Social Europe

Recommendation from the Scientific Committee on Occupational Exposure Limits for carbon tetrachloride

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8 hour TWA	:	1 ppm (6.4 mg/m^3)
STEL (15 mins)	:	5 ppm (32 mg/m^3)
Notation	:	"skin"
SCOEL carcinogen group	:	D (non-genotoxic carcinogen for which a threshold can be established)

Substance identification

Carbon tetrachloride, CCl4

Synonyms	:	tetrachloromethane; carbon chloride; methane tetrachloride; perchloromethane; tetrachlorocarbon; Freon 10
EC N°	:	200-262-8
INDEX N°	:	602-008-00-5
EU Classification:		Carc. Cat. 3; R40; T; R23/24/25-48/23;R52-53 N; R59
CAS N°	:	56-23-5
MWt	:	153.8

Conversion factor (20°C, 101kPa) : 6.40 mg/m^3 = 1 ppm

1. Occurrence/use

Carbon tetrachloride is a colourless, dense, volatile, non-flammable liquid with a sweetish odour. It has a MPt of -23°C, a BPt of 76.7°C and a vapour pressure of 12 kPa at 20°C. It has a vapour density of 5.3 times that of air. The odour threshold is about 20 ppm (about 130 mg/m^3).

Carbon tetrachloride is manufactured as a co-product with tetrachloroethylene and as a by-product in the manufacture of chloroform. It is used in the manufacture of CFC 10 and CFC 11. It is also used in the production of chlorinated rubber, as a reaction solvent in the production of pharmaceuticals and pesticides, as a catalyst sweetener in hydro-reformers, and in the production of anti-knock agents. In the 1980's, the production rate in the EEC was in excess of 100,000 tonnes per annum, but as it is an ozone-depleting agent covered by international agreements, its production has decreased considerably. Its use as a solvent is restricted or banned in many countries.

2. Health Significance

Carbon tetrachloride causes death from CNS depression following acute exposure to very high levels. The critical effects of carbon tetrachloride occur in the liver of experimental animals and humans following repeated exposure.

2.1 Toxicokinetics

Carbon tetrachloride is well-absorbed by inhalation, orally and percutaneously, in animals and humans (Tsurata, 1975; Stewart and Dodd, 1964). A substantial proportion is eliminated, either unchanged or as carbon dioxide, by exhalation. It is mainly metabolised *via* an initial cytochrome P450-dependent dechlorination (mainly via CYP2E1) and subsequent free radical reactions. A metabolic scheme is shown in Fig. 1. For further details, see DFG (2002).

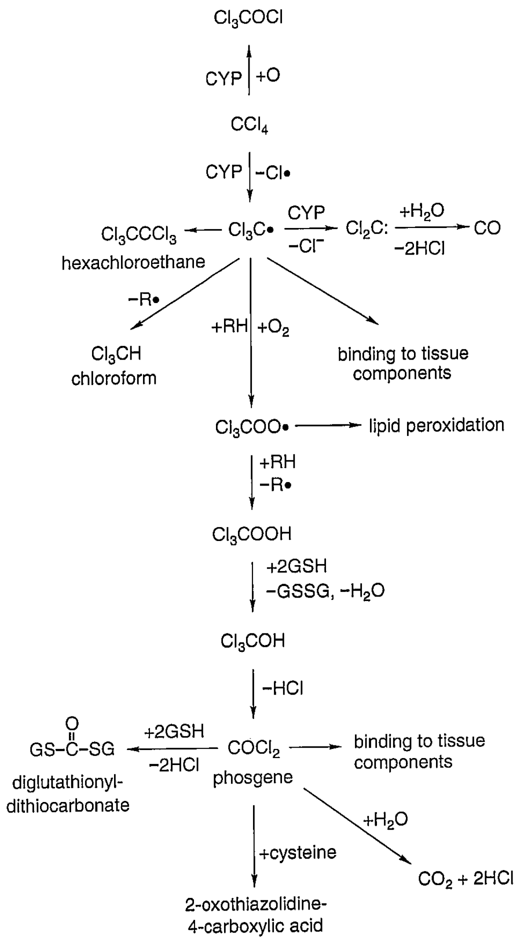

Figure 1. Metabolism of carbon tetrachloride (from WHO 1999)

Dermal absorption

The level of dermal absorption from the gaseous phase is low. The rate of dermal absorption of liquid carbon tetrachloride by intact mouse skin was found to be 8.3 microg/cm^2 and minute (WHO 1999). The application of 1 ml to the shaved skin of guinea pigs (3.1 cm^2) yielded a blood carbon tetrachloride level of 1 mg/1 after one hour. Although exposure was continued, the concentration in blood decreased over the course of the next hour. Local vasoconstriction, rapid transport from the blood to adipose tissue, or metabolism are thought to be responsible (WHO 1999). On the skin of guinea pigs, complete absorption occurred within a few days after application of 0.5 to 2 ml carbon tetrachloride to 3.1 cm2 skin in a closed glass container (WHO 1999). After 3 volunteers had dipped a thumb into carbon tetrachloride for 30 minutes, concentrations of 3.8 mg/m^3 were detected in the exhaled air. The concentration decreased with a half-time of 2.5 hours (WHO 1999).

Biological monitoring

Carbon tetrachloride can be determined in biological materials. However, because of its rapid metabolism the sampling time is critical. DFG has proposed a biological limit (BAT value) of 70 µg/liter blood, correlated with an airborne exposure level of 0.5 ppm. Because of the restricted use of carbon tetrachloride, the relevance of biological monitoring of this compound is low in general.

2.2 Acute toxicity

2.2.1 Human data

Regardless of the route of absorption, the clinical picture of carbon tetrachloride intoxication is dominated in the first 24 hours by gastrointestinal and neurological symptoms. Liver damage occurs at the earliest after 24 hours. In severe cases ascites and hepatic coma develop, and are often accompanied by haemorrhage. Kidney damage is first manifest after 2-3 days, often not until 2-3 weeks after the intoxication (WHO 1999). Abnormal liver function values were reported in 10 of 25 workers accidentally poisoned with carbon tetrachloride after exposure to concentrations of 300 to 500 ml/m^3 (Deng *et al.* 1987).

2.2.2 Animal data

After inhalation exposure, LC_{50} values for the mouse of 45000 to 50000 mg/m^3 were determined. The acute inhalation toxicity of carbon tetrachloride is, therefore, low. In the Jungs of rats and mice exposed to carbon tetrachloride concentrations between 70000 and 600000 mg/m^3, Clara cell lesions, decreased levels of cytochrome P450, thickening of the septa, alveolar collapse and atypical type 11 pneumocyte configuration were observed.
In the serum, the levels of aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase and glutamate dehydrogenase were increased; necrosis was observed in the liver (WHO 1999).

2.3 Irritation and corrosivity

2.3.1 Human data

Three volunteers dipped a thumb into carbon tetrachloride for 30 minutes; this led to slight erythema, which regressed 1 or 2 hours after exposure. The volunteers reported a burning feeling in the thumb within the first 10 minutes of exposure (WHO 1999).

2.3.2 Animal data

Epicutaneous application of 1 ml carbon tetrachloride for periods between 15 minutes and 16 hours caused degenerative changes in the epidermis (WHO 1999). Application of 0.5 ml carbon tetrachloride to the shaved skin of rabbits and guinea pigs caused moderate irritation (WHO 1999). In a Draize skin Irritation test, 0.5 ml carbon tetrachloride was applied occlusively to the shaved and scarified skin of rabbits. After 3 days irritative effects were observed (WHO 1999). 10 .i1 carbon tetrachloride was applied to the skin non-occlusively 3 times a day for 3 days. A skin reaction was observed after 2 days and reddening after 4 days (WHO 1999). Aliquots of 0.1 ml (159 mg) carbon tetrachloride were rubbed into the skin of rabbits and guinea pigs daily for 10 days. Oedema and erythema were observed (WHO 1999).
0.1 ml carbon tetrachloride caused mild irritation in the rabbit eye. The eyes were examined 24, 48 and 72 hours after exposure and after a follow-up period of 14 days (WHO 1999).

2.4. Sensitisation

2.4.1. Human data

After a woman used an anti-grease lotion for the scalp containing 100 g carbon tetrachloride, dermatitis of the scalp developed. The woman was treated with a test substance containing 10 % carbon tetrachloride, 10 % carbon disulphide and 10 % acetone, corresponding to the amounts in the Lotion, and produced a marked reaction after 48 hours (no other details) (Romaguera and Grimalt 1980, cited by WHO 1999).

2.4.2. Animal data

There are no indications of a sentitisation potential of carbon tetrachloride based on animal experiments.

2.5. Repeated dose toxicity

2.5.1 Human data

Workers were exposed to carbon tetrachloride concentrations of 1 ml/m^3 or less (n = 40), 1 to 3 ml/m^3 (n = 54) or 4 ml/m^3 and more (n = 61). The workers were also subdivided into three groups depending on the duration of the activity: < 1 year, 1 to 5 years, > 5 years. The level of alcohol consumption was the Same in all exposed workers. The workers were questioned about their age, height, weight, workplace situation, firm, hobbies, state of health and alcohol consumption. Haematological parameters such as haemoglobin level, number of red blood cells and the haematocrit were significantly changed in the middle exposure group, as were the activities of gamma-glutamyltransferase and alkaline phosphatase in serum. In the low exposure group only the haematocrit was significantly decreased. No other parameters were investigated. In the high exposure group, however, no changes were observed (Tomenson *et al.* 1995).

17 workers were exposed for about 2 years to carbon tetrachloride concentrations of 290 to 620 mg/m^3. 15 workers complained of nausea, 12 of loss of appetite, 7 of vomiting and flatulence, 10 of feeling unwell and dizzy. Clinical examination did not yield any abnormal findings (Kazantzis and Bomfort 1960).

In 16 workers exposed to carbon tetrachloride concentrations of about 490 to 3900 mg/m^3 (no data for duration of exposure), increased values for serum bilirubin and serum transaminases, and increased amounts of protein in urine were found (Barnes and Jones 1967).

2.5.2 Animal data

The text which follows is a selection of studies performed, which appear relevant for establishing an OEL (DFG 2002). The IPCS report describes all the studies which have been carried out (WHO 1999).

Groups of 10 BDF1 mice were exposed to carbon tetrachloride concentrations of 10, 30, 90, 270 or 810 ml/m^3 (64, 192, 577, 1731 or 5192 mg/m^3) 6 hours/day, 5 days a week, for 13 weeks. Microscopic examination of the liver revealed cytological changes in the animals of the lowest concentration group only in the males. With increasing concentration the effects increased in severity. The incidence of mitosis was increased; focus formation and increased proliferation were observed. From concentrations of 30 ml/m3 the body weights of the male animals were reduced. An increase was observed in the liver enzymes in blood from concentrations of 90 ml/m^3. From 270 ml/m3 haematological changes developed, after concentrations of 810 ml/m^3 the urinary pH decreased (Japan Bioassay Research Centre 1998; Nagano et al. 2007). From this study, a no observed effect level (NOEL) of 10 ml/m3 can be derived only for the female animals.

Groups of 50 BDF1 mice were exposed to carbon tetrachloride concentrations of 5, 25, or 125 ml/m^3 (32.05, 160.25, or 801.25 mg/m^3), 6 hours/day, 5 days a week for 104 weeks. In the lowest concentration group no effects were observed. Survival and body weights were significantly reduced from concentrations of 25 ml/m^3, and haematological and urinary parameters were changed. Microscopic changes developed from 25 rnl/m^3 in the liver (thrombus, necrosis, cysts, degeneration), in the kidneys and in the spieen (increasing deposition of haemosiderin and extramedullary haematopoiesis) (Japan Bioassay Research Centre 1998, Nagano *et al*. 1998, 2007). From this study, a NOEL of 5 m1/m3 can be derived for the mouse.

Groups of 50 F344 rats were exposed to carbon tetrachloride for 13 and 104 weeks under the same experimental conditions as were the mice. In the 13-week study body weights were reduced in the highest concentration group; in the low concentration group, granulation was seen in the livers of male and female animals. Haematological changes developed in the female animals from concentrations of 30 m1/m^3 and in the males from 90 ml/m^3. From 90 ml/m^3, the levels of liver enzymes in blood were increased and urinalysis revealed changes. Microscopic examination of the liver revealed fat deposition and cytological changes from concentrations of 30 ml/m^3. There were increases in mitosis, fibrosis, focus formation and proliferation in the female animals from 90 ml/m^3 and in the male animals from 270 ml/m^3. In the high concentration group, vacuolation of the tubules, hyaline degeneration of the glomeruli and accumulation of protein in the kidneys were found (Japan Bioassay Research Centre 1998, Nagano *et al*. 2007).

The effects seen in the 2-year study are described below. Survival was reduced in the high concentration group. The animals died of liver tumours or chronic nephropathy. The effects in male and female animals exposed to 5 m1/m^3 were changed nitrate and protein vahies in the urine. Increasing deposition of haemosiderin in the spieen was observed in the male animals of all concentration groups. In the female animals of all concentration groups, eosinophilia developed in the nasal cavity, and in male animals from 25 ml/m^3. From 25 ml/m^3 body weights were reduced, and the levels of liver enzymes *in* blood and other haematological parameters were changed. Microscopic changes in the liver (see 13-week study) were observed at the two highest concentrations. The female animals developed chronic nephropathy from concentrations of 25 ml/m^3 and the male animals from 125 mUm^3 (Japan Bioassay Research Centre 1998, Nagano *et al.* 1998, 2007).

After inhalation exposure to carbon tetrachloride concentrations of 5 to 400 ml/m^3 a NOEL of 5 ml/m^3 was found for rats, rabbits and male guinea pigs. In the female guinea pigs, increased relative liver weights were observed at this concentration, but no histopathological changes were found (Adams *et al.* 1952).

Ingestion

Groups of 12 male and 12 female CD-1 mice were given carbon tetrachloride doses of 1.2, 12 or 120 mg/kg body weight in corn oil or in 1 % Tween 60 solution 5 times a week for 90 days. This study was carried out to investigate the effects of carbon tetrachloride administered with different vehicles. From 12 mg/kg body weight (vehicle corn oil) and 120 mg/kg body weight (vehicle Tween) the liver enzymes alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase increased significantly in serum. Hepatomegaly, large fat deposits and necrosis with acute inflammation (only in the male animals) were observed after administration of the substance in corn oil. From this study, a no observed adverse effect level (NOAEL) for hepatotoxic effects of 1.2 mg/kg body weight can be derived for male and female mice (Condie *et al.* 1986).

Groups of 15 to 16 male rats were given carbon tetrachloride doses of 1, 10, or 33 mg/kg body weight an 5 days a week for 12 weeks. In the middle dose group the sorbitol dehydrogenase activity in serum was significantly increased and slight centri-lobular vacuolation was observed in the liver. In the high dose group in addition the levels of ornithine carbamyl transferase and alanine aminotransferase were increased. Liver cirrhosis, bile duct proliferation, fibrosis, parenchymal regeneration, hyperplastic nodules and single cell necrosis were found. There were no effects an the kidneys. From this study, a NOEL of 1 mg/kg body weight can be derived for the male rat (Bruckner *et al.* 1986).

Dose-response relationship

After administration of carbon tetrachloride doses of 2 or 10 mg/kg body weight in corn oil for 1, 7 and 14 days to CD-1 mice, there was no increase in the incidence of cell proliferation, in the DNA repair in hepatocytes or in the levels of aspartate aminotransferase and alanine aminotransferase in serum. From doses of 20 mg/kg body weight, an day 7 the levels of aspartate aminotransferase and alanine aminotransferase in serum increased, and from 100 mg/kg body weight also the incidence of cell proliferation was increased. After 14 days the aspartate aminotransferase and alanine aminotransferase levels had returned to the control values, the increase in cell proliferation was not, however, reversible. The study shows that the hepatocyte cytotoxicity increases in parallel with the increase in cell proliferation (Doolittle *et al.* 1987).

2.6 Genotoxicity

The studies of genotoxicity *in vitro* and *in* vivo are described in detail in the tables of the IPCS monograph (WHO 1999). Relevant results *in vitro* and *in vivo* are summarised below (DFG 2000). There are no human data concerning the genotoxicity of carbon tertrachloride.

2.6.1 In vitro

Carbon tetrachloride was not found to be mutagenic in the tests for gene mutations or for an SOS response in *Salmonella typhimurium* or *Escherichia coli* either in the absence of a metabolic activation system or in the presence of S9 from the rat, mouse or rabbit. In the yeast *Saccharomyces cerevisiae,* in the absence of an activation system, carbon tetra-chloride induced intrachromosomal and mitotic recombination. In V79 cells, a high carbon tetrachloride concentration (2500 microg/ml, about 16 mM) produced aneuploidy, which can probably be attributed to the lipophilia of the substance. Studies of the induction of aneuploidy and micronuclei by carbon tetrachloride in various genetically engineered human lymphoblastoid cell lines which express different cytochrome P450 forms, revealed that lower concentrations (2 mM) are genotoxic only in cells which express CYP2E1. Whether clastogenic effects were involved in the formation of micronuclei was not investigated.

In primary hepatocyte cultures of the rat and mouse, and in human epithelial liver cell lines, carbon tetrachloride led to the induction of DNA strand breaks. In most of the studies strand breaks occurred only at cytotoxic concentrations.

From the available data it was concluded that *in vitro* studies do not provide any clear evidence of the formation of DNA-reactive metabolites of carbon tetrachloride (DFG 2002). More recently, Araki et al. (2004) described further mutagenicity experiments in bacteria with both chloroform and carbon tetrachloride, using a gas exposure method. It was claimed that carbon tetrachloride was mutagenic in the *S. typhimurium* strain TA98 without S9 mix, and in *E. coli* WP2/pKM101 and WP2uvrA/pKM101 with and without S9 mix, but not in *S. typhimurium* TA100, TA1535 or TA1537. However the alledged positive effects were seen only at high doses (5000 ppm or more in the gas phase) and, under these conditions, were only slight (i.e. below or not significantly exceeding a doubling of the spontaneous mutation rates in the control experiments). Hence, the data of Araki et al. (2004) do not contradict the statement of DFG (2002), as mentioned above.

2.6.2 In vivo

In *Drosophila melanogaster,* carbon tetrachloride did not lead to sex-specific recessive lethal mutations (SLRL mutations) after feeding or injection.

In rats given oral doses of carbon tetrachloride, no induction of DNA strand breaks or DNA repair synthesis was detected. In rats and mice, no sister chromatid exchange (SCE) or chromosomal aberrations were found. Micronucleus tests with mice also yielded negative results.

Barrows and Shank (1981, cited by WHO 1999) described the formation of 0^6-methyl guanine and 7-methyl guanine in the liver DNA of rats after oral administration of carbon tetrachloride doses of 1000 mg/kg body weight. The formation of the methylated bases was attributed to the hepatotoxicity caused by this dose, but the mechanism could not be explained or the results confirmed in later studies.

Two research groups showed that the lipid peroxidation occurring *in vivo* after administration of high doses of carbon tetrachloride is accompanied by the increased formation of certain DNA adducts also found in untreated animals and attributed to the reaction of lipid peroxidation products with DNA bases. Wang and Liehr (1995) found an increase in the concentration of lipid peroxides and polar endogenous DNA adducts in the kidneys and liver of hamsters after oral administration of carbon tetrachloride in doses of 0.1 ml/kg body weight (about 159 mg). In the kidneys the incidence of adducts was increased by about 120 %, in the liver by about 50 %. The higher dose of 1.0 mg/kg body weight led in the kidneys to a slight increase in both parameters, in the liver the levels were found to be below those in the untreated animals. This was ascribed to the inactivation of carbon tetrachloride-activating cytochrome P450 forms. Independent of the treatment conditions, there was a linear correlation between the concentration of lipid peroxides and the level of adducts in the organs (Wang and Liehr 1995). The polar DNA adducts were found to be two reaction products of malondialdehyde with deoxyadenosine and one DNA base modification of unknown structure induced by free radicals.

After intraperitoneal administration of carbon tetrachloride doses of 3200 mg/kg body weight to rats, the concentrations of various 1,N^2-propanoguanine adducts in the liver DNA, which may also be detected in untreated animals, were investigated. These exocyclic adducts are derived from the reaction with DNA of □□□-unsaturated aldehydes (enals), formed during the lipid peroxidation of polyunsaturated fatty acids. Formation of these adducts is markedly increased when glutathione levels are reduced. 24 and 72 hours after administration of the substance, significantly increased concentrations of an acrolein-deoxyguanosine adduct were detected, which were about 3.7 and 4.4 times the control value. The concentrations of two crotonaldehyde-deoxyguanosine adducts were increased, but the increase was not statistically significant. 24 hours after administration of the substance, marked zonal necrosis was found, and after 72 hours developed into fibrosis. After 24 hours the binding of 4-hydroxynonenal to liver proteins was increased (Chung *et al.* 1999).

After intragastric administration of carbon tetrachloride doses of 1 ml/kg body weight to rats, the amounts of 4-hydroxynonenal-protein and malondialdehyde-protein adducts (products of lipid peroxidation) in the liver were found by immunochemical analysis to increase with time (Hartley *et al.* 1999).

Twice-weekly administration of carbon tetrachloride doses of 0.75 ml/kg body weight (about 1190 mg) led in mice to a decrease in the frequency of endogenous DNA modifications (so-called I-compounds of type 1) in the liver DNA, which persisted for at least 22 weeks (Nath *et al.* 1990, cited by WHO 1999; Randerath *et al.* 1999). The structures and biological importance of these modifications are unknown. They are thought to have a physiological function. Their frequency is decreased by numerous hepatocarcinogens and is reduced in tumours (Randerath *et al.* 1999).

2.7 Carcinogenicity

2.7.1 Human data

There are several epidemiological studies of the carcinogenicity of carbon tetrachloride. In all studies, however, the persons were exposed to a mixture of carbon tetrachloride and other halogenated solvents or other substances. In addition, data for the exposure levels are lacking in these studies. For these reasons the following studies are not included in the present assessment: Blair *et al*. 1990, Bond *et al*. 1986, Chekoway *et al*. 1984, Heinemann *et al*. 1994, Ott *et al*. 1985.
In an epidemiological study carried out in the rubber industry (about 7000 exposed workers studied for 10 years) it was suspected that carbon tetrachloride at the workplace may be responsible for an increased incidence of cancer (Wilcosky *et al*. 1984). It was demonstrated that the incidence of lymphatic leukaemia and lymphosarcomas in workers from these firms correlated with the exposure to carbon tetrachloride. For other solvents there was no correlation. However, other possible carcinogenic substances, e.g. nitrosamines, were not taken into consideration. In a case-control study, the exposure of 342 persons with chronic lymphatic leukaemia to various substances was investigated. A significantly increased risk of developing leukaemia was not observed (Linet *et al*. 1987).

2.7.2 Animal data

After both oral and inhalation exposure to carbon tetrachloride, the incidence of hepatocellular carcinomas and phaeochromocytomas in mice was increased in a dose-dependent manner. In mice which had inhaled carbon tetrachloride concentrations of 5 ml/m^3, the lowest concentration tested, no tumours were found. After 2 years a complete histopathological examination of all organs was carried out. Therefore from this study a NOEL of 5 ml/m^3 for the formation of hepatocarcinomas in the mouse can be derived (Japan Bioassay Research Centre 1998, Nagano *et al*. 1998). In female rats, oral administration of the substance led to the formation (not dose-dependent) of neoplastic nodules in the liver and hepatocellular carcinomas, while in the male animals only neoplastic nodules were found. After inhalation exposure, hepatocellular adenomas and carcinomas were observed only at the highest concentration tested of 125 ml/m^3. Therefore from this study a NOEL of 25 ml/m^3 for the formation of hepatocarcinomas in the rat can be derived (Japan Bioassay Research Centre 1998, Nagano *et al*. 1998). Data for the incidence of tumours and from this and other studies are listed in *Table 1*.

There are several mechanisms which could explain the formation of the tumours. Under discussion for a long time was whether the trichloromethyl radical formed during metabolism can bind covalently to DNA. The studies carried out did not yield conclusive results. Also the mainly negative results in genotoxicity tests, above all *in vivo*, speak against this mechanism. In view of the short lifetime of the trichloromethyl radical, which is mainly found covalently bound to proteins and lipids, binding to the DNA in the cell nucleus is also improbable.

Products of lipid peroxidation, such as malondialdehyde or 4-hydroxynonenal can in principle bind to DNA and cause mutations. They occur, however, also without specific exposure in healthy persons in various organs, including the liver (Marnett 1999).

Table 1. Studies of the carcinogenicity of carbon tetrachloride (DFG 2002) *(m=male; f=female)*

Author:	Reuber and Glover 1970, cited by WHO 1999
Substance:	carbon tetrachloride (purity not specified)
Species:	rat (Japanese, Osborne-Mendel, Wistar, Black Rat, Sprague-Dawley), 12-17 m, 12 controls/strain
Administration route:	subcutaneous, vehicle corn oil
Concentration:	1000 mg/kg body weight
Duration:	70-105 weeks, 2/week
Toxicity:	moderate to severe liver cirrhosis, body weights decreased, changed liver weights, hepatic venous thrombosis, cholangiofibrosis

	Japanese	Osborne-Mendel	Wistar	Black Rat	Sprague-Dawley
Survivors week 78	12/15	8/13	0	0	0
Average survival	47 weeks	44 weeks	33 weeks	13 weeks	11 weeks

Findings:[1]		0 (vehicle controls)	1000 mg/kg body weight
Japanese	liver carcinomas	0/12	12/15
	spleen haemangiomas	not specified	2/15
	thyroid carcinomas	not specified	3/15
	kidney cysts	not specified	3/15
Osborne-Mendel	liver carcinomas	0/13	8/13
	spleen haemangiomas	not specified	1/13
	thyroid carcinomas	not specified	3/13
	kidney cysts	not specified	2/13
	lung metastases	not specified	1/13
Wistar	liver carcinomas	0/12	4/12

[1] no effects in Black rat or Sprague-Dawley rat

Author:	Weisburger 1977
Substance:	carbon tetrachloride (purity tested, no other details)
Species:	rat (Osborne-Mendel), 99 m, 99 f, controls: 20 m,18 f, mouse ($B6C3F_1$), 97 m, 87 f, controls: 18 m, 18 f

Administration route:	gavage, vehicle corn oil
Dose:	rat: 50-160 mg/kg body weight (exact details not given) mouse: 1250-2500 mg/kg body weight (exact details not given)
Duration:	78 weeks, 5 days/week
Toxicity:	not specified, carbon tetrachloride served as positive control

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		control	low dose	high dose
Osborne-Mendel rats				
thyroid adenomas and carcinomas	m	1/20	1/49	1/50
	f	2/20	2/50	4/49
haemangiosarcomas	m	0/20	4/49	4/50
	f	0/20	3/50	1/50
neoplastic nodules in the liver	m	0/20	9/49	3/50
	f	1/20	11/50	9/49
hepatocellular carcinomas	m	0/20	2/49	2/50
	f	1/20	4/50	2/49
B6C3F$_1$ mice				
hepatocellular carcinomas	m	3/18	49/49	47/47
	f	1/18	40/40	43/43
adrenal adenomas and chromocytomas	m	0/18	28/49	28/47
	f	0/18	15/40	10/43

Author:	Nagano *et al.* 1998, 2007; Japan Bioassay Research Centre 1998
Substance:	carbon tetrachloride (purity > 99.9 %)
Species:	F344 rat 50 m, 50 f; BDF$_1$ mouse 50 m, 50 f
Administration route:	inhalation
Concentration:	5, 25, 125 ml/m^3
Duration:	6 hours/day, 5 days/week, 104 week
Toxicity:	see Section 5.2.1

		control	5 ml/m³	25 ml/m³	125 ml/m³
F344 rats					
survivors	m	22/50	29/50	19/50	3/50
	f	39/50	43/50	39/50	1/50
hepatocellular adenomas	m	0/50	1/50	1/50	21/50
	f	0/50	0/50	0/50	40/50
hepatocellular carcinomas	m	1/50	0/50	0/50	32/50
	f	0/50	0/50	3/50	15/50

		control	5 ml/m³	25 ml/m³	125 ml/m³
BDF_1 mice					
survivors	m	35/50	36/50	25/50	1/50
	f	26/50	24/49	10/50	1/49
hepatocellular adenomas	m	9/50	9/50	27/50	16/50
	f	2/50	8/50	17/50	5/50
hepatocellular carcinomas	m	17/50	12/50	42/50	48/50
	f	2/50	1/49	33/50	48/49
phaeochromocytomas	m	0/50	0/50	16/50	31/50
	f	0/50	0/49	0/50	22/49

The products of lipid peroxidation, also of that caused by carbon tetrachloride, induce the expression of proto-oncogenes such as c-fos and c-jun, which was also detected for carbon tetrachloride (Schiaffonati and Tiberio 1997). The DNA damage (deletion via intrachromosomal recombination) observed in vitro in yeast cells (Brennan and Schiestl 1998) is similar to the damage normally observed after oxidative stress (Wang et al. 1998). Increased levels of 8-hydroxy-2'-desoxyguanosine, a parameter for DNA changes resulting from oxidative stress, were detected in the liver tissue of rats after administration of carbon tetrachloride (Takahashi et al. 1998).

Investigations by Camandola et al. (1999) indicate that the lipid peroxidation resulting from exposure to carbon tetrachloride is responsible for the "upregulation" of activator protein-1 (AP-1). It could be completely inhibited by the antioxidant, vitamin E. In addition, it was found that Kupffer's cells play an important role in this gene activation. The authors suspect that products of lipid peroxidation activate the Kupffer's cells, which in turn stimulate gene activation and proliferation in the liver cells.

In all the mechanisms named above, with the exception of the direct alkylation of DNA by trichloromethyl radicals, which is, however, an unlikely mechanism, the effects correlate with carbon tetrachloride-induced lipid peroxidation. The dose-response relationship for lipid peroxidation, however, has a "threshold value", as it takes place in the organism also without specific activation and is inhibited by various antioxidative protective mechanisms. It is also involved to a high degree in the liver toxicity induced by carbon tetrachloride. It can therefore be assumed that in the absence of liver toxicity no liver tumours are formed. The course of the dose-response relationship for the induction of liver tumours in rats and mice confirms this hypothesis (DFG 2002).

2.8 Reproductive toxicity

2.8.1 Human data

Data for man has been found in only one publication (Dowty *et al.* 1976). The authors analysed 11 blood samples taken from mothers and their offspring after normal births. Carbon tetrachloride was found in both the mothers and offspring; the concentrations determined in the offspring were of the same level as those found in the mothers, or in some cases even higher. Thus it must be assumed that carbon tetrachloride can pass the placental barrier in man. Other data for man are not available (Barlow and Sullivan 1982).

2.8.2 Animal data

Fertility

After oral administration of 0.1 to 1.5 mg/kg body weight for 5 days to CD-1 mice, no sperm anomalies were detected (WHO 1999).

Groups of 6 male and 6 female rats were given single intraperitoneal doses of carbon tetrachloride of 3 ml/kg body weight (2378 mg/kg body weight) in coconut oil. After 15 days, the pituitary gland weights in the male animals were significantly increased and the testis weights reduced. Histological examination revealed testis atrophy and changes in spermatogenesis. After 10 days the female cycle stopped, the ovary and Uterus weights were reduced and the adrenal gland and pituitary gland weights increased (Chatterjee 1966, 1968).

In an early 3-generation study, groups of 24 albino rats were exposed to carbon tetrachloride concentrations of 50, 100, 200 and 400 m1/m^3 on 5 days a weck. The authors reported that fertility in the animals exposed to 200 and 400 ml/m^3 was impaired. Embryotoxic, foetotoxic and teratogenic effects were not, however, mentioned (Smyth et *al.* 1936, Smyth and Smyth 1935).

Developmental toxicity

In Sprague-Dawley rats which had inhaled carbon tetrachloride concentrations of 334 or 1004 ml/m^3 for 7 hours a day an days 6 to 15 of gestation, food consumption and body weights were reduced. In the two dose groups the alanine aminotransferase and S-glutamyl transpeptidase activities increased in a manner not dependent on the dose- an indication of liver toxicity. The relative liver weights were significantly increased. Carbon tetrachloride had no influence on the number of implantations, resorptions or pups per litter. The body weights of the foetuses decreased and the body lengths were shorter. Subcutaneous oedema was observed at concentrations of 334 ml/m^3 significantly more often than in the control animals. In the high concentration group, anomalies of the breastbone were significantly more frequent (Schwetz *et al.* 1974).

F344 rats were given gavage doses of carbon tetrachloride of 25, 50 or 75 mg/kg body weight in corn oil or 10 % Emulphor from days 6 to 15 of gestation. Independent of the vehicle, dose-dependent piloerection was seen in the dams from doses of 50 mg/kg body weight. Reduced body weights and kyphosis were observed in the high dose group after administration of the substance in corn oil and from doses of 50 mg/kg body weight in aqueous solution. In the groups given 50 and 75 mg/kg doses in corn oil, 42 and 67 of the litters were resorbed, with the aqueous solution 14 and B. After administration of 25 mg/kg body weight, the prenatal and postnatal body weights and survival were not affected. No morphological changes were found in the foetuses. The authors give a NOAEL for both the dams and the foetuses of 25 mg/kg body weight after administration in either corn oil or aqueous solution (Narotsky *et al.* 1997).

On 5 consecutive days of gestation, beginning an day 1, 6 or 11, groups of 31 $B6D2F_1$ mice were given carbon tetrachloride doses of 82.6 or 826.3 mg/kg body weight by gavage. No effects were observed in either the dams or the foetuses (Hamlin *et al.* 1993). However, because of the small number of animals investigated and the inadequate documentation of the findings, the study is of only limited use for the evaluation of the developmental toxicity of the substance.

Recommendation

In view of the predominantly negative genotoxicity data and the specificity of carcinogenicity, it is considered that the tumours observed in carbon tetrachloride-treated animals are associated with chronic tissue damage. Thus, carbon tetrachloride is not likely to be carcinogenic under occupational exposure conditions providing protection from toxicity. Accordingly, carbon tetrachloride is categorised into the *SCOEL carcinogen group D*, as a non-genotoxic carcinogen with a threshold based on its mode of action (Bolt and Huici-Montagud 2007).

In the earlier recommendation of SCOEL, the study of Adams *et al* (1952), establishing a NOAEL of 5 ppm (32 mg/m^3) for liver damage in animals, was considered a basis for proposing an Occupational Exposure Limits of 1 ppm (SCOEL Recommendation, 1993). In the meantime, this has been further supported by published data both in animals (Nagano et al. 2007) and in humans (Tomenson et al. 1995). In rats, the publication of Nagano et al. (2007) has confirmed a NOAEL of 5 ppm in mice after exposure for 2 years (fom concentrations of 25 ppm on toxicity occurred in the liver, spleen and kidneys). In the occupational field study of Tomenson et al. (1995), serum parameters of hepatotoxicity were not altered in a low-exposure group (exposed up to 1 ppm). [A slight change of packed blood cell volume, statistically significant in this low-dose group, was not dose-dependent and therefore not considered to be an adverse effect.] At higher exposure concentrations (1-3 ppm; 4 ppm and more) effects were not consistent. From this study SCOEL concludes that an airborne level 1 ppm carbon tetrachloride represents an established NOAEL for humans under industrial exposure conditions, which very likely also includes a further margin of safety.

Hence, the recommended Occupational Exposure Limit (OEL; 8-hour TWA) is 1 ppm (6.4 mg/m^3). In view of a report of increased serum enzymes in rats treated with 10 ppm (63 mg/m^3) carbon tetrachloride for 1 h/d (McSheehy et al, 1984), a STEL (15 mins) of 5 ppm (32 mg/m^3) can be proposed to limit peaks of exposure which could result in hepatotoxicity.

A "skin" notation was recommended as dermal absorption may contribute substantially to the total body burden (see chapter 2.1).

According to the available experimental data, effects on the progeny are avoided at the proposed OEL.
Because of the nowadays very much restricted use of carbon tetrachloride and a critical sampling time, a BLV is not recommended.

At the levels recommended, no measurement difficulties are foreseen.

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This revision of SCOEL/SUM/31 was based on criteria documents published by WHO/IPCS (1999) and DFG (2002). This was supplemented by a search of the recent literature. Comments made during Public Consultation were incorporated.