in the United States were potentially exposed to carbon tetrachloride (see General Remarks). Occupational exposure to carbon tetrachloride may occur in the chemical industry, in laboratories, and during degreasing operations.

#### 1.3.2 *Environmental occurrence*

The major source of carbon tetrachloride in air is industrial emissions. Carbon tetrachloride has been detected in surface water, groundwater and drinking-water as a result of industrial and agricultural activities. Carbon tetrachloride has also been found in wastewater from iron and steel manufacturing, foundries, metal finishing, paint and ink formulations, petroleum refining and nonferrous metal manufacturing industries (United States National Library of Medicine, 1997).

### 1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 31 mg/m$^3$ as the 8-h time-weighted average threshold limit value, with a skin notation, for occupational exposures to carbon tetrachloride in workplace air. Values of 10–65 mg/m$^3$ have been used as standards or guidelines in other countries (International Labour Office, 1991).

The World Health Organization has established an international drinking-water guideline for carbon tetrachloride of 2 μg/L (WHO, 1993).

## 2. Studies of Cancer in Humans

### 2.1 Industry-based studies (Table 1)

Ott *et al*. (1985) conducted a cohort mortality study of 1919 men employed for one or more years between 1940 and 1969 at a chemical manufacturing facility in the United States. This cohort included 226 workers assigned to a unit which produced chlorinated methanes (methyl chloride (see this volume), dichloromethane (see this volume), chloroform (IARC, 1987b), and carbon tetrachloride) and, recently, tetrachloroethylene (IARC,

**Table 1. Epidemiological results from industry-based studies relevant to the evaluation of carbon tetrachloride**

| Reference | Country | Cohort size/ no. of deaths | Cancer site[a] | Observed | RR | 95% CI | Comment |
|---|---|---|---|---|---|---|---|
| Ott *et al*. (1985) | United States | 226/42 | All cancers | 9 | 0.7 | 0.3–1.3 | Expected from US rates |
| | | | | | [0.8] | [0.4–1.5] | Expected from company rates |
| | | | Respiratory | 3 | [0.7] | [0.1–2.0] | |
| | | | Digestive | 6 | [1.8] | [0.7–4.0] | |
| | | | Pancreas | 3 | [3.3] | [0.7–9.7] | |
| Blair *et al*. (1990) | United States | 5365/1129 | All cancers | 294 | [1.2] | 1.0–1.3 | |
| | | | Lung | 47 | 1.3 | 0.9–1.7 | |
| | | | Oesophagus | 13 | 2.1 | 1.1–3.6 | |
| | | | Pancreas | 15 | 1.2 | 0.7–1.9 | |
| | | | Lympho/reticulosarcoma | 7 | 1.7 | 0.7–3.4 | |
| | | | Hodgkin's disease | 4 | 2.1 | 0.6–5.3 | |
| | | | Leukaemia | 7 | 0.9 | 0.4–1.8 | |
| | | | Other lymphatic | 4 | 0.7 | 0.2–1.8 | |
| | | | Breast | 36 | 1.0 | 0.7–1.4 | |
| Blair *et al*. (1998) | United States | 14475/3832 | All cancers[b] | 641 | 0.90 | 0.83–0.97 | SMR, full cohort |
| | | | Non-Hodgkin lymphoma, women | 8 exposed | 3.3 | 0.9–12.7 | Incident cancer, RR from Poisson regression |
| | | | Non-Hodgkin lymphoma, men | 14 exposed | 1.2 | 0.4–3.3 | |
| | | | Multiple myeloma, women | 4 exposed | 2.0 | 0.4–9.1 | |
| | | | Multiple myeloma, men | 10 exposed | 1.2 | 0.4–3.7 | |
| | | | Breast, women | 18 exposed | 1.3 | 0.7–2.5 | |
| Wilcosky *et al*. (1984) | United States | 6678 | Lymphocytic leukaemia (white men) | 8 exposed | 15.3 | $p < 0.0001$ | Odds ratios from nested case–control analysis |
| | | | Lymphosarcoma (white men) | 6 exposed | 4.2 | $p < 0.05$ | |
| Bond *et al*. (1986) | United States | 19608 | Lung cancer | | 0.8 | 0.6–1.1 | Odds ratio from nested case–control analysis |

[a] Results are presented for all cancers, lung, oesophagus, pancreas, lymphatic and haematopoietic cancers, and breast when reported.
[b] Includes entire cohort regardless of potential exposure to dichloromethane.

1995). Exposure levels were not reported. The follow-up period was from 1940 to 1979 and follow-up was 94% complete. Expected numbers were based on national rates for white males in the United States for the full cohort and on the rates for the full cohort for sub-cohort analyses. There were 42 deaths observed among the 226 workers (standardized mortality ratio (SMR), 0.6, based on national rates) [SMR, 0.8, based on company rates]. Nine cancers were observed [SMR, 0.8; 95% confidence interval (CI), 0.4–1.5, based on company rates], including three pancreatic cancers [SMR, 3.3; 95% CI, 0.7–9.7, based on company rates]. Two of the three workers who died of pancreatic cancer had been employed for less than five years. All three were first assigned to the chlorinated methane unit between 1942 and 1946, and the interval between first assignment to the unit and death was between 20 and 31 years. [The Working Group noted that the mix of exposures and the lack of information regarding exposure levels limits the ability to draw conclusions regarding the carcinogenicity of carbon tetrachloride.]

Blair *et al*. (1990) studied the risk of cancer and other causes of death among a cohort of 5365 members of a dry-cleaners union in the United States. The cohort consisted of persons who were union members for one year or more before 1978 and had been employed in dry-cleaning establishments. Carbon tetrachloride was used extensively in dry-cleaning between 1930 and 1960, although other solvents, such as Stoddard solvent, were also widely used. The mean year at entry into the cohort was 1956. Follow-up was from 1948 through 1978 and was 88% complete. For individuals lost to follow-up, person-years were counted only until last date known alive. The exposure assessment classified members by level of exposure to solvents, but not type of solvent. Three time-weighted average (TWA) exposure categories for solvents (none, medium, high) were assigned weights of 0, 7, 40 for cumulative exposure analysis. Expected deaths were calculated from national rates for the United States and the overall SMR (based on 1129 deaths) was 0.9. Cancer deaths amounted to 294 (SMR, 1.2). A significant excess of oesophageal cancer (SMR, 2.1; 95% CI, 1.1–3.6, based on 13 cases) and non-significant excesses of several other cancers were found. However, only the risk of lymphatic and haematopoietic cancers appeared to be related to level of solvent exposure (SMR, 4.0 for high exposure, based on five cases). The authors state that mortality patterns among those entering the union after 1960, when the use of tetrachloroethylene was predominant, were similar to those in people entering before 1960.

Blair *et al*. (1998) performed a retrospective cohort mortality study of 14 457 workers employed for at least one year between 1952 and 1956 at an aircraft maintenance facility in the United States. Among this cohort were 6737 workers who had been exposed to carbon tetrachloride (Stewart *et al*., 1991). The methods used for this study are described in greater detail in the monograph on dichloromethane. An extensive exposure assessment was performed to classify exposure to trichloroethylene quantitatively and to classify exposure (ever/never) to other chemicals qualitatively (Stewart *et al*., 1991). Risks from chemicals other than trichloroethylene were examined in a Poisson regression analysis of cancer incidence data. Among women, exposure to carbon tetrachloride was associated with an increased risk of non-Hodgkin lymphoma (relative risk (RR), 3.3; 95% CI,

0.9–12.7; 8 exposed cases) and multiple myeloma (RR, 2.0; 95% CI, 0.4–9.1; 4 exposed cases), but among men the corresponding risks were lower (non-Hodgkin lymphoma: RR, 1.2; 95% CI, 0.4–3.3; 14 exposed cases and multiple myeloma: RR, 1.2; 95% CI, 0.4–3.7; 10 exposed cases). No association was observed with breast cancer and no other site-specific results for carbon tetrachloride were presented. Exposure levels for carbon tetrachloride were not reported. [The Working Group noted that overlapping exposures limit the ability to draw conclusions regarding carbon tetrachloride.]

A nested case–control study within a cohort of rubber workers in the United States was performed to examine the relationship between exposure to solvents and the risk of cancer (Checkoway *et al*., 1984; Wilcosky *et al*., 1984). The cohort consisted of 6678 male rubber workers who either were active or retired between 1964 and 1973. The cases comprised all persons with fatal stomach cancer ($n$ = 30), respiratory system cancer ($n$ = 101), prostate cancer ($n$ = 33), lymphosarcoma ($n$ = 9) or lymphocytic leukaemia ($n$ = 10). These sites were chosen because they were those at which cancers had been found to be in excess in an earlier cohort analysis (McMichael *et al*., 1976). The controls were a 20% age-stratified random sample of the cohort ($n$ = 1350). Exposure was classified from a detailed work history and production records. An association was observed between exposure for one year or more to carbon tetrachloride and lymphocytic leukaemia (odds ratio (OR), 15.3; $p < 0.0001$, based on eight exposed cases) and lymphosarcoma (OR, 4.2; $p < 0.05$, based on six exposed cases) after adjusting for year of birth. The relative risk associated with 24 solvents was examined and levels of exposure were not reported. [The Working Group noted that overlapping exposures limit the ability to draw conclusions regarding carbon tetrachloride.]

Bond *et al*. (1986) conducted a nested case–control study of lung cancer among a large cohort of chemical workers in the United States. The cohort consisted of 19 608 white male workers employed for one year or more between 1940 and 1980 at a large facility which produced chlorinated solvents, plastics, chlorine, caustic soda, ethylene (IARC, 1994a), styrene (IARC, 1994b), epoxy latex, magnesium metal, chlor-nitrogen agricultural chemicals and glycols (Bond *et al*., 1985). The cases were 308 lung cancer deaths that occurred among cohort members between 1940 and 1981. Two control groups, one consisting of other deaths ($n$ = 308) and the other a 'living' series ($n$ = 97), were matched on race, year of birth, and year of hire. Occupational exposures were classified on the basis of work history records and information regarding exposure to chemical and physical agents collected for each work area [levels of exposure to carbon tetrachloride were not reported], while information on smoking and other potential confounders was collected by interview. No association was observed between having been exposed to carbon tetrachloride (ever versus never) and lung cancer (OR, 0.8; 95% CI, 0.6–1.1).

### 2.2 Community-based studies

Linet *et al*. (1987) performed an analysis to compare two different methods for determining occupational exposure in a population-based case–control study of chronic

lymphocytic leukaemia. Incident cancers were identified using hospital records, and controls matched on age, race and sex were selected from among patients with nonmalignant diseases from the same hospitals. The study included 342 cases and an equal number of controls [participation rates were not reported]. Relative risks derived from exposures classified on the basis of the job–exposure matrix developed by Hoar *et al.* (1980) were compared with those derived from a classification of exposure based on the National Occupational Hazard Survey (NOHS). The prevalence of exposure among cases and controls using the job–exposure matrix developed by Hoar *et al*. (1980) was 10.5% and 10.2%, respectively. The prevalence of exposure among cases and controls using the job–exposure matrix based on the NOHS was 3.8% and 5.2%, respectively. No association between chronic lymphocytic leukaemia and carbon tetrachloride exposure was observed in either set of analyses (odds ratio, 1.1; 95% CI, 0.6–2.0 for the Hoar method; and odds ratio, 0.8; 95% CI, 0.4–1.9 for the NOHS method). [The Working Group expressed concern regarding the sensitivity and specificity of the exposure assessment used.]

Heineman *et al*. (1994) performed a case–control study to examine the relationship between occupational exposure to six chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. The study was conducted in three areas of the United States, and 300 cases and 320 controls were included in the analysis. The methods used for this study are described in greater detail in the monograph on dichloromethane. Exposure was assessed using a semi-quantitative job–exposure matrix developed for the study (Gomez *et al*., 1994), and probability of exposure, duration of exposure, average intensity and cumulative exposure were examined. There were 137 cases and 123 controls classified as ever exposed. The odds ratios for the highest-exposure categories were 0.8 (95% CI, 0.4–1.9; 13 exposed cases) for high probability of exposure, 1.6 (95% CI, 0.9–2.8; 36 exposed cases) for more than 21 years of exposure, 2.9 (95% CI, 1.2–7.1; 22 exposed cases) for high average intensity, and 1.6 (95% CI, 0.8–3.2; 24 exposed cases) for high cumulative exposure.

Cantor *et al*. (1995) performed a case–control study to examine the relationship between occupational exposures and female breast cancer mortality in 24 states of the United States. The methods used for this study are described in greater detail in the monograph on dichloromethane. Probability and level of workplace exposure to 31 chemical and physical agents were estimated using a job–exposure matrix. No association was found with probability of exposure to carbon tetrachloride. After adjustment for age and socioeconomic status, a slightly elevated risk was observed for the highest exposure level among white women (odds ratio, 1.2; 95% CI, 1.1–1.3) but not among black women. [The Working Group noted that the usual occupation from death certificate in combination with a job–exposure matrix may be a poor indicator of exposure to carbon tetrachloride.]

Holly *et al*. (1996) performed a case–control study of intraocular melanoma to examine the role of chemical exposures. Cases were white male patients referred to the Ocular Oncology Unit at the University of California San Francisco (United States) between 1978 and 1987. Two white males matched on age and geographical area were selected for each case using random-digit dialling. A total of 221 cases and 447 control

(93% and 85% participation rates, respectively) were interviewed for the study. An association with exposure (ever versus never) to 'carbon tetrachloride and other cleaning fluids' was observed (odds ratio, 2.3; 95% CI, 1.3–4.1). [The Working Group expressed concern regarding the potential for recall bias from exposures based on self-reporting. The broad category of 'carbon tetrachloride and other cleaning fluids' limits the ability to draw inferences regarding carbon tetrachloride alone.]

In the Montreal case–control study carried out by Siemiatycki *et al.* (1991) (see the monograph on dichloromethane in this volume), the investigators estimated the associations between 293 workplace substances and several types of cancer. Carbon tetrachloride was one of the substances. About 4% of the study subjects had ever been exposed to carbon tetrachloride. Among the main occupations to which carbon tetrachloride exposure was attributed were fire fighters, machinists and electricians. For most types of cancer examined (oesophagus, stomach, colon, pancreas, prostate, kidney, skin melanoma), there was no indication of an excess risk. For non-Hodgkin lymphoma, based on three cases exposed at any level, the odds ratio was 0.4 (90% CI, 0.1–1.0). For rectal cancer, based on 16 cases exposed at any level, the odds ratio was 2.0 (90% CI, 1.2–3.3). For bladder cancer, in the population subgroup of French Canadians (the majority ethnic group in this region), based on nine cases exposed at the 'substantial' level, the odds ratio was 2.5 (90% CI, 1.2–5.1). [The interpretation of null results has to take into account the small numbers and presumed low levels of exposure.]

## 3. Studies of Cancer in Experimental Animals

Carbon tetrachloride was tested for carcinogenicity in several experiments in mice by oral and intrarectal administration and in rats by oral and subcutaneous administration and by inhalation exposure; it was also tested in one experiment in hamsters and one experiment in trout by oral administration. In various strains of mice, it produced liver tumours, including hepatocellular carcinomas. In various strains of rats, it produced benign and malignant liver tumours; and in one experiment with subcutaneous injection, an increased incidence of mammary adenocarcinomas was observed. In hamsters and trout, increased incidences of liver tumours were observed; however, these studies were considered to be inadequate (IARC 1979).

### 3.1 Oral administration

*Rat*: A group of 20 female Sprague-Dawley rats, weighing 200 ± 20 g, was administered 0.08–1.6 mL/rat carbon tetrachloride [purity unspecified] by gavage once a week for 30 weeks. The initial dose was 0.08 mL/rat for six weeks followed by 1.1 mL/rat for four weeks and then increasing to 1.6 mL/rat. Animals were killed at the end of 30 weeks and the livers were examined histologically. Hepatocellular carcinomas occurred in 6/20 rats (Frezza *et al.*, 1994). [The Working Group noted that no controls were used in this study.]

### 3.2 Inhalation exposure

#### 3.2.1 *Mouse*

Groups of 50 male and 50 female $BDF_1$ (C57BL/6 × DBA/2) mice, six weeks of age, were exposed by whole-body inhalation to 0, 5, 25 or 125 ppm [0, 32, 157 or 787 mg/m$^3$] carbon tetrachloride (purity, > 99%) for 6 h per day on five days a week for 104 weeks. The incidence of hepatocellular adenomas (9/50, 10/50, 27/50 and 16/50 males; 2/50, 8/49, 17/50 and 5/49 females) was significantly increased in mid- and high-dose males and in low-dose and mid-dose females. The incidence of hepatocellular carcinomas (17/50, 12/50, 44/50 and 47/50 males; 2/50, 1/49, 33/50 and 48/49 females) was increased in mid- and high-dose males and females. Incidence of phaeochromocytomas of the adrenal gland (0/50, 0/50, 16/50 and 31/50 males; 0/50, 0/49, 0/50 and 22/49 females) was increased in mid- and high-dose males and in high-dose females (Nagano *et al.*, 1998).

#### 3.2.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were exposed by whole-body inhalation to 0, 5, 25 or 125 ppm [0, 32, 15 or 787 mg/m$^3$] carbon tetrachloride (purity, > 99.8%) for 6 h per day on five days per week for 104 weeks. The incidence of hepatocellular adenomas (0/50, 1/50, 1/50 and 21/50 males; 0/50, 0/50, 0/50 and 40/50 females) and of hepatocellular carcinomas (1/50, 0/50, 0/50 and 32/50 males; 0/50, 0/50, 3/50 and 15/50 females) was significantly increased in high-dose rats of each sex (Nagano *et al.*, 1998).

### 3.3 Multistage protocols and preneoplastic lesions

#### 3.3.1 *Mouse*

Three groups of 30 male and 30 female C57BL/6 mice, six to eight weeks old, received a single-dose irradiation with 0, 170 or 330 rad of fast neutrons. Nine weeks later all mice received a single subcutaneous injection of 3 g/kg bw carbon tetrachloride [purity unspecified] dissolved in corn oil. Animals were observed for lifetime and were necropsied after death. Histological examinations were performed on the livers of all animals and on all other organs or tissues with macroscopic lesions. The incidence of liver carcinomas was increased in high-dose females (330 rad neutrons + carbon tetrachloride, 11/27; 330 rad neutrons + corn oil, 1/17; 330 rad neutrons alone, 2/14) [statistical significance unspecified]. No liver carcinomas were observed in females receiving carbon tetrachloride alone (0/30) (Habs *et al.*, 1983).

Groups of 8–12 female $B6C3F_1$ mice were administered 1.6 g/kg bw carbon tetrachloride [purity unspecified] dissolved in corn oil by gavage once every other week (four or eight times, starting at 4, 18 or 26 weeks of age) after a single dose of 15 mg/kg bw *N*-nitrosodiethylamine (NDEA) given at seven days of age. Gross and histological examinations were performed on the liver of all surviving mice killed at 36 weeks of age. An increased number and volume of the hepatocellular nodules [lesion histology not described] was observed compared with mice administered NDEA alone ($p < 0.01$ by

Scheffe's test). No hepatocellular nodules were observed in mice receiving carbon tetrachloride alone (Dragani *et al*., 1986).

3.3.2 *Rat*

Groups of 12 male Fischer rats, weighing approximately 150 g, were given 200 mg/kg of diet [ppm] 2-acetylaminofluorene for two weeks and received by gavage a single dose of 1.6 g/kg bw carbon tetrachloride dissolved in olive oil at the end of week 1. Subsequently, phenobarbital was added to the diet at a concentration of 500 mg/kg of diet for six weeks and a two-thirds partial hepatectomy was performed at the end of week 3. Animals were killed at the end of week 8. Quantitative analysis of hyperplastic nodules of the liver [lesion histology not described] was carried out. The number and area of hyperplastic nodules per $cm^2$ ($1.44 \pm 1.05$ and $0.77 \pm 0.71$ $mm^2$, respectively) were significantly higher in animals receiving carbon tetrachloride than in animals that did not receive carbon tetrachloride treatment ($0.30 \pm 0.30$ and $0.18 \pm 0.17$ $mm^2$, respectively) (number, $p < 0.01$; area, $p < 0.05$) [statistical method unspecified]. No hyperplastic nodules were observed in the group not given 2-acetylaminofluorene (Takano *et al*., 1980).

A group of 24 male and 21 female inbred ACI rats [age unspecified] was administered 0.5 mL/kg bw carbon tetrachloride [purity unspecified] by gavage followed 24 h later by intraperitoneal injections of 25 mg/kg bw methylazoxymethanol acetate once a week for four weeks and animals were observed until they were killed 30 weeks later. A group of 15 males and 15 females received the methylazoxymethanol acetate treatment alone. Organs [unspecified] were examined histologically. There was no significant difference in the number of animals bearing tumours of the whole intestine (males: carbon tetrachloride + methylazoxymethanol acetate, 18/19; methylazoxymethanol acetate alone, 10/15; females: 13/17 and 10/14, respectively). No intestinal tumours were observed in a group receiving carbon tetrachloride only (0/15 males). However, in males, the multiplicity of tumours in the small intestine (3.4; no. of tumours/no. of tumour-bearing rats) was significantly higher in the carbon tetrachloride + methylazoxymethanol acetate group than that in rats receiving methylazoxymethanol acetate alone (1.4; $p < 0.025$ by *t*-test) (Kazo *et al*., 1985).

A group of 17 male Fischer 344 rats, weighing 160–170 g, received thrice-weekly intraperitoneal injections of 10 mg/kg bw NDEA dissolved in 0.9% saline up to a total dose of 200 mg/kg bw (treatment lasted six weeks). Starting two weeks later, the rats were administered 0.2 mL/kg bw carbon tetrachloride [purity unspecified] dissolved in corn oil by gavage twice a week for three months. All animals were killed eight months after the start of the experiment and a complete necropsy was performed. The incidence of hepatocellular carcinomas in the group receiving NDEA + carbon tetrachloride (17/17) was significantly higher than in a group that received NDEA only (9/17) ($p < 0.005$, by chi-square test). No hepatocellular carcinomas were observed in a group of 15 rats receiving carbon tetrachloride only (Zalatnai *et al*., 1991).

Newborn Sprague-Dawley rats received a single intraperitoneal injection of 15 mg/kg bw NDEA dissolved in 0.1 mL normal saline one day after parturition. From

three weeks of age, female rats received twice-weekly intraperitoneal injections of a 33% solution of carbon tetrachloride [purity unspecified] in 0.25 mL mineral oil for nine weeks. Animals were killed at week 12 and the livers were examined histologically by staining with haematoxylin and eosin and by glutathione *S*-transferase placental form (GST-P) staining. The incidence of foci of cellular alterations and of neoplastic nodules was 15/20 and 13/20, respectively, in the NDEA + carbon tetrachloride group compared with 10/10 and 0/10 in the group not receiving carbon tetrachloride treatment (NDEA group). Most of the nodular lesions were GST-P-positive. The number and area of GST-P-positive neoplastic nodules and/or foci per $cm^2$ were significantly larger in the NDEA + carbon tetrachloride group (7.27 ± 3.18 and 4.34 ± 4.41 $mm^2$, respectively) than in the NDEA group (3.97 ± 1.86 and 0.29 ± 0.16 $mm^2$, respectively ($p < 0.001$, Student's *t*-test) (Cho & Jang, 1993).

3.3.3 *Hamster*

Groups of 11–15 male Syrian hamsters, six weeks of age, were administered carbon tetrachloride by gavage at a dose of 0 or 0.1 mL/animal every two weeks for 30 weeks alone or beginning one week after a single intraperitoneal injection of 6 mg/kg bw NDEA. At the end of the study at 30 weeks, carbon tetrachloride alone produced no liver tumours compared with 1/15 (7%) in hamsters given NDEA and 11/13 (85%) in hamsters given NDEA followed by carbon tetrachloride (Tanaka *et al*., 1987).

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

## 4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The absorption, distribution, metabolism and excretion have previously been reviewed (IARC, 1979; McGregor & Lang, 1996).

Liquid carbon tetrachloride on intact mouse skin was absorbed at a rate of 8.3 µg/$cm^2$/minute (Tsuruta, 1975). Jakobson *et al.* (1982) examined the percutaneous uptake by guinea-pigs of liquid carbon tetrachloride (1 mL in a glass depot, covering 3.1 $cm^2$ of clipped skin). A peak blood level of about 1 mg carbon tetrachloride/L was reached within 1 h. Despite continuation of the exposure, the blood levels declined during the following hours, possibly due to local vasoconstriction, rapid transport from blood to adipose tissues or biotransformation processes. McCollister *et al.* (1951) exposed the clipped skin of one male and one female monkey to [$^{14}$C]carbon tetrachloride vapour (whole body exposure). After exposure to 3056 mg/$m^3$ for 3 h, the blood of the female ained radioactivity equivalent to a carbon tetrachloride level of 12 µg/100 g and the

expired air contained 0.8 µg/L. After exposure to 7230 mg/m$^3$ for 3.5 h, the blood of the male contained a carbon tetrachloride-equivalent level of 30 µg/100 g and the expired air contained 3 µg/L.

Many early studies examining hepatotoxicity of carbon tetrachloride used corn oil as a dosing vehicle for laboratory animals, but corn oil has been found to markedly delay the absorption of carbon tetrachloride from the gastrointestinal tract (Kim *et al.*, 1990). More recent studies have used Emulphor®, a polyethoxylated oil, in concentrations up to 10% in an aqueous vehicle for carbon tetrachloride. Aqueous solutions of carbon tetrachloride in Emulphor® were administered to Sprague-Dawley rats both as a bolus and during gastric infusion at a constant rate over a 2-h period (Sanzgiri *et al.*, 1997). Uptake and tissue levels of carbon tetrachloride after gastric infusion were less than after bolus dosing. When the concentration of Emulphor® was varied up to 10%, absorption (and distribution) of carbon tetrachloride was not affected (Sanzgiri & Bruckner, 1997).

Following inhalation exposure of rats to 406 ppm [2600 mg/m$^3$] carbon tetrachloride for 4 h, the blood level was 10.5 mg/L, but dropped to 50% of this value in less than 30 min (Frantik & Benes, 1984). Carbon tetrachloride, administered by inhalation to rats, mice or monkeys, is distributed to most tissues, including fat, liver, brain, bone marrow and kidney (McCollister *et al.*, 1951; Bergman, 1984; Paustenbach *et al.*, 1986). In mice exposed to [$^{14}$C]carbon tetrachloride, much of the radioactivity became non-volatile and a portion appeared to be non-extractable (Bergman, 1984).

The discrepancy between bolus oral administration of carbon tetrachloride (the route used for most toxicity and mechanistic studies) and inhalation exposure, the route most representative of human exposure, has been addressed by Sanzgiri *et al.* (1995), who studied the kinetics of carbon tetrachloride in rats at doses of (1) 100 and 1000 ppm [630 and 6300 mg/m$^3$] by inhalation for 2 h (equivalent to a systemically administered dose of 17.5 and 179 mg/kg bw), (2) as a gavage bolus emulsion of 17.5 and 179 mg/kg bw and (3) as a gastric infusion emulsion at these dose levels over a period of 2 h. The concentration of carbon tetrachloride in arterial blood were considerably higher in the bolus-administered groups. In the groups administered 17.5 and 179 mg/kg bw, respectively, $C_{max}$ and AUC values were approximately six- and 16-fold higher in the bolus-administered groups than the inhalation-exposed groups. $C_{max}$ and AUC values were slightly lower following gastric infusion than after inhalation, probably due to first-pass metabolism effects. A pharmacokinetic model has been developed for carbon tetrachloride in order to study its interaction with methanol (Evans & Simmons, 1996). The metabolic rate ($V_{max}$) for carbon tetrachloride was 0.11 mg/h, and increased about 4.5-fold 24 h after exposure to methanol (10 000 ppm, 6 h), but < 2-fold 48 h after methanol treatment. The $K_m$ value was 1.3 mg/L.

Known metabolites of carbon tetrachloride include chloroform, carbon monoxide, carbon dioxide, hexachloroethane and phosgene (Poyer *et al.*, 1978; Shah *et al.*, 1979; Ahr *et al.*, 1980; Kubic & Anders, 1980; Nastaincyzyk *et al.*, 1991). Metabolism of carbon tetrachloride is initiated by cytochrome P450-mediated transfer of an electron to the C–Cl

bond, forming an anion radical that eliminates chloride, thus forming the trichloromethyl radical. The isoenzymes implicated in this process are CYP2E1 and CYP2B1/2B2 (Raucy *et al*., 1993; Gruebele *et al*., 1996).

## 4.2 Toxic effects

The toxicity of carbon tetrachloride has been reviewed (Recknagel *et al*., 1989; McGregor & Lang, 1996).

### 4.2.1 *Humans*

Numerous poisonings and fatalities have occurred due to ingestion or inhalation of carbon tetrachloride. The major pathological changes have been seen in the liver and kidney (IARC, 1979). Minor changes in enzyme levels reflecting hepatic effects were observed among workers exposed to carbon tetrachloride levels that were generally below 5 ppm [32 mg/m$^3$] (Tomenson *et al*., 1995). In a case series of carbon tetrachloride-exposed workers, fulminant hepatic damage was observed only in the two individuals who were heavy users of alcoholic beverages, suggesting a synergistic effect between ethanol and carbon tetrachloride (Manno *et al*., 1996).

### 4.2.2 *Experimental systems*

High doses of carbon tetrachloride kill animals within hours by central nervous system depression; smaller doses produce death by liver damage after several days. Repeated administration of carbon tetrachloride induces liver cirrhosis (IARC, 1979). This observation of liver damage was substantiated in a carcinogenicity study comparing responses in different strains of rats (Reuber & Glover, 1970). Severe cirrhosis was observed in all (16/16) Sprague-Dawley rats at 5–16 weeks (the time of death of the animals) and in 13/17 Black rats at 7–18 weeks. In Wistar rats, 6/12 rats developed moderate and 6/12 severe cirrhosis by 17–68 weeks, while the cirrhosis was mild in 2/13, moderate in 7/13 and severe in 4/13 Osborne-Mendel rats at 10–105 weeks; in Japanese rats, the cirrhosis was mild in 9/15, moderate in 5/15 and severe in 1/15 rats at 8–78 weeks. Lipid peroxidation, presumably initiated by a free-radical metabolite of carbon tetrachloride, seems to be the most important factor in carbon tetrachloride-induced liver toxicity. Similar events may be responsible for tissue damage in lung, kidney, testes, adrenals and placenta. Induction and inhibition of drug-metabolizing enzymes alters the hepatotoxicity of carbon tetrachloride (IARC, 1979).

A single oral bolus of carbon tetrachloride (17.5 or 179 mg/kg) to male Sprague-Dawley rats induced a dose-dependent increase in serum sorbitol dehydrogenase and alanine aminotransferase activities, and a decrease in the hepatic cytochrome P-450 content and glucose-6-phosphatase activity. When the same dose was given as a gastric infusion for 2 h, or by inhalation, the effects were much smaller (Sanzgiri *et al*., 1995). In contrast, continuous inhalation exposure (16 ppm [100 mg/m$^3$]) for four weeks was more hepatotoxic to rats than a fluctuating, but similar cumulative exposure (87 ppm [550 mg/m$^3$] 6 h per day, five days per week) (Plummer *et al*., 1990). No significant

difference was observed in the toxicity of carbon tetrachloride administered orally in either corn oil, Emulphor or Tween-85 (Raymond & Plaa, 1997).

Carbon tetrachloride induced hepatic cell proliferation, increasing the frequency of cells in S-phase from < 1% in control animals to about 10% in male and female B6C3$F_1$ mice 48 h after dosing with 100 mg/kg by gavage; in male Fischer 344 rats, a similar increase was observed after a dose of 400 mg/kg (Mirsalis *et al*., 1985) to about 30%. In CD-1 mice, an increase to about 30% was observed 48 h after a single oral dose of 50 mg/kg (Doolittle *et al*., 1987). In male Fischer 344 rats, the frequency of S-phase cells was elevated in one study to 30% 24 h after administration of 0.4 mL/rat, the only dose tested (Cunningham & Matthews, 1991). In male Fischer 344 rats administered 400 mg/kg carbon tetrachloride orally, it was increased to 3% in animals fed *ad libitum* and to 15% in fasting rats (Asakura *et al*., 1994). Twenty-four hours after an intraperitoneal dose of 400 mg/kg carbon tetrachloride to male Fischer 344 rats fed *ad libitum*, an increase to 5% was observed (Mirsalis *et al*., 1985). An even lower response, to approximately 2%, was observed in male Tif:RAIf rats 24 h or 48 h after treatment with 400 mg/kg by gavage (Puri & Müller, 1989). In Sprague-Dawley rats, an increase in DNA synthesis was observed 48 h after an intragastric dose (0.25 mL/100 g [4000 mg/kg bw]) of carbon tetrachloride, and the number of *ras* transcripts was elevated 36–48 h after dosing (Goyette *et al*., 1983). After a single intraperitoneal dose (1.25 mL/kg [2000 mg/kg] bw) of carbon tetrachloride to female Sprague-Dawley rats, sequential transient expression of c-*fos* (peak at 1 h in pericentral hepatocytes and at 1–12 h in mesenchymal cells), c-*jun* (1 h), c-*myc* (3–12 h), c-Ha-*ras* (12–24 h), and c-Ki-*ras* (12–24 h) RNA transcripts was observed; the pattern of proto-oncogene expression spread later to the peripheral parts of the hepatic lobulus (Herbst *et al*., 1991). A rapid transient increase of 8–10-fold in c-*fos* and c-*jun* mRNA (1–2 h after treatment) was also observed in the liver of male Sprague-Dawley rats after a single dose of 160 mg/kg carbon tetrachloride (Zawaski *et al*., 1993). An increase in c-*fos*, c-*jun* and c-*myc* mRNA was also observed in male Wistar rats after a single intragastric dose of carbon tetrachloride (2 mL/kg [3200 mg/kg] bw) (Coni *et al*., 1990, 1993). These authors also concluded that elevations in c-*fos* and c-*myc* RNA are not inevitably linked with liver hyperplasia. Concentrations of *ras* and *myc* proteins were assessed by immunohistochemical techniques in periportal areas of rat liver after a dose of 0.25 mL/100 g [4000 mg/kg] bw carbon tetrachloride; staining throughout the lobule was greatest 96 h after dosing (Richmond *et al*., 1992). The sequence of *fos*, *myc* and Ha-*ras* mRNA expression, followed by hepatocyte proliferation, was observed also in Fischer 344 rats after a single intraperitoneal dose of 2000 mg/kg carbon tetrachloride by gavage (Goldsworthy *et al*., 1994). Injection of a polyclonal antiserum to murine tumour necrosis factor α (TNF-α) 1 h before a challenge with carbon tetrachloride (0.1 mL/kg [0.15 mg/kg bw]) blocked the increase in c-*fos* and c-*jun* mRNA expression, DNA binding of the activator protein-1 (AP-1) nuclear transcription factor and the subsequent increase of S-phase cells, while at the same time delaying liver repair, as shown by the prolonged elevation of serum alanine and aspartate aminotransferases and sorbitol dehydrogenase in female B6C3$F_1$ mice. When recombinant TNF-α was injected into mice, rapid expression

of c-*jun* and c-*fos* proto-oncogene mRNA was observed (Bruccoleri *et al.*, 1997). This result supports the notion, formulated after the demonstration of increased expression of TNF-α after administration of a hepatotoxic dose of carbon tetrachloride, that TNF-α has a role in hepatocellular regeneration after carbon tetrachloride administration (Czaja *et al*., 1989). It has also been demonstrated, however, that injection of a soluble TNF-α receptor preparation to rats had a protective effect against a single, 2.5 mL/kg [4000 mg/kg] bw dose of carbon tetrachloride by reducing serum aminotransferase levels and the extent of histological liver damage, as well as reducing mortality following a single 6000 mg/kg bw dose (Czaja *et al*., 1995)

Like several naturally occurring tumour promoters, carbon tetrachloride (at millimolar concentrations) increased 43 kDa protein phosphorylation by rabbit platelets *in vitro*, and activated protein kinase C in a cell-free system (Roghani *et al*., 1987). Carbon tetrachloride (≥ 15 mg/kg) greatly enhanced hepatic ornithine decarboxylase activity, even at dose levels that also decreased the hepatic total cytochrome P450 concentrations but did not induce elevated serum alanine aminotransferase levels (Kitchin & Brown, 1989). Electrical and dye coupling between hepatocytes *in vitro* was reversibly blocked by carbon tetrachloride (650 μmol/L); this activity was substantially reduced by the cytochrome P450 inhibitor SKF 525-A and by β-mercaptoethanol (Sáez *et al*., 1987). Injection of carbon tetrachloride (1 mL/kg [1600 mg/kg] bw) to male Sprague-Dawley rats caused a transient decrease in hepatic connexin 32 content (Miyashita *et al*., 1991). Repeated administration of carbon tetrachloride (0.5 mL/kg bw injections twice a week for 12 weeks), which led to liver cirrhosis, also decreased the connexin 32 content of the liver in male Sprague-Dawley rats (Nakata *et al*., 1996).

Oral dosage of carbon tetrachloride (2.5 mL/kg [4000 mg/kg] bw) decreased ATP-dependent calcium uptake of liver microsomes within 30 min in Sprague-Dawley rats (Moore *et al*., 1976). The cytosolic calcium concentration increased 100-fold in hepatocytes exposed to carbon tetrachloride (1 mmol/L [1500 μg/mL]), and this was paralleled by inhibition of the endoplasmic reticulum Ca-Mg ATPase (Long & Moore, 1986). The inhibition of the ATPase by carbon tetrachloride exposure has been confirmed (Srivastava *et al*., 1990), and has led to the hypothesis that this is the specific mechanism by which radical intermediates from carbon tetrachloride cause cell death. The calcium-chelating agents, Calcion and alizarin sodium sulfonate, administered 6 or 10 h after a necrogenic intraperitoneal dose of carbon tetrachloride (1 mL/kg [1600 mg/kg] bw), markedly decreased the necrotizing effect of carbon tetrachloride on the liver, and decreased the hepatic calcium concentration, but did not affect carbon tetrachloride-induced lipid peroxidation *in vitro* or lipid accumulation in the liver (de Ferreyra *et al*., 1989, 1992). Carbon tetrachloride (0.01–0.12 mmol/L) induced complete release of calcium from calcium-loaded microsomes in the presence of NADPH; this release was blocked by adding the spin-trapping agent, phenyl-*tert*-butylnitrone (PBN) after a lag period that was dependent on the concentration of carbon tetrachloride. The lag period was shortened in microsomes from pyrazole-treated rats, which showed elevated activity for *para*-nitrophenol oxidation, and was lengthened in the presence of the CYP2E1 inhibitor,

methylpyrazole, or an anti-CYP2E1 antibody. Calcium release was practically complete at concentrations of carbon tetrachloride that had no effect on the Ca-Mg ATPase activity. Ruthenium red, a specific ryanodine receptor inhibitor, completely blocked the carbon tetrachloride-induced calcium release at a concentration (0.02 mmol/L) which had no effect on *para*-nitrophenol hydroxylation or on formation of PBN–carbon tetrachloride adducts (Stoyanovsky & Cederbaum, 1996). These results support the notions that the hepatotoxicity of carbon tetrachloride requires metabolism to the trichloromethyl radical, and that it is mediated by calcium release from intracellular stores, most likely from the ryanodine-sensitive calcium store.

Several studies have demonstrated that ethanol, methanol and other alcohols potentiate the hepatic toxicity of carbon tetrachloride (Traiger & Plaa, 1971; Cantilena *et al*., 1979; Harris & Anders, 1980; Ray & Mehendale, 1990; Simko *et al*., 1992). Dietary ethanol (2 g/80 mL liquid diet for three weeks) potentiated the hepatoxicity of carbon tetrachloride (inhalation exposure to 10 ppm [63 mg/m$^3$] for 8 h), measured by serum aminotransferases and liver malonaldehyde concentrations, in male Wistar rats (Ikatsu *et al*., 1991; Ikatsu & Nakajima, 1992). Only a minor potentiating effect on weight gain, but no potentiating effect on carbon tetrachloride-induced hepatotoxicity was observed, when rats were treated simultaneously with ≤ 0.5 mL/kg ethanol and 20 mg/kg carbon tetrachloride by gavage for 14 days (Berman *et al*., 1992). Micronodular cirrhosis was observed in all treated male black-headed Wistar rats after 10 weeks of inhalation exposure to carbon tetrachloride (80 ppm [500 mg/m$^3$], 6 h per day, 5 days per week) when the animals were simultaneously given ethanol as a part of a liquid diet, whereas no animal treated with either ethanol or carbon tetrachloride alone developed cirrhosis (Hall *et al*., 1991). Similar cirrhosis was observed also in male Porton rats treated with carbon tetrachloride and ethanol (Hall *et al*., 1994). Inhalation exposure to methanol (10 000 ppm for 6 h) increased the hepatotoxicity of carbon tetrachloride (a single gavage dose of 0.075 mL/kg [120 mg/kg] bw after 24 h) (Simmons *et al*., 1995). Similar exposure to methanol also increased the toxicity of inhaled carbon tetrachloride (100, 250 or 1000 ppm [630, 1550, 6300 mg/m$^3$] for 6 h, 26–27 h after the beginning of the methanol exposure). This potentiation subsided when the interval between methanol and carbon tetrachloride exposures was increased by 24 h (Evans & Simmons, 1996). Malonaldehyde generation induced by carbon tetrachloride *in vitro* was enhanced by prior exposure of the rats to methanol (10 000 ppm for 6 h); this enhancement coincided with increased microsomal activity of *para*-nitrophenol hydroxylase, used as a marker of CYP2E1; inhibition of CYP2E1 by allyl sulfone abolished the carbon tetrachloride-induced lipid peroxidation (Allis *et al*., 1996). Malonaldehyde–DNA adducts have been detected in livers of rats and Syrian hamsters treated with carbon tetrachloride (Chaudhary *et al*., 1994; Wang & Liehr, 1995). Imidazole and pyrazole, inducers of CYP2E1, caused 3–25-fold enhanced rates of carbon tetrachloride-induced lipid peroxidation (and chloroform production from carbon tetrachloride); the increase was directly related to the microsomal concentration of CYP2E1 (Johansson & Ingelman-Sundberg, 1985).

Acetone, methyl ethyl ketone (2-butanone) and methyl isobutyl ketone (4-methylpentan-2-one) (6.8 mmol/kg bw for 3 days) increased the hepatotoxicity of carbon tetrachloride to Sprague-Dawley rats (Raymond & Plaa, 1995a); this enhancement of toxicity was coincident with increased microsomal aniline hydroxylase activity (Raymond & Plaa, 1995b). In addition to the effect on cytochrome P450, acetone, but not the other ketones, increased basal canalicular membrane fluidity, as measured by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene or 1-[4-(trimethylammoniumphenyl)-6-phenyl]-1,3,5-hexatriene (Raymond & Plaa, 1996).

Treatment of male athymic nude rats, male and female Sprague-Dawley rats, and male Fischer 344 rats with vitamin A (75 mg/kg per day for seven days) greatly enhanced the hepatotoxicity of carbon tetrachloride (0.2 or 0.1 (Fischer 344 rats) mL/kg [320 or 160 mg/kg] bw intraperitoneally), while it protected BALB/c, C3H/HeJ, athymic nude and Swiss-Webster mice against carbon tetrachloride hepatotoxicity (0.0125, 0.015, 0.015 and 0.02 mL/kg [20, 24, 24 and 32 mg/kg] bw, respectively) (Hooser *et al*., 1994). In male Sprague-Dawley rats, vitamin A (≥ 100 000 IU/kg/day for three weeks or 250 000 IU/kg/day for ≥ 1 week) greatly increased the hepatotoxicity of carbon tetrachloride (0.15 mL/kg [240 mg/kg] intraperitoneally) (ElSisi *et al*., 1993c). There was a simultaneous six- to eight-fold increase in the amount of exhaled ethane and a less than two-fold increase in covalent binding to liver proteins in rats treated with vitamin A (250 000 IU [75 mg]/kg/day for one week) and [$^{14}$C]carbon tetrachloride (0.15 mL/kg [240 mg/kg bw]) in comparison with rats treated with carbon tetrachloride alone, but no increase in exhaled $^{14}CO_2$, exhaled organics or metabolites excreted in the urine, or in covalent binding to hepatic lipids (ElSisi *et al.*, 1993a). Aminobenzotriazole (50 mg/kg intraperitoneally, 2 h before carbon tetrachloride), an inhibitor of cytochrome P450, blocked the vitamin A-induced potentiation of the hepatotoxicity of carbon tetrachloride (ElSisi *et al*., 1993b). A single dose of vitamin A (75 mg/kg orally) 24 h before carbon tetrachloride also very significantly potentiated carbon tetrachloride hepatotoxicity. While the total cytochrome P450 content of the liver was not affected by retinol treatment, the concentration (Western blot analysis) and activity (aniline hydroxylase) of CYP2E1 were both elevated. Isolated hepatocytes from retinol-treated rats were more susceptible to carbon tetrachloride (Badger *et al*., 1996).

An intravenous injection of gadolinium chloride (10 mg/kg) 24 h before an intragastric dose of carbon tetrachloride (4000 mg/kg) nearly completely protected rats against hepatic necrosis, as measured by serum aspartate aminotransferase levels and trypan blue exclusion, without having any effect on CYP2E1 (Edwards *et al*., 1993). This was interpreted to indicate a role of Kupffer cells in carbon tetrachloride-induced hepatic damage, since gadolinium chloride at this concentration strongly inhibits Kupffer cell phagocytosis (Husztik *et al*., 1980). A similar dose of gadolinium chloride was, however, reported to decrease the total amount of hepatic cytochrome P450 in rats, as well as the activity of aniline *para*-hydroxylase (Badger *et al*., 1997). In support of the role of Kupffer cells in carbon tetrachloride-induced hepatic damage, it was reported that gadolinium chloride (10 mg/kg intravenously 24 h before carbon tetrachloride administration) prevented and

methyl palmitate (another Kupffer cell inhibitor) attenuated the periportal oedema observed using proton magnetic imaging 1–2 h after carbon tetrachloride administration (0.8 mL/kg [1280 mg/kg] intraperitoneally) (Towner *et al*., 1994). In-vivo spin trapping using PBN and subsequent electron paramagnetic resonance study of the liver indicated that gadolinium chloride did not affect the generation of trichloromethyl radical from carbon tetrachloride (Towner *et al*., 1994). Gadolinium chloride (10 mg/kg intravenously), methyl palmitate, polyethylene glycol-coupled superoxide dismutase and polyethylene glycol-coupled catalase protected Sprague-Dawley rats against vitamin A-induced potentiation of carbon tetrachloride hepatotoxicity, both after a single oral dose and after daily oral dosing for seven days with 75 mg/kg bw retinol (ElSisi *et al*., 1993a; Sauer & Sipes, 1995; Badger *et al*., 1996). Dietary α-tocopherol (250 mg/kg diet) partly protected male Wistar rats against hepatic damage induced by carbon tetrachloride (0.15 mL [240 mg] injected intraperitoneally three times per week for five weeks) (Parola *et al*., 1992). A single intraperitoneal dose of α-tocopheryl hemisuccinate (0.19 mmol, about 100 mg/kg) gave partial protection against the hepatotoxicity of carbon tetrachloride (1.0 g/kg bw by gavage) administered 18 h later (Tirmenstein *et al*., 1997). However, a much more pronounced protection, apparent as a decrease in mortality, less pronounced histological damage, and lower serum aminotransferase levels, resulted from intravenous administration of α-tocopherol as a suspension or in liposomes, which are accumulated in Kupffer cells (Yao *et al*., 1994; Liu *et al*., 1995). If incorporated into liposomes, other antioxidants, such as butylated hydroxytoluene and ascorbic acid palmitate, also protected mice against carbon tetrachloride toxicity (Yao *et al*., 1994).

Carbon tetrachloride (intraperitoneally, daily for seven days) affected both humoral and cell-mediated immune responses in female $B6C3F_1$ mice; the most sensitive parameters were the T-cell-dependent antibody-forming cell response to sheep red blood cells (effect observed at ≥ 500 mg/kg), mixed lymphocyte response (≥ 1000 mg/kg) and the proliferative response to concanavalin A and lipopolysaccharide (≥ 1000 mg/kg) (Kaminski *et al*., 1989). The effects were prevented by treatment of the animals with aminoacetonitrile, a competitive inhibitor of cytochrome P450, but enhanced by treatment with ethanol, an inducer of CYP2E1 (Kaminski *et al*., 1990). Incubation of serum from carbon tetrachloride-treated mice with neutralizing monoclonal antibodies towards transforming growth factor (TGF) β1 reversed the immunosuppression, indicating that TGF β1 at least in part mediates the immunosuppression induced by carbon tetrachloride (Delaney *et al*., 1994).

### 4.3 Reproductive and developmental effects

#### 4.3.1 *Humans*

No data were available to the Working Group.

#### 4.3.2 *Experimental systems*

Carbon tetrachloride increased fetal mortality in mice after a single intraperitoneal or subcutaneous dose of 150 mg/kg late in gestation (IARC, 1979).

### 4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 2 for references)

Carbon tetrachloride was not mutagenic in bacteria. It induced intra-chromosomal and mitotic recombination but not aneuploidy in *Saccharomyces cerevisiae*; aneuploidy was detected in another single study in *Aspergillus nidulans*. *In vivo*, in a single study with *Drosophila melanogaster*, no sex-linked recessive mutations were observed.

In mammalian in-vitro systems, in single studies, carbon tetrachloride induced cell transformation in Syrian hamster cells and kinetochore-positive micronuclei (which are indicative of aneuploidy) and kinetochore-negative micronuclei in human MCL-5 cells that stably express cDNAs encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1 and epoxide hydrolase and in h2E1 cells, which contain a cDNA for CYP2E1. AHH-1 cells constitutively expressing CYP1A1 showed neither an increase in total micronucleus frequencies nor kinetochore-staining micronuclei.

Neither sister chromatid exchanges nor chromosomal aberrations were induced in cultured human lymphocytes.

*In vivo* in rat hepatocytes, unscheduled DNA synthesis was not induced, and no DNA repair intermediate products were found after exposure to carbon tetrachloride; neither micronuclei nor polyploidy were induced in a single study with the same experimental system. Carbon tetrachloride did not induce micronuclei in mouse bone-marrow cells or peripheral erythrocytes.

*In vitro*, carbon tetrachloride binds covalently to DNA. Inhibition of intercellular communication was observed *in vivo* in rats and induction of TNF-α expression *in vivo* in mice.

## 5. Summary of Data Reported and Evaluation

### 5.1 Exposure data

Exposure to carbon tetrachloride may occur in its production, in the production of refrigerants, in laboratories and during degreasing operations. It has been detected at low levels in ambient air and water.

### 5.2 Human carcinogenicity data

The risk of cancer from carbon tetrachloride has been examined in five occupational populations. In three of four studies that collected information on non-Hodgkin lymphoma (two cohort investigations and one independent nested case–control study), associations with exposure to carbon tetrachloride were suggested. However, not all of these studies distinguished exposure to carbon tetrachloride specifically, and the associations were not strong statistically. In the fourth study (another cohort investigation), few men were exposed to carbon tetrachloride and the risk of non-Hodgkin lymphoma was not reported.

**Table 2. Genetic and related effects of carbon tetrachloride**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| PRB, SOS response, *Salmonella typhimurium* TA1535/pSK1002, *umu* test | – | NT | 5300 | Nakamura *et al*. (1987) |
| SAF, *Salmonella typhimurium* BA13, Ara forward mutation | ? | – | 190 | Roldán-Arjona & Pueyo (1993) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 5000 | McCann *et al*. (1975) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 1400 | Barber *et al*. (1981) |
| SA5, *Salmonella typhimurium* TA1535, reverse mutation | – | – | 5000 | McCann *et al*. (1975) |
| SA5, *Salmonella typhimurium* TA1535, reverse mutation | – | – | 1400 | Barber *et al*. (1981) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 1400 | Barber *et al*. (1981) |
| ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation | NT | (+) | 160 | Norpoth *et al*. (1980) |
| SCG, *Saccharomyces cerevisiae* D7, gene conversion | + | NT | 5200 | Callen *et al*. (1980) |
| SCH, *Saccharomyces cerevisiae* D7, homozygosis | + | NT | 5200 | Callen *et al*. (1980) |
| SCH, *Saccharomyces cerevisiae* RS112, intra-chromosomal recombination | + | NT | 4000 | Schiestl *et al*. (1989) |
| SCH, *Saccharomyces cerevisiae* AGY3, intra-chromosomal recombination | + | NT | 2000 | Galli & Schiestl (1996) |
| ANG, *Aspergillus nidulans*, crossing-over | (+) | NT | 8000 | Gualandi (1984) |
| SCR, *Saccharomyces cerevisiae*, reverse mutation | + | NT | 5200 | Callen *et al*. (1980) |
| ANF, *Aspergillus nidulans*, forward mutation | (+) | NT | 8000 | Gualandi (1984) |
| SCN, *Saccharomyces cerevisiae* D61-M, aneuploidy | – | NT | 5000 | Whittaker *et al*. (1989) |
| ANN, *Aspergillus nidulans*, aneuploidy | + | NT | 0.02% (v:v) | Benigni *et al*. (1993) |
| DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations | – | | 25000 ppm feed | Foureman *et al*. (1994) |
| DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations | – | | 2000 ppm inj | Foureman *et al*. (1994) |
| DIA, DNA strand breaks/cross-links, rat hepatocytes *in vitro* | (+) | NT | 462 | Sina *et al*. (1983) |

**Table 2 (contd)**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| SIR, Sister chromatid exchange, rat epithelial-type RL1 cells *in vitro* | – | NT | 0.02 | Dean & Hodson-Walker (1979) |
| CIR, Chromosomal aberrations, rat epithelial-type RL1 cells *in vitro* | – | NT | 0.02 | Dean & Hodson-Walker (1979) |
| AIA, Aneuploidy, Chinese hamster ovary CHO cells *in vitro* | + | NT | 8000 | Coutino (1979) |
| TCS, Cell transformation, Syrian hamster embryo cells, clonal assay | + | NT | 3 | Amacher & Zelljadt (1983) |
| SHL, Sister chromatid exchange, human lymphocytes *in vitro* | – | – | 48 | Garry *et al.* (1990) |
| MIH, Micronucleus test, AHH-1 (CYP1A1 native) *in vitro* | – | NT | 1540 | Doherty *et al*. (1996) |
| MIH, Micronucleus test, MCL-5 (cDNAs for CYP1A2, 2A6, 3A4, 2E1 and epoxide hydrolase) *in vitro* | +[c] | NT | 770 | Doherty *et al*. (1996) |
| MIH, Micronucleus test, h2E1 (cDNA for CYP2E1) *in vitro* | +[c] | NT | 308 | Doherty *et al*. (1996) |
| CHL, Chromosomal aberrations, human lymphocytes *in vitro* | – | – | 38 | Garry *et al.* (1990) |
| DVA, DNA strand breaks/cross-links, NMRI mouse liver *in vivo* | – | | 4000 po × 1 | Schwarz *et al*. (1979) |
| DVA, DNA strand breaks/cross-links, Fischer 344 rat liver *in vivo* | – | | 400 po × 1 | Bermudez *et al*. (1982) |
| DVA, DNA strand breaks/cross-links, BD-VI rat liver *in vivo* | – | | 4000 ip × 1 | Barbin *et al*. (1983) |
| DVA, DNA strand breaks/cross-links, Sprague-Dawley rat liver *in vivo* | – | | 200 ip × 1 | Brambilla *et al*. (1983) |
| RVA, DNA repair intermediates, Wistar rat hepatocytes *in vivo* | – | | 800 ip × 1 | Stewart (1981) |
| UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo* | – | | 100 po × 1 | Mirsalis & Butterworth (1980) |
| UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo* | – | | 400 po × 1 | Bermudez *et al*. (1982) |
| MVM, Micronucleus test, $BDF_1$ mouse bone marrow *in vivo* | – | | 2000 po × 1 | Suzuki *et al.* (1997) |
| MVM, Micronucleus test, $BDF_1$ mouse peripheral erythrocytes *in vivo* | – | | 3000 ip × 1 | Suzuki *et al.* (1997) |

**Table 2 (contd)**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| MVM, Micronucleus test, CBA × C57BL/6 mouse hepatocytes *in vivo* | – | | 0.05–0.1 mL/5L inh | Uryvaeva & Delone (1995) |
| CBA, Chromosomal aberrations, 101/H and C57BL/6 mouse bone marrow *in vivo* | – | | 8000 im × 1 | Lil'p (1983) |
| AVA, Aneuploidy, CBA × C57BL/6 mouse hepatocyte polyploidy *in vivo* | – | | 0.05–0.1 mL/5L inh | Uryvaeva & Delone (1995) |
| BVD, Binding (covalent) to DNA, A/J mouse liver *in vivo* | + | | 1.4 ip × 1 | Diaz Gomez & Castro (1980) |
| BVD, Binding (covalent) to DNA, Sprague-Dawley rat liver *in vivo* | + | | 1.4 ip × 1 | Diaz Gomez & Castro (1980) |
| BVD, Binding (covalent) to DNA, Syrian hamster liver *in vivo* | + | | 1200 ip × 1 | Castro *et al*. (1989) |
| BVD, Binding (covalent) to DNA, C3H mouse liver *in vivo* | + | | 1200 ip × 1 | Castro *et al*. (1989) |
| BVD, Binding (covalent) to DNA, Sprague-Dawley rat liver *in vivo* | + | | 1200 ip × 1 | Castro *et al*. (1989) |
| Decreased connexin 32 expression, Sprague-Dawley rat liver *in vivo* | + | | 800 ip × 24 | Nakata *et al*. (1996) |
| Induction of TNF-α expression, $B6C3F_1$ mouse liver *in vivo* | + | | 160 ip × 1 | Bruccoleri *et al*. (1997) |

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; po, oral; ip, intraperitoneal; im, intramuscular

[c] Greater number of kinetochore-positive micronuclei than kinetochore-negative micronuclei

A nested case–control study of lung cancer in a cohort of chemical workers showed no association with exposure to carbon tetrachloride.

Four population-based case–control studies have examined associations of carbon tetrachloride with chronic lymphocytic leukaemia, brain cancer, female breast cancer and intraocular melanoma. Findings were generally unremarkable. In a fifth case–control study, which examined several cancers, no association was found with non-Hodgkin lymphoma, although the power to detect an increased risk was low.

### 5.3 Animal carcinogenicity data

Carbon tetrachloride was tested for carcinogenicity by various routes of administration. It produced liver neoplasms in mice and rats and mammary neoplasms in rats following subcutaneous injection. In one study in mice by inhalation, an increased incidence of phaeochromocytomas was reported. In experiments involving administration of carbon tetrachloride after known carcinogens, the occurrence of tumours and/or preneoplastic lesions of the liver in mice, rats and hamsters was enhanced.

### 5.4 Other relevant data

Carbon tetrachloride is metabolized by CYP2 enzymes; several reactive metabolites have been postulated, including radicals and phosgene. *In vitro*, DNA binding of carbon tetrachloride is observed in several cellular systems; no such binding *in vivo* has been reported.

Carbon tetrachloride induces hepatic cell proliferation and DNA synthesis.

Carbon tetrachloride has a mutagenic effect and induces aneuploidy in several in-vitro systems.

### 5.5 Evaluation

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

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

### Overall evaluation

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

## 6. References

Ahr, H.J., King, L.J., Nastainczyk, W. & Ullrich, V. (1980) The mechanism of chloroform and carbon monoxide formation from carbon tetrachloride by microsomal cytochrome P-450. *Biochem. Pharmacol.*, **29**, 2855–2861

Allis, J.W., Brown, B.L., Simmons, J.E., Hatch, G.E., McDonald, A. & House, D.E. (1996) Methanol potentiation of carbon tetrachloride hepatotoxicity: the central role of cytochrome P450. *Toxicology*, **112**, 131–140

Amacher, D.E. & Zelljadt, I. (1983) The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to *Salmonella typhimurium*. *Carcinogenesis*, **4**, 291–295

American Conference of Governmental Industrial Hygienists (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th Ed., Vol. 1, Cincinnati, OH, pp. 233–236

American Conference of Governmental Industrial Hygienists (1997) *1997 TLVs® and BEIs®*, Cincinnati, OH, p. 18

Asakura, S., Sawada, S., Daimon, H., Fukuda, T., Ogura, K., Yamatsu, K. & Furihata, C. (1994) Effects of dietary restriction on induction of unscheduled DNA synthesis (UDS) and replicative synthesis (RDS) in rat liver. *Mutat. Res.*, **322**, 257–264

Badger, D.A., Sauer, J.-M., Hoglen, N.C., Jolley, C.S. & Sipes, I.G. (1996) The role of inflammatory cells and cytochrome P450 in the potentiation of $CCl_4$-induced injury by a single dose of retinol. *Toxicol. appl. Pharmacol.*, **141**, 507–519

Badger, D.A., Kuester, R.K., Sauer, J.M. & Sipes, I.G. (1997) Gadolinium chloride reduces cytochrome P450: relevance to chemical-induced hepatotoxicity. *Toxicology*, **121**, 143–153

Barber, E.D., Donish, W.H. & Mueller, K.R. (1981) A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames *Salmonella*/microsome assay. *Mutat. Res.*, **90**, 31–48

Barbin, A., Bereziat, J.C. & Bartsch, H. (1983) Evaluation of DNA damage by the alkaline elution technique in liver, kidneys and lungs of rats and hamsters treated with *N*-nitrosodialkylamines. *Carcinogenesis*, **4**, 541–545

Benigni, R., Andreoli, C., Conti, L., Tafani, P., Cotta-Ramusino, M., Carere, A. & Crebelli, R. (1993) Quantitative structure–activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in *Aspergillus nidulans*. *Mutagenesis*, **8**, 301–305

Bergman, K. (1984) Application and results of whole-body radiography in distribution studies of organic solvlents. *Crit. Rev. Toxicol.*, **12**, 59–119

Berman, E., House, D.E., Allis, J.W. & Simmons, J.E. (1992) Hepatotoxic interactions of ethanol with allyl alcohol or carbon tetrachloride in rats. *J. Toxicol. environ. Health*, **37**, 161–176

Bermudez, E., Mirsalis, J.C. & Eales, H.C. (1982) Detection of DNA damage in primary cultures of rat hepatocytes following *in vivo* and *in vitro* exposure to genotoxic agents. *Environ. Mutag.*, **4**, 667–679

Blair, A., Stewart, P.A., Tolbert, P.E., Grauman, D., Moran, F.X., Vaught, J. & Rayner, J. (1990) Cancer and other causes of death among a cohort of dry cleaners. *Br. J. ind. Med.*, **47**, 162–168

Blair, A., Hartge, P., Stewart, P.A., McAdams, M. & Lubin, J. (1998) Mortality and cancer incidence of workers at an aircraft maintenance facility exposed to trichloroethylene and other organic solvents and chemicals: Extended follow-up. *Occup. environ. Med.*, **55**, 161–171

Bond, G.G., Shellenberger, R.J., Fishbeck, W.A., Cartmill, J.B., Lasich, B.J., Wymer, S.T. & Cook, R.R. (1985) Mortality among a large cohort of chemical manufacturing employees. *J. natl Cancer Inst.*, **75**, 859–869

Bond, G.G., Flores, G.H., Shellenberger, R.J., Cartmill, J.B., Fishbeck, W.A. & Cook, R.R. (1986) Nested case–control study of lung cancer among chemical workers. *Am. J. Epidemiol.*, **124**, 53–66

Brambilla, G., Carlo, P., Finollo, R., Bignone, F.A., Ledda, A. & Cajelli, E. (1983) Viscometric detection of liver DNA fragmentation in rats treated with minimal doses of chemical carcinogens. *Cancer Res.*, **43**, 202–209

Bruccoleri, A., Gallucci, R., Germolec, D.R., Blackshear, P., Simeonova, P., Thurman, R.G. & Luster, M.I. (1997) Induction of early-immediate genes by tumor necrosis factor α contribute to liver repair following chemical-induced hepatotoxicity. *Hepatology*, **25**, 133–141

Budavari, S., ed. (1996) *The Merck Index*, 12th Ed., Whitehouse Station, NJ, Merck & Co., p. 297

Callen, D.F., Wolf, C.R. & Philpot, R.M. (1980) Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in *Saccharomyces cerevisiae*. *Mutat. Res.*, **77**, 55–63

Cantilena, L.R., Cagen, S.Z. & Klaassen, C.D. (1979) Methanol potentiation of carbon tetrachloride-induced hepatotoxicity. *Proc. Soc. exp. Biol. Med.*, **162**, 90–95

Cantor, K.P., Stewart, P.A., Brinton, L.A. & Dosemeci, M. (1995) Occupational exposures and female breast cancer mortality in the United States. *J. occup. environ. Med.*, **37**, 336–348

Castro, G.D., Díaz Gómez, M.I. & Castro, J.A. (1989) Species differences in the interaction between $CCl_4$ reactive metabolites and liver DNA or nuclear protein fractions. *Carcinogenesis*, **10**, 289–294

Chaudhary, A.K., Nokubo, M., Reddy, G.R., Yeola, S.N., Morrow, J.D., Blair, I.A. & Marnett, L.J. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. *Science*, **265**, 1580–1582

Checkoway, H., Wilcosky, T., Wolf, P. & Tyroler, H. (1984) An evaluation of the associations of leukemia and rubber industry solvent exposures. *Am. J. ind. Med.*, **5**, 239–249

Chemical Information Services (1995) *Directory of World Chemical Producers 1995/96 Edition*, Dallas, TX

Cho, K.-J. & Jang, J.-J. (1993) Effects of carbon tetrachloride, ethanol and acetaldehyde on diethylnitrosamine-induced hepatocarcinogenesis in rats. *Cancer Lett.*, **70**, 33–39

Coni, P., Pichiri-Coni, G., Ledda-Columbano, G.M., Rao, P.M., Rajalakshmi, S., Sarma, D.S.R. & Columbano, A. (1990) Liver hyperplasia is not necessarily associated with increased expression of c-*fos* and c-*myc* RNA. *Carcinogenesis*, **11**, 835–839

Coni, P., Simbula, G., de Prati, A.C., Menegazzi, M., Suzuki, H., Sarma, D.S.R., Ledda-Columbano, G.M. & Columbano, A. (1993) Differences in the steady-state levels of c-*fos*, c-*jun* and c-*myc* messenger RNA during mitogen-induced liver growth and compensatory regeneration. *Hepatology*, **17**, 1109–1116

Coutino, R.R. (1979) Analysis of anaphase in cell culture: an adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus. *Environ. Health Perspect.*, **31**, 131–136

Cunningham, M.L. & Matthews, H.B. (1991) Relationship of hepatocarcinogenicity and hepatocellular proliferation induced by mutagenic noncarcinogens vs carcinogens. II. 1- vs 2-nitropropane. *Toxicol. appl. Pharmacol.*, **110**, 505–513

Czaja, M.J., Flanders, K.C., Biempica, I., Klein, C., Zern, M.A. & Weiner, F.R. (1989) Expression of tumor necrosis factor-α and transforming factor β1 in acute liver injury. *Growth Factors*, **1**, 219–226

Czaja, M.J., Xu, J. & Alt, E. (1995) Prevention of carbon tetrachloride-induced rat liver injury by soluble tumor necrosis factor receptor. *Gastroenterology*, **108**, 1849–1854

Dean, B.J. & Hodson-Walker, G. (1979) An in vitro chromosome assay using cultured rat-liver cells. *Mutat. Res.*, **64**, 329–337

Delaney, B., Strom, S.C., Collins, S. & Kaminski, N.E. (1994) Carbon tetrachloride suppresses T-cell-dependent immune responses by induction of transforming growth factor-β1. *Toxicol. appl. Pharmacol.*, **126**, 98–107

Diaz Gomez, M.I. & Castro, J.A. (1980) Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids. *Toxicol. appl. Pharmacol.*, **56**, 199–206

Doherty, A.T., Ellard, S., Parry, E.M. & Parry, J.M. (1996) An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells. *Mutagenesis*, **11**, 247–274

Doolittle, D.J., Muller, G. & Scribner, H.E. (1987) Relationship between hepatotoxicity and induction of replicative DNA synthesis following single or multiple doses of carbon tetrachloride. *J. Toxicol. environ. Health*, **22**, 63–78

Dragani, T.A., Manenti, G. & Della Porta, G. (1986) Enhancing effects of carbon tetrachloride in mouse hepatocarcinogenesis. *Cancer Lett.*, **31**, 171–179

Edwards, M.J., Keller, B.J., Kauffman, F.C. & Thurman, R.G. (1993) The involvement of Kupffer cells in carbon tetrachloride toxicity. *Toxicol. appl. Pharmacol.*, **119**, 275–279

ElSisi, A.E., Earnest, D.L. & Sipes, I.G. (1993a) Vitamin A potentiation of carbon tetrachloride hepatotoxicity: enhanced lipid peroxidation without enhanced biotransformation. *Toxicol. appl. Pharmacol.*, **119**, 289–294

ElSisi, A.E., Earnest, D.L. & Sipes, I.G. (1993b) Vitamin A potentiation of carbon tetrachloride hepatotoxicity: role of liver macrophages and active oxygen species. *Toxicol. appl. Pharmacol.*, **119**, 295–301

ElSisi, A.E., Hall, P., Sim, W.-L. W., Earnest, D.L. & Sipes, I.G. (1993c) Characterization of vitamin A potentiation of carbon tetrachloride-induced liver injury. *Toxicol. appl. Pharmacol.*, **119**, 280–288

Evans, M.V. & Simmons, J.E. (1996) Physiologically based pharmacokinetic estimated metabolic constants and hepatotoxicity of carbon tetrachloride after methanol pretreatment in rats. *Toxicol. appl. Pharmacol.*, **140**, 245–253

de Ferreyra, E.C., Villarruel, M.C., Bernacchi, A.S., Fernández, G., de Fenos, O.M. & Castro, J.A. (1989) Late preventive effects against carbon tetrachloride-induced liver necrosis of the calcium chelating agent Calcion. *Arch. Toxicol.*, **63**, 450–455

de Ferreyra, E.C., Villarruel, M.C., Bernacchi, A.S., de Fenos, O.M. & Castro, J.A. (1992) Prevention of carbon tetrachloride-induced liver necrosis by the chelator alizarin sodium sulfonate. *Exp. mol. Pathol.*, **56**, 197–207

Foureman, P., Mason, J.M., Valencia, R. & Zimmering, S. (1994) Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. *Environ. mol. Mutag.*, **23**, 208–227

Frantik, E. & Benes, V. (1984) Central nervous effect and blood level regressions on exposure time paralleled in solvents (toluene, carbon tetrachloride and chloroform). *Act. nerv. super.* (Praha), **26**, 131–133

Frezza, E.E., Gerunda, G.E., Farinati, F., DeMaria, N., Galligioni, A., Plebani, F., Giacomin, A & Van Thiel, D.H. (1994) $CCl_4$-induced liver cirrhosis and hepatocellular carcinoma in rats: relation to plasma zinc, copper and estradiol levels. *Hepato-Gastroenterology*, **41**, 367–369

Galli, A. & Schiestl, R.H. (1995) *Salmonella* test positive and negative carcinogens show different effects on intrachromosomal recombination in $G_2$ cell cycle arrested yeast cells. *Carcinogenesis*, **16**, 659–663

Galli, A. & Schiestl, R.H. (1996) Effects of *Salmonella* assay negative and positive carcinogens on intrachromosomal recombination in $G_1$-arrested yeast cells. *Mutat. Res.*, **370**, 209–221

Garry, V.F., Nelson, R.L., Griffith, J. & Harkins, M. (1990) Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants. *Teratog. Carcinog. Mutag.*, **10**, 21–29

Goldsworthy, T.L., Goldsworthy, S.M., Sprankle, C.S. & Butterworth, B.E. (1994) Expression of *myc*, *fos* and Ha-*ras* associated with chemically induced cell proliferation in the rat liver. *Cell Prolif.*, **27**, 269–278

Gomez, M.R., Cocco, P., Dosemeci, M. & Stewart, P.A. (1994) Occupational exposure to chlorinated aliphatic hydrocarbons: job exposure matrix. *Am. J. ind. Med.*, **26**, 171–183

Goyette, M., Petropulos, C.J., Shank, P.R. & Fausto, N. (1983) Expression of cellular oncogene during liver regeneration. *Science*, **219**, 510–512

Gruebele, A., Zawaski, K., Kaplan, D. & Novak, R.F. (1996) Cytochrome P4502E1- and cytochrome P4502B1/2B2-catalysed carbon tetrachloride metabolism. *Drug Metab. Dispos.*, **24**, 15–22

Gualandi, G. (1984) Genotoxicity of the free-radical producers $CCl_4$ and lipoperoxide in *Aspergillus nidulans*. *Mutat. Res.*, **136**, 109–114

Habs, H., Künstler, K., Schmähl, D. & Tomatis, L. (1983) Combined effects of fast neutron irradiation and subcutaneously applied carbon tetrachloride or chloroform in C57Bl/6 mice. *Cancer Lett.*, **20**, 13–20

Hall, P. de la M., Plummer, J.L., Ilsley, A.H. & Cousins, M.J. (1991) Hepatic fibrosis and cirrhosis after chronic administration of alcohol and 'low-dose' carbon tetrachloride vapor in the rat. *Hepatology*, **13**, 815–819

Hall, P. de la M., Plummer, J.L., Ilsley, A.H., Ahern, M.J., Cmielewski, P.L. & Williams, R.A. (1994) The pathology of liver injury induced by the chronic administration of alcohol and 'low-dose' carbon tetrachloride in Porton rats. *J. Gastroenterol. Hepatol.*, **9**, 250–256

Harris, R.N. & Anders, M.W. (1980) Effect of fasting, diethyl maleate, and alcohols on carbon tetrachloride-induced hepatotoxicity. *Toxicol. appl. Pharmacol.*, **56**, 191–198

Heineman, E.F., Cocco, P., Gómez, M.R., Dosemeci, M., Stewart, P.A. Hayes, R.B., Zahm, S.H., Thomas, T.L. & Blair, A. (1994) Occupational exposure to chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. *Am. J. ind. Med*., **25**, 155–169

Herbst, H., Milani, S., Schuppan, D. & Stein, H. (1991) Temporal and spatial patterns of proto-oncogene expression at early stages of toxic liver injury in the rat. *Lab. Invest.*, **65**, 324–333

Hoar, S.K., Morrison, A.S., Cole, P. & Silverman, D.T. (1980) An occupation and exposure linkage system for the study of occupational carcinogenesis. *J. occup. Med*., **22**, 722–726

Holly, E.A., Aston, D.A., Ahn, D.K. & Smith, A.H. (1996) Intraocular melanoma linked to occupations and chemical exposures. *Epidemiology*, **7**, 55–61

Hooser, S.B., Rosengren, R.J., Hill, D.A., Mobley, S.A. & Sipes, I.G. (1994) Vitamin A modulation of xenobiotic-induced hepatotoxicity in rodents. *Environ. Health Perspect.*, **102** (Suppl. 9), 39–43

Husztik, E., Lázár, G. & Párducz, A. (1980) Electron microscopic study of Kupffer-cell phagocytosis blockade induced by gadolinium chloride. *Br. J. exp. Pathol.*, **61**, 624–630

IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 20, *Some Halogenated Hydrocarbons*, Lyon, pp. 371–399

IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 143–144

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 152–154

IARC (1994a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 60, *Some Industrial Chemicals*, Lyon, pp. 45–71

IARC (1994b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 60, *Some Industrial Chemicals*, Lyon, pp. 233–320

IARC (1995a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 63, *Dry Cleaning, Some Chlorinated Solvents and Other Industrial Solvents*, Lyon, pp. 159–221

IARC (1995a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 63, *Dry Cleaning, Some Chlorinated Solvents and Other Industrial Solvents*, Lyon, pp. 332–334

Ikatsu, H. & Nakajima, T. (1992) Hepatotoxic interaction between carbon tetrachloride and chloroform in ethanol treated rats. *Arch. Toxicol.*, **66**, 580–586

Ikatsu, H., Okino, T. & Nakajima, T. (1991) Ethanol and food deprivation induced enhancement of hepatotoxicity in rats given carbon tetrachloride at low concentration. *Br. J. ind. Med.*, **48**, 636–642

International Labour Office (1991) *Occupational Exposure Limits for Airborne Toxic Substances*, 3rd Ed. (Occupational Safety and Health Series No. 37), Geneva, pp. 76–77

Jakobson, I., Wahlberg, J.E., Holmberg, B. & Johansson, G. (1982) Uptake via the blood and elimination of 10 organic solvents following epicutaneous exposure of anesthetized guinea pigs. *Toxicol. appl. Pharmacol.*, **63**, 181–187

Johansson, I. & Ingelman-Sundberg, M. (1985) Carbon tetrachloride-induced lipid peroxidation dependent on an ethanol-inducible form of rabbit liver microsomal cytochrome P-450. *FEBS Lett.*, **183**, 265–269

Kaminski, N.E., Jordan, S.D. & Holsappe, M.P. (1989) Suppression of humoral and cell-mediated immuno responses by carbon tetrachloride. *Fundam. appl. Toxicol.*, **12**, 117–128

Kaminski, N.E., Barnes, D.W., Jordan, S.D. & Holsappe, M.P. (1990) The role of metabolism in carbon tetrachloride-mediated immunosuppression: *in vivo* studies. *Toxicol. appl. Pharmacol.*, **102**, 9–20

Kauppinen, T., Toikkanen, J., Pedersen, D., Young, R., Kogevinas, M., Ahrens, W., Boffetta, P., Hansen, J., Kromhout, H., Maqueda Blasco, J., Mirabelli, D., de la Orden-Rivera, V., Plato, N., Pannett, B., Savela, A., Veulemans, H. & Vincent, R. (1998) *Occupational Exposure to Carcinogens in the European Union in 1990–93*, Carex (International Information System on Occupational Exposure to Carcinogens), Helsinki, Finnish Institute of Occupational Health

Kazo, K., Kawai, T., Fujii, M., Bunai, Y., Shima, H. & Takahashi, M. (1985) Enhancing effect of preadministration of carbon tetrachloride on methylazoxymethanol acetate-induced intestinal carcinogenesis. *J. toxicol. Sci.*, **10**, 289–293

Kim, H.J., Bruckner, J.V., Dallas, C.E. & Gallo, J.M. (1990) Effect of dosing vehicles on the pharmacokinetics of orally administered carbon tetrachloride in rats. *Toxicol. appl. Pharmacol.*, **102**, 50–60

Kitchin, K.T. & Brown, J.L. (1989) Biochemical effects of three carcinogenic chlorinated methanes in rat liver. *Teratog. Carcinog. Mutag.*, **9**, 61–69

Kubic, V.L. & Anders, M.W. (1980) Metabolism of carbon tetrachloride to phosgene. *Life Sci.*, **26**, 2151–2155

Lewis, R.J., Jr (1993) *Hawley's Condensed Chemical Dictionary*, 12th Ed., New York, Van Nostrand Reinhold, pp. 221–222

Lide, D.R., ed. (1997) *CRC Handbook of Chemistry and Physics*, 78th Ed., Boca Raton, FL, CRC Press, p. 3–207

Lil'p, I.G. (1983) Instability of the chromosomes in 101/H and C57BL/6 mice during aging. *Sov. Genet.*, **18**, 1467–1472

Linet, M.S., Stewart, W.F., Van Natta, M.L., McCaffrey, L.D. & Szklo, M. (1987) Comparison of methods for determining occupational exposure in a case–control interview study of chronic lymphocytic leukemia. *J. occup. Med.*, **29**, 136–141

Liu, S.-L., Esposti, S.D., Yao, T., Diehl, A.M. & Zern, M.A. (1995) Vitamin E therapy of acute $CCl_4$-induced hepatic injury in mice is associated with inhibition of nuclear factor kappa B binding. *Hepatology*, **22**, 1474–1481

Long, R.M. & Moore, L. (1986) Elevated cytosolic calcium in rat hepatocytes exposed to carbon tetrachloride. *J. Pharmacol. exp. Ther.*, **238**, 186–191

Manno, M., Rezzadore, M., Grossi, M. & Sbrana, C. (1996) Potentiation of occupational carbon tetrachloride toxicity by ethanol abuse. *Hum. exp. Toxicol.*, **15**, 294–300

McCann, J., Choi, E., Yamasaki, E. & Ames, B.N. (1975) Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. *Proc. natl Acad. Sci. USA*, **72**, 5135–5139

McCollister, D.D., Beamer, W.H., Atchison, G.J. & Spencer, H.C. (1951) The absorption, distribution and elimination of radioactive carbon tetrachloride by monkeys upon exposure to low vapor concentrations. *J. Pharmacol. exp. Ther.*, **102**, 112–124

McGregor, D. & Lang, M. (1996) Carbon tetrachloride: genetic effects and other modes of action. *Mutat. Res.*, **366**, 181–195

McMichael, A.J., Andjelkovic, D.A. & Tyroler, H.A. (1976) Cancer mortality among rubber workers: an epidemiologic study. *Ann. N.Y. Acad. Sci.*, **271**, 125–137

Mirsalis, J.C. & Butterworth, B.E. (1980) Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents. An in vivo–in vitro assay for potential carcinogens and mutagens. *Carcinogenesis*, **1**, 621–625

Mirsalis, J.C., Tyson, C.K., Loh, E.N., Steinmetz, K.L., Bakke, J.P., Hamilton, C.M., Spak, D.K. & Spalding, J.W. (1985) Induction of hepatic cell proliferation and unscheduled DNA synthesis in mouse hepatocytes following in vivo treatment. *Carcinogenesis*, **6**, 1521–1524

Miyashita, T., Takeda, A., Iwai, M. & Shimazu, T. (1991) Single administration of hepatotoxic chemicals transiently decreases the gap-junction-protein levels of connexin32 in rat liver. *Eur. J. Biochem.*, **196**, 37–42

Moore, L., Davenport, G.R. & Landon, E.J. (1976) Calcium uptake of a rat liver microsomal subcellular fraction in response to in vivo administration of carbon tetrachloride. *J. biol. Chem.*, **251**, 1197–1201

Nagano, K., Nishizawa, T., Yamamoto, S. & Matsushima, T. (1998) Inhalation carcinogenesis studies of six halogenated hydrocarbons in rats and mice. In: Chiyotani, K., Hosoda, Y. & Aizawa, Y., eds, *Advances in the Prevention of Occupational Respiratory Diseases*, Amsterdam, Elsevier, pp. 741–746

Nakamura, S.L., Oda, Y., Shimada, T., Oki, I. & Sugimoto, K. (1987) SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: examination with 151 chemicals. *Mutat. Res.*, **192**, 239–246

Nakata, Y., Iwai, M., Kimura, S. & Shimazu, T. (1996) Prolonged decrease in hepatic connexin 32 in chronic liver injury induced by carbon tetrachloride in rats. *J. Hepatol.*, **25**, 529–537

Nastainczyk, W., Ahr, H. & Ulrich, V. (1991) The mechanism of the reductive dehalogenation of polyhalogenated compounds by cytochrome P450. *Adv. exp. Med. Biol.*, **136**, 799–808

NOES (1997) *National Occupational Exposure Survey 1981–83*, Unpublished data as of November 1997. Cincinnati, OH, United States, Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health

Norpoth, K.H., Reisch, A. & Heinecke, A. (1980) Biostatistics of Ames-test data. In: Norpoth, K.H. & Garmer, R.C., eds, *Short-term Systems for Detecting Carcinogens*, Berlin, Springer-Verlag, pp. 312–322

Ott, M.G., Carlo, G.L., Steinberg, S. & Bond, G.G. (1985) Mortality among employees engaged in chemical manufacturing and related activities. *Am. J. Epidemiol.*, **122**, 311–322

Parola, M., Leonarduzzi, G., Biasi, F., Albano, E., Biocca, M.E., Poli, G. & Dianzani, M.U. (1992) Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis. *Hepatology*, **16**, 1014–1021

Paustenbach, D.J., Carlson, G.P., Christian, J.E. & Born, G.S. (1986) A comparative study of the pharmacokinetics of carbon tetrachloride in the rat following repeated inhalation exposures of 8 and 11.5-hr/day. *Fundam. appl. Toxicol.*, **6**, 484–497

Plummer, J.L., Hall, P. de la M., Ilsley, A.H., Jenner, M.A. & Cousins, M.J. (1990) Influence of enzyme induction and exposure profile on liver injury due to chlorinated hydrocarbon inhalation. *Pharmacol. Toxicol.*, **67**, 329–337

Poyer, J.L., Floyd, R.A., McCay, P.B., Janzen, E.G. & Davis, E.R. (1978) Spin-trapping of the trichloromethyl radical produced during enzymic NADPH oxidation in the presence of carbon tetrachloride or bromotrichloromethane. *Biochim. biophys. Acta*, **539**, 402–409

Puri, E.C. & Müller, D. (1989) Testing of hydralazine in in vivo-in vitro hepatocyte assays for UDS and stimulation of replicative DNA synthesis. *Mutat. Res.*, **218**, 13–19

Raucy, J.L., Kraner, J.C. & Lasker, J.M. (1993) Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit. Rev. Toxicol.*, **23**, 1–20

Ray, S. & Mehendale, H.M. (1990) Potentiation of $CCl_4$ and $CHCl_3$ hepatotoxicity and lethality by various alcohols. *Fundam. appl. Toxicol.*, **15**, 429–440

Raymond, P. & Plaa, G.L. (1995a) Ketone potentiation of haloalkane-induced hepato- and nephrotoxicity. I. Dose–response relationships. *J. Toxicol. environ. Health*, **45**, 465–480

Raymond, P. & Plaa, G.L. (1995b) Ketone potentiation of haloalkane-induced hepato- and nephrotoxicity. II. Implication of monooxygenases. *J. Toxicol. environ. Health*, **46**, 317–328

Raymond, P. & Plaa, G.L. (1996) Ketone potentiation of haloalkane-induced hepatotoxicity: $CCl_4$ and ketone treatment on hepatic membrane integrity. *J. Toxicol. environ. Health*, **49**, 285–300

Raymond, P. & Plaa, G.L. (1997) Effect of dosing vehicle on the hepatotoxicity of $CCl_4$ and nephrotoxicity of $CHCl_3$ in rats. *J. Toxicol. environ. Health*, **51**, 463–476

Recknagel, R.O., Glende, E.A.J., Dolak, J.A. & Waller, R.L. (1989) Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Ther.*, **43**, 139–154

Reuber, M.D. & Glover, E.L. (1970) Cirrhosis and carcinoma of the liver in male rats given subcutaneous carbon tetrachloride. *J. natl Cancer Inst.*, **44**, 419–427

Richmond, R.E., DeAngelo, A.B. & Daniel, F.B. (1992) Immunohistochemical detection of *ras* and *myc* oncogene expression in regenerating rat liver. *Toxicol. Lett.*, **60**, 119–129

Roghani, M., Da Silva, C. & Castagna, M. (1987) Tumor promoter chloroform is a potent protein kinase C activator. *Biochem. biophys. Res. Commun.*, **142**, 738–744

Roldán-Arjona, T. & Pueyo, C. (1993) Mutagenic and lethal effects of halogenated methanes in the Ara test of *Salmonella typhimurium*: quantitative relationship with chemical reactivity. *Mutagenesis*, **8**, 127–131

Sáez, J.C., Bennett, M.V.L. & Spray, D.C. (1987) Carbon tetrachloride at hepatotoxic levels blocks reversibly gap junctions between rat hepatocytes. *Science*, **236**, 967–969

Sanzgiri, U.Y. & Bruckner, J.V. (1997) Effect of Emulphor, an emulsifier, on the pharmacokinetics and hepatotoxicity of oral carbon tetrachloride in the rat. *Fundam. appl. Toxicol.*, **36**, 54–61

Sanzgiri, U.Y., Kim, H.J., Muralidhara, S., Dallas, C.E. & Bruckner, J.V. (1995) Effect of route and pattern of exposure on the pharmacokinetics and acute hepatotoxicity of carbon tetrachloride. *Toxicol. appl. Pharmacol.*, **134**, 148–154

Sanzgiri, U.Y., Srivatasan, V., Muralidhara, S., Dallas, C.E. & Bruckner, J.V. (1997) Uptake, distribution, and elimination of carbon tetrachloride in rat tissues following inhalation and ingestion exposures. *Toxicol. appl. Pharmacol*., **143**, 120–129

Sauer, J.-M. & Sipes, I.G. (1995) Modulation of chemical-induced lung and liver toxicity by all-*trans*-retinol in the male Sprague-Dawley rat. *Toxicology*, **105**, 237–249

Schiestl, R.H., Gietz, R.D., Mehta, R.D. & Hastings, P.J. (1989) Carcinogens induce intrachromosomal recombination in yeast. *Carcinogenesis*, **10**, 1445–1455

Schwarz, M., Hummel, J., Appel, K.E., Rickert, R. & Kunz, W. (1979) DNA damage induced *in vivo* evaluated with a non-radioactive alkaline elution technique. *Cancer Lett.*, **6**, 221–226

Shah, H., Hartman, S.P. & Weinhouse, S. (1979) Formation of carbonyl chloride in carbon tetrachloride metabolism by rat liver *in vitro*. *Cancer Res*., **39**, 3942–3947

Siemiatycki, J., Gérin, M., Dewar, R., Nadon, L., Lakhani, R., Bégin, D. & Richardson, L. (1991) Associations between occupational circumstances and cancer. In: Siemiatycki, J., ed., *Risk Factors for Cancer in the Workplace*, Boca Raton, FL, CRC Press, pp. 141–294

Simko, V., Michael, S., Katz, J., Oberstein, E. & Popescu, A. (1992) Protective effect of oral acetylcysteine against the hepatorenal toxicity of carbon tetrachloride potentiated by ethyl alcohol. *Alcohol. clin. exp. Res.*, **16**, 795–799

Simmons, J.E., McDonald, A., Seely, J.C. & Sey, Y.M. (1995) Potentiation of carbon tetrachloride hepatotoxicity by inhaled methanol: Time course of injury and recovery. *J. Toxicol. environ. Health*, **46**, 203–216

Sina, J.F., Bean, C.L., Dysart, G.R., Taylor, V.I. & Bradley, M.O. (1983) Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat. Res.*, **113**, 347–391

Srivastava, S.P., Chen, N. & Holtzman, J.L. (1990) The in vitro NADPH-dependent inhibition by $CCl_4$ of the ATP-dependent calcium uptake of hepatic microsomes from male rats. *J. biol. Chem.*, **265**, 8392–8399

Stewart, B.W. (1981) Generation and persistence of carcinogen-induced repair intermediates in rat liver DNA *in vivo*. *Cancer Res.*, **41**, 3238–3243

Stewart, P.A., Lee, J.S., Marano, D.E., Spirtas, R., Forbes, C.D. & Blair, A. (1991) Retrospective cohort mortality study of workers at an aircraft maintenance facility: II. Exposures and their assessment. *Br. J. ind. Med.*, **48**, 531–537

Stoyanovsky, D.A. & Cederbaum, A.I. (1996) Thiol oxidation and cytochrome P450-dependent metabolism of $CCl_4$ triggers $Ca^{2+}$ release from liver microsomes. *Biochemistry*, **35**, 15839–15845

Suzuki, H., Hirano, N., Watanabe, C. & Tarumoto, Y. (1997) Carbon tetrachloride does not induce micronucleus in either mouse bone marrow or peripheral blood. *Mutat. Res.*, **394**, 77–80

Takano, T., Tatematsu, M., Hasegawa, R., Imaida, K. & Ito, N. (1980) Dose–response relationship for the promoting effects of phenobarbital on the induction of liver hyperplastic nodules in rats exposed to 2-fluorenylacetamide and carbon tetrachloride. *Gann*, **71**, 580–581

Tanaka, T., Mori, H. & Williams, G.M. (1987) Enhancement of dimethylnitrosamine-initiated hepatocarcinogenesis in hamsters by subsequent administration of carbon tetrachloride but not phenobarbital or *p,p'*-dichlorodiphenyltrichloroethane. *Carcinogenesis*, **8**, 1171–1178

Tirmenstein, M.A., Leraas, T.L. & Fariss, M.W. (1997) α-Tocopherol hemisuccinate administration increases rat liver subcellular α-tocopherol levels and protects against carbon tetrachloride-induced hepatotoxicity. *Toxicol. Lett.*, **92**, 67–77

Tomenson, J.A., Baron, C.E., O'Sullivan, J.J., Edwards, J.C., Stonard, M.D., Walker, R.J. & Fearnley, D.M. (1995) Hepatic function in workers occupationally exposed to carbon tetrachloride. *Occup. environ. Med.*, **52**, 508–514

Towner, R.A., Reinke, L.A., Janzen, E.G. & Yamashiro, S. (1994) In vivo magnetic resonance imaging study of Kupffer cell involvement in $CCl_4$-induced hepatotoxicity in rats. *Can. J. Physiol. Pharmacol.*, **72**, 441–446

Traiger, G.J. & Plaa, G.L. (1971) Differences in the potentiation of carbon tetrachloride in rats by ethanol and isopropanol pretreatment. *Toxicol. appl. Pharmacol.*, **20**, 105–112

Tsuruta, H. (1975) Percutaneous absorption of organic solvents. I. Comparative study of the in vivo percutaneous absorption of chlorinated solvents in mice. *Ind. Health*, **13**, 227–236

United States International Trade Commission (1993) *Synthetic Organic Chemicals: US Production and Sales*, *1991* (USITC Publ. 2607), Washington DC, United States Government Printing Office, p. 3-85

United States National Library of Medicine (1997*) Hazardous Substances Data Bank (HSDB)*, Bethesda, MD [Record No. 53]

Uryvaeva, I.V. & Delone, G.V. (1995) An improved method of mouse liver micronucleus analysis: an application to age-related genetic alteration and polyploidy study. *Mutat. Res.*, **334**, 71–80

Wang, M.Y. & Liehr, J.G. (1995) Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis. *Arch. Biochem. Biophys.*, **316**, 38–46

Whittaker, S.G., Zimmermann, F.K., Dicus, B., Piegorsch, W.W., Fogel, S. & Resnick, M.A. (1989) Detection of induced mitotic chromosome loss in *Saccharomyces cerevisiae*—an interlaboratory study. *Mutat. Res.*, **224**, 31–78

WHO (1993) *Guidelines for Drinking-Water Quality*, 2nd Ed., Vol. 1, *Recommendations*, Geneva, p. 175

Wilcosky, T.C., Checkoway, H., Marshall, E.G. & Tyroler, H.A. (1984) Cancer mortality and solvent exposures in the rubber industry. *Am. ind. Hyg. Assoc. J.*, **45**, 809–811

Yao, T., Degli Esposito, S., Huang, L., Arnon, R., Spangenberger, A. & Zern, M.A. (1994) Inhibition of carbon tetrachloride-induced liver injury by liposomes containing vitamin E. *Am. J. Physiol.*, **267**, G476–G484

Zalatnai, A., Sárosi, I., Rot, A. & Lapis, K. (1991) Inhibition and promoting effects of carbon tetrachloride-induced liver cirrhosis on the diethylnitrosamine hepatocarcinogenesis in rats. *Cancer Lett.*, **57**, 67–73

Zawaski, K., Gruebele, A., Kaplan, D., Reddy, S., Mortensen, A. & Novak, R.F. (1993) Evidence for enhanced expression of c-*fos*, c-*jun*, and the $Ca^{2+}$-activated neutral protease in rat liver following carbon tetrachloride administration. *Biochem. biophys. Res. Commun.*, **197**, 585–590