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GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

# Vinyl chloride

## MAK Value Documentation, supplement – Translation of the German version from 2019

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**Keywords**

vinyl chloride; liver; hepatic angiosarcomas; hepatocellular carcinomas; human carcinogen; exposure–risk relationship; tumor risk; genotoxicity

## Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated vinyl chloride [75-01-4] considering all toxicological end points. Vinyl chloride is a genotoxic liver carcinogen in humans and animals. The re-evaluation showed that a maximum concentration at the workplace (MAK value) cannot be derived and vinyl chloride remains classified in Carcinogen Category 1. However, the Commission has, for the first time, established an exposure–risk relationship for a carcinogen using an approach similar to that of the Dutch Expert Committee on Occupational Safety. Pre-defined excess risks for hepatic angiosarcomas after occupational exposure to vinyl chloride were calculated from two large epidemiological studies. The exposure–risk relationships for both studies were similar. Concentrations of 40, 4, and 0.4 ml/m$^3$ for a 40-year exposure correspond to risks of 4:1000, 4:10 000, and 4:100 000, respectively. A 40-year exposure to 1 ml/m$^3$ thus results in a risk of 1:10 000. These risk values also cover the risks for hepatocellular carcinomas. Vinyl chloride is a mutagen in vitro and in vivo. It can reach the testes of animals, but does not lead to dominant lethal mutations in mice and rats and is therefore not classified as a germ cell mutagen. Vinyl chloride from the gas phase is not taken up via the skin in toxicologically relevant amounts. Studies on sensitization are not available and there are no reports on sensitization in humans.

Citation Note:
Hartwig A, MAK Commission. Vinyl chloride. MAK Value Documentation, supplement – Translation of the German version from 2019. MAK Collect Occup Health Saf. 2023 Jun;8(2):Doc038. https://doi.org/10.34865/mb7501e8_2ad

Manuscript completed:
12 Dec 2017

Publication date:
30 Jun 2023

| | |
|---|---|
| **MAK value** | – |
| **Peak limitation** | – |
| **Absorption through the skin** | – |
| **Sensitization** | – |
| **Carcinogenicity (1977)** | **Category 1** |
| **Prenatal toxicity** | – |
| **Germ cell mutagenicity** | – |

**BAR** **1.5 mg thiodiglycolic acid/l urine**

**EKA**

| vinyl chloride (air) | thiodiglycolic acid (urine) |
|---|---|
| 1 ml/m³ | 1.8 mg/24 hours |
| 2 ml/m³ | 2.4 mg/24 hours |
| 4 ml/m³ | 4.5 mg/24 hours |
| 8 ml/m³ | 8.2 mg/24 hours |
| 16 ml/m³ | 10.6 mg/24 hours |

**1 ml/m³ (ppm) ≙ 2.59 mg/m³** **1 mg/m³ ≙ 0.385 ml/m³ (ppm)**

Since the 1986 documentation was published (Henschler 1993), new data for the mechanism of action, genotoxicity, carcinogenicity and toxicity after repeated exposure and several reviews (Bolt 2005; IARC 2008; Kielhorn et al. 2000; OECD 2001; Sherman 2009; WHO 1999) have become available, making a re-evaluation necessary. The EU has set a binding limit value of 1 ml/m³ for vinyl chloride, valid as from 2020 (European Parliament and European Council 2017).

## 1 Toxic Effects and Mode of Action

See the documentation from 1986 (Henschler 1993).

## 2 Mechanism of Action

Vinyl chloride is a genotoxic carcinogen. Exposure to the substance primarily induces angiosarcomas (haemangioendotheliomas) of the liver both in humans and in rats. These tumours are very rare in humans and make up only about 2% of all primary liver tumours (Weihrauch et al. 2002 b).

Vinyl chloride is metabolized by CYP2E1 to form chloroethylene oxide and chloroacetaldehyde. This metabolic pathway is saturated at concentrations above 100 ml/m³. The two reactive metabolites form covalent bonds with proteins, DNA and RNA. Chloroethylene oxide was found to react rapidly with DNA and chloroacetaldehyde to react slowly with proteins (Bolt 2005). Vinyl chloride induces gene mutations and chromosomal aberrations in bacteria, yeasts, mammalian cells, Drosophila, rodents and humans (WHO 1999). The most prevalent mutagenic effects are DNA base-pair substitutions. Both chloroethylene oxide and chloroacetaldehyde react with DNA bases to form the main adduct 7-(2′-oxoethyl)guanine and 4 exocylic etheno adducts, 1,$N^6$-ethenoadenine (εA), 3,$N^4$-ethenocytosine (εC), $N^2$,3-ethenoguanine ($N^2$,3-εG) and 1,$N^2$-ethenoguanine (1,$N^2$-εG). 1,$N^6$-Ethenoadenine (εA) and 3,$N^4$-ethenocytosine (εC) are weakly mutagenic in vitro and in vivo; $N^2$,3-ethenoguanine ($N^2$,3-εG) is a strong mutagen (Hang et al. 1996, 1997, 1998). All 4 etheno adducts are premutagenic, primarily inducing base-pair substitutions and frameshift

mutations. The induction of the following base transitions was observed in test systems in vitro, in Escherichia coli and in mammalian cells: εG and εC lead to G:C⟶A:T transitions, εC leads to C:G⟶A:T transversions. εA leads to the misincorporation of G, C or A during DNA synthesis, resulting in the sequence changes A:T⟶C:G, A:T⟶G:C and A:T⟶T:A. The most prevalent DNA adduct is 7-(2′-oxoethyl)guanine, making up 98% of the adducts. 7-(2′-Oxoethyl) guanine is at most weakly premutagenic. It is primarily eliminated by chemical depurination; however, repair by DNA glycosylase activity is also possible (Swenberg et al. 2011). Etheno-DNA adducts are repaired by various DNA glycosylases (Bartsch 1999). It was found that 3,$N^4$-ethenocytosine is repaired by human mismatch-specific thymine-DNA glycosylase, while 1,$N^6$-ethenoadenine is repaired by the enzyme alkylpurine-DNA-*N*-glycosylase. Therefore, the two adducts 1,$N^6$-ethenoadenine and 3,$N^4$-ethenocytosine are repaired by two different gene products (Hang et al. 1996, 1997, 1998).

The three etheno adducts εA, εC and $N^2$,3-εG were found in the DNA of rats and mice exposed to vinyl chloride. However, these adducts were identified also in humans and rats without exposure to the substance (Nair et al. 1995). Several authors (Albertini et al. 2003; Nair et al. 2007) suggested that endogenous etheno adducts are formed during fatty acid metabolism, even though the individual molecular mechanisms have yet to be explained. The main products of lipid peroxidation of unsaturated fatty acids are malondialdehyde and *trans*-4-hydroxy-2-nonenal. These are expected to form not only propano adducts, but also substituted ethenoadenine, ethenoguanine, and ethenocytosine adducts (Chung et al. 1996). One of these endogenous adducts, 7-(1′,2′-dihydroxyheptyl)-1,$N^6$-ethenoadenine, which is formed with the epoxide of *trans*-4-hydroxy-2-nonenal, was found in relatively high concentrations in the tissues of rats and humans (Fu et al. 2014). A study determined etheno-DNA adducts in the nuclei from human liver samples using immunohistochemical methods. Ethenoadenine (εA) made up 3.1% of the adducts in healthy persons (number of positively stained cells in relation to the total number of cells), 15% in patients with fatty liver, and 50% in patients with liver fibrosis (Frank et al. 2004).

The etheno adducts are responsible for the development of specific point mutations (G:C⟶A:T transitions and A:T⟶T:A transversions, see above) in oncogenes and tumour suppressor genes, which are in turn associated with angiosarcomas (see Tables 1 and 2). In 3 of 5 examined cases, analyses of liver angiosarcomas from workers exposed to vinyl chloride revealed A:T⟶T:A transversions at different codons of the *TP53* gene (Boivin et al. 1997; Hollstein et al. 1996; Marion and Boivin-Angele 1999). Hepatocellular carcinomas in rats induced by exposure of the animals to vinyl chloride demonstrated that the *Hras* gene was activated at codon 61 by A:T⟶T:A transversion (see Tables 1 and 2; Kielhorn et al. 2000).

**Tab. 1** Comparison of the mutation spectra at the *TP53* gene in the livers of humans and rats following exposure to vinyl chloride (Kielhorn et al. 2000; Smith et al. 1998)

| Species | Tumours | Number of mutations/number of cases | Number and kind of mutation |
|---|---|---|---|
| human | angiosarcomas | 3/6 | 3 A:T→T:A<br>CAT→CTT, codon 179;<br>AGG→TGG, codon 249;<br>ATC→TTC, codon 255 |
| rat | angiosarcomas | 11/25 | 5 A:T→T:A<br>2 A:T→T:C<br>2 A:T→C:G<br>3 G:C→A:T<br>one 12-base pair deletion<br>1 deletion |
| | hepatocellular carcinomas | 1/8 | 1 A:T→T:A |

**Tab. 2** Mutations of the RAS proto-oncogene in human and rat liver tumours following exposure to vinyl chloride (Kielhorn et al. 2000)

| Species | Tumour | Gene | Codon | mutations/ tumours | Base-pair mutations | Codon mutations |
|---|---|---|---|---|---|---|
| human | angiosarcomas | *KRAS* | 13 | 15/18 | G→A | GGC→GAC |
| rat | angiosarcomas | *Kras* | | 0/10 | | |
| | hepatocellular carcinomas | *Hras* | 61 | 5/8 | A:T→T:A | A:T→T:A |

The mutation spectra of the liver tumours associated with exposure to vinyl chloride, that is, of angiosarcomas and hepatocellular carcinomas, are clearly different from the mutation spectra of sporadically occurring liver tumours or of liver tumours induced by other carcinogens (Kielhorn et al. 2000). *KRAS* mutations in the DNA of tumours and p21 protein in the blood were detected in 4 of 5 cases of angiosarcomas in persons exposed to vinyl chloride, but in none of 18 control persons without exposure to the substance. In addition, mutant p53 protein in serum was found in 3 of 21 workers exposed to vinyl chloride and in only 1 of 18 persons without exposure. As mutant *TP53* genes form anomalous protein products and anti-p53 antibodies have been found in the serum of patients with hepatocellular carcinomas, several authors attempted to use these kinds of serum antibodies as a biomarker for the early diagnosis of haemoangiosarcomas. However, to date, this has not been possible with the requisite reliability (Marion et al. 1996; Trivers et al. 1995).

A review found that liver tumours associated with exposure to vinyl chloride reveal a specific mutation spectrum. This comprises mutations in the *KRAS* gene of human angiosarcomas, mutations in the *Hras* gene of hepatocellular carcinomas in rats and mutations in the *TP53* gene of human and rat angiosarcomas (Barbin 2000).

The production of mutations in cellular tumour suppressor genes such as *p53* is involved in the development of many types of cancer. These mutations result in the expression of "mutant" forms of p53 protein. Vinyl chloride induces specific *TP53* mutations in persons with angiosarcomas. As mentioned above, a number of authors suggested that seropositivity for "mutated" p53 protein may have a predictive value (Smith et al. 1998; Trivers et al. 1995; see Section 4.6). However, other authors (Sherman 2009) are sceptical that the occurrence of altered p53 proteins has the requisite specificity and sensitivity for use as evidence of vinyl chloride exposure or even for quantifying the risk of developing an angiosarcoma. Even though the evidence clearly demonstrates that *KRAS* and *TP53* mutations occur in angiosarcomas, they can be found also in tumour types that are not attributable to vinyl chloride exposure. Mutations in the *KRAS* gene are observed at codon 13, or less commonly at codon 12 of the same gene. As the mutations in the *TP53* gene of angiosarcomas occur in several and different positions on the gene after exposure to vinyl chloride, they cannot be regarded as characteristic of vinyl chloride exposure. In addition, the general occurrence of "mutated" KRAS and p53 proteins in the blood of vinyl chloride workers does not appear to offer the requisite specificity (Sherman 2009).

Other studies investigated the involvement of the gene locus *INK4a-ARF* (official gene symbol *CDKN*$^2$*A*) in the development of angiosarcomas in the liver (Chaubert et al. 1997; Weihrauch et al. 2002 b).

A brief explanation to aid in the understanding of these studies. The gene locus *INK4a-ARF* is located on chromosome 9p21. The transcripts differ primarily in their start codons, as these are produced by two different exon 1 regions (of a total of 3 exons). In the case of *p14(ARF)*, this opens an alternate open reading frame (ARF). The resulting gene products are two cell cycle regulating proteins with varying sequence lengths: *p16(INK4a)* contains 156 amino acids, while *p14(ARF)* contains 132 amino acids. *p14(ARF)* functions as a stabilizer of the tumour suppressor protein p53 because it inhibits an ubiquitin ligase that is responsible for the degradation of p53. By contrast, *p16(INK4a)* inhibits the onset of cell division by blocking the key enzyme for its activation, cyclin-dependent protein kinase 4/6. Even though both proteins differ in structure and function, they both keep the cell from entering the cell division cycle. This suppression does not take place if the underlying (tumour suppressor) genes are damaged or silenced by (over)methylation. The authors mentioned above examined the gene locus *INK4a-ARF* in primary liver angiosarcomas of 19 patients using a series of analysis techniques such as methylation-specific polymerase chain reaction, a restriction enzyme-based (that is, exon-related) polymerase chain reaction (REPCR), microsatellite analysis and DNA sequencing. The control group consisted of 12 angiosarcomas from other organs of other patients. Promoter methylation of *p14(ARF)* was determined in 5 of the 19 cases (26%), and anomalous promoter methylation of *p16(INK4a)* was observed in 12 of the 19 cases (63%). Homozygous deletion of the *INK4a-ARF* locus was found in one tumour (5%). Methylation and deletion correlated with

the loss of mRNA transcription. In 3 of 5 angiosarcomas, methylation was found at the *p14(ARF)* gene and concurrently at the *p16(INK4a)* promoter. By contrast, anomalous methylation of *p14(ARF)* was not linked with the occurrence of *TP53* mutations. In 6 of 19 cases (32%), the only mutation found was anomalous methylation of *p14(ARF)*. These data indicate that the *INK4a-ARF* locus is frequently inactivated in angiosarcomas of the liver and that promoter methylation is the primary cause of the inactivation of this gene locus.

In addition, it was demonstrated that gene polymorphisms of enzymes responsible for the metabolism of vinyl chloride, in particular during phase I (that is, CYP2E1) and possibly during phase II (that is, GSTT1 = glutathione S-transferase theta 1), influence the risk of mutations induced by vinyl chloride, irrespective of the level of exposure to vinyl chloride (Schindler et al. 2007; see also Section 4.6).

Even though vinyl chloride is primarily metabolized in the hepatocytes, the target cells for carcinogenicity are the sinusoidal cells of the liver. A study investigated possible causes for the cell specificity in the development of angiosarcomas after exposure to vinyl chloride (Dragani and Zocchetti 2008). CYP2E1 is responsible for the metabolism of vinyl chloride to chloroethylene oxide in hepatocytes (Henschler 1993), while the sinusoidal endothelial cells, from which angiosarcomas develop, exhibited only low metabolic activity for vinyl chloride. Premutagenic $N^2$,3-ethenoguanine is regarded as the primary cause for the development of angiosarcomas. Studies in vitro and in vivo have shown that the metabolites of vinyl chloride are sufficiently stable to diffuse from the hepatocytes to the endothelial cells mentioned above and into the liver capillaries, where they induce DNA damage. In addition, the studies demonstrated that the enzymes epoxide hydrolase and GST, which are involved in the detoxification of vinyl chloride metabolites in mammalian cells, occur predominantly in the parenchymal cells. In rats, the basal proliferation rate of non-parenchymatous cells was more than 2 times higher than that of the hepatocytes; this in turn may lead to a higher number of mutations at the same adduct concentration. Also the DNA repair capacity may contribute to the cell specificity. The studies thus found that the content of 3-methylpurine-DNA-glycosylase is more than 4 times as high in hepatocytes as in non-parenchymatous cells (Dragani and Zocchetti 2008; Swenberg et al. 1999; see also Figure 1). After exposure to vinyl fluoride, an analogue to vinyl chloride, non-parenchymatous cells contained only an eighth of the (induced) amount of 3-methylpurine-DNA-glycosylase-mRNA found in hepatocytes (Swenberg et al. 1999).

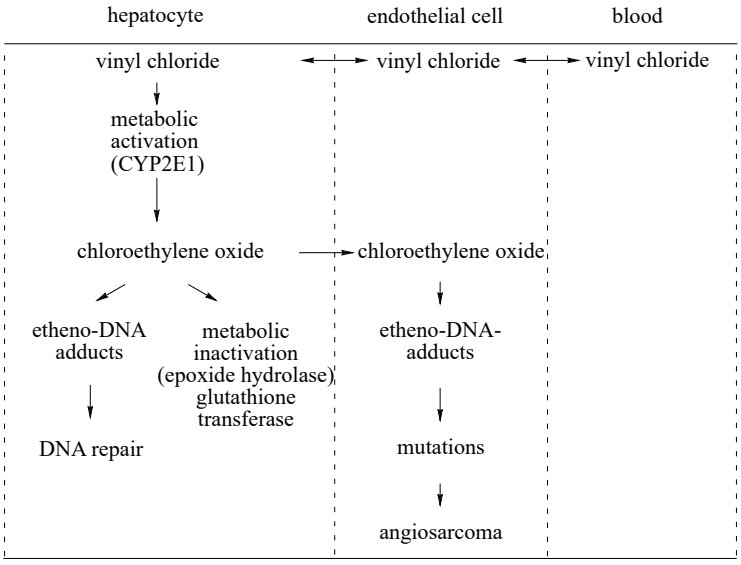


**Fig. 1** Carcinogenic mechanism of vinyl chloride taking into account the higher sensitivity of liver endothelial cells compared with that of hepatocytes (Dragani and Zocchetti 2008)

The likelihood that a DNA adduct will cause neoplastic transformations depends on such factors as its persistence and how accurately DNA damage is repaired. Even though all etheno adducts are repaired by the same DNA glycosylase, specifically 3-methyladenine-DNA-glycosylase, this does not occur at the same rate. In particular, the $N^2$,3-etheno

adduct of guanine has a long retention time in comparison with that of 1,$N^6$-adenine (half-life εG: about 150 days in comparison with that of εA: about 24 hours) (Dosanjh et al. 1994; Mutlu et al. 2010). However, in humans, the thymine-DNA glycosylase responsible for base-pair mismatches contributes to the excision of 3,$N^4$-ethenocytosine (Saparbaev and Laval 1998).

In addition to angiosarcomas of the liver, vinyl chloride may induce also hepatocellular carcinomas in exposed persons. Vinyl chloride is causally associated with the development of a form of non-cirrhotic portal hypertension, which exacerbates the damage caused to the sinusoidal endothelium and thus contributes indirectly to the formation of rare angiosarcomas of the liver. Hepatocellular carcinomas, however, are not a rare form of cancer. Therefore, persons exposed to vinyl chloride may develop hepatocellular carcinomas even if these were not actually caused by vinyl chloride. Nevertheless, the IARC (2008) concluded that there was "sufficient evidence" that hepatocellular carcinomas are induced by vinyl chloride. In spite of this, Sherman (2009) does not consider the evidence for the induction of hepatocellular carcinomas by vinyl chloride to be convincing.

There is no evidence that a cytotoxic mechanism is involved in the development of liver tumours after exposure to vinyl chloride (Albertini et al. 2003). CYP2E1 metabolism became saturated in humans, monkeys and rats at the same level of exposure at which a plateau was reached in the development of angiosarcomas in rats (Albertini et al. 2003).

In summary, the mechanism of action of vinyl chloride is based on the mutagenic potential of the induced DNA base modifications, which result from the metabolism of vinyl chloride to chloroethylene oxide and chloroacetaldehyde and the formation of DNA adducts. This is supported by the coherent mutation spectrum that was demonstrated for the genes responsible for angiosarcomas (Dogliotti 2006). However, a recent publication suggested that the endothelial cells of the liver may require a proliferative stimulus for the induction of mutations after exposure to vinyl chloride. After exposure to high concentrations of vinyl chloride (1000 ml/m$^3$), steatohepatitis, a type of fatty liver disease characterized by inflammation of the liver, was determined in 25% of the workers with angiosarcomas. The authors suggested that, should steatohepatitis be necessary for the induction of liver angiosarcomas after exposure to vinyl chloride, there would have to be a "threshold value" for this mechanism of action (Cohen et al. 2009).

After inhalation exposure, foci of altered hepatocytes develop prior to the formation of liver tumours. These are regarded as preneoplastic lesions (Bannasch 1986; Goodman et al. 1994; Williams et al. 2000). An increase in basophilic and mixed cell foci, that is, liver foci containing eosinophilic and basophilic cells, was determined in rats also after oral administration of vinyl chloride (Til et al. 1991). In general, a certain percentage of liver foci exhibit phenotypic reversibility depending on the dose and other experimental conditions (Bannasch 1986). Less than 1 in 1000 foci develop into neoplasms by promotion. The development of preneoplastic liver foci is supralinear at high dose levels which induce compensatory cell proliferation; this has been demonstrated using diethylnitrosamine as an example (Williams 1989; Williams et al. 2000).

## 3 Toxicokinetics and Metabolism

See the documentation from 1986 (Henschler 1993).

Only a small amount of the epoxide produced during the metabolism of vinyl chloride was found to be available for DNA alkylation in vivo; the main fraction of the epoxide is detoxified via conjugation with glutathione (Bolt 2005).

A study detected two etheno adducts of guanine, 1,$N^2$-ethenoguanine and $N^2$,3-ethenoguanine, in the urine of 13 persons without exposure using two-dimensional liquid chromatography in combination with electrospray tandem mass spectrometry and gas chromatography–mass spectrometry (Gonzalez-Reche et al. 2002). The concentration of ethenoguanine isomers excreted in the urine was in the lower nmol/l range (< 0.3–8 nmol/l). These ethenoguanines may have been produced during DNA repair, but it first needs to be established that the various RNA, which also may contain etheno products, do not come into question as a potential source (Chung et al. 1996).

The $N^2$,3-ethenoguanine adduct was detected in DNA hydrolysates from the liver, lungs and kidneys of rats exposed to vinyl chloride using similar methods of analysis (liquid chromatography quadrupole mass spectrometry). A half-life of

150 days was determined for the adduct in the liver and lungs and of 75 days in the kidneys (Mutlu et al. 2010). The half-life of 7-(2-oxoethyl)guanine was calculated to be 4 days, that of 1,$N^6$-ethenoadenine 24 hours (Swenberg et al. 2011).

Gaseous vinyl chloride is absorbed through the skin in negligible amounts. In a study in rhesus monkeys exposed whole-body (excluding the head) to radioactively-labelled vinyl chloride at concentrations of 800 or 7000 ml/m$^3$ for 2 to 2.5 hours, absorption through the skin was determined to be 0.031% and 0.023%, respectively, of the amount of vinyl chloride present in the chamber. At a concentration of 7000 ml/m$^3$, this was equivalent to 0.787 mg. The authors estimated that a man weighing 90 kg would absorb 4.75 mg of vinyl chloride through the skin after being exposed to a concentration of 7000 ml/m$^3$ for 2 hours. This is equivalent to approximately the amount that would be absorbed after inhalation exposure to 0.2 ml/m$^3$ for 8 hours assuming 100% absorption by inhalation (Hefner et al. 1975). Assuming an exposure period of 8 hours for absorption through the skin, the fraction absorbed through the skin in comparison with the amount absorbed by inhalation is about 0.01% and thus negligible.

The concentration of vinyl chloride in an aqueous film on the skin can be estimated based on the body surface of monkeys (3450 cm$^2$), an exposure period of 2 hours, a concentration of 7000 ml/m$^3$, the Henry's constant of 0.0278 atm-m$^3$/mol (NCBI 2023) and a molar mass of 62.5 g/mol. By applying the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), absorption through the skin is calculated to be in a range from 1.15 to 17 mg. The level of absorption calculated by applying the models is thus somewhat higher than that determined experimentally.

# 4 Effects in Humans

## 4.1 Single exposures

There are no new data available.

## 4.2 Repeated exposure

A study investigated the liver function parameters of 347 workers exposed to vinyl chloride from 1994 to 1997. The enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT), the hepatitis B virus antigen (protein fragment with 57 amino acids; HBsAg) and anti-hepatitis C virus antibodies (protein fragment with 543 amino acids; anti-HCV) were determined in the serum. Liver and spleen were examined by ultrasound. Precirrhotic and cirrhotic cases were assigned to the general category of liver fibrosis. The concentrations of vinyl chloride were determined using personal air monitoring or stationary determinations and ranged from below 1 ml/m$^3$ to 80 ml/m$^3$. The odds ratio (OR: 5.5, 95% CI: 1.7–25.4) determined for liver fibrosis in 194 workers with high levels of cumulative exposure (2400 ml/m$^3$ × month) was high in comparison with that for 153 persons without exposure. The incidence of liver fibrosis discovered by ultrasound increased significantly with the cumulative dose; 2% of the cases were determined in the low exposure group (n = 153), 5.2% in the moderate exposure group (n = 97) and 12.4% in the high exposure group (n = 97). In the high exposure group, hepatitis B and C virus infections increased the OR above from 5.5 to 40.8, which was statistically significant. Another risk factor for liver fibrosis was being overweight. As liver function tests did not result in unusual findings in half of the persons diagnosed with liver fibrosis by ultrasound, the authors recommended the use of ultrasonography as an additional monitoring instrument (Hsiao et al. 2004).

Another study found a correlation between liver cirrhosis and cumulative exposure to vinyl chloride. The livers of 320 workers employed in 5 different vinyl chloride production plants were investigated by ultrasound. The highest levels of cumulative exposure were greater or equal to 800 ml/m$^3$ × year. Liver fibrosis was diagnosed in 13 workers (4.1%) (Hsieh et al. 2007).

By contrast, the study of Maroni and Fanetti (2006) did not find a correlation between exposure to vinyl chloride and changes in liver function parameters. In this study with 757 workers from vinyl chloride production plants, liver

function parameters in the blood were determined and the liver was examined by ultrasound. Mean annual exposure to vinyl chloride ranged from 1 to 500 ml/m$^3$.

A case–control study with 18 patients with liver carcinomas and 68 control persons investigated a possible relationship between viral hepatitis B infection and the development of liver cancer after exposure to vinyl chloride. A higher risk of developing liver cancer was calculated for HbsAg carriers with occupational exposure to vinyl chloride in comparison with the risk determined for HbsAg-negative patients (Wong et al. 2003). However, the number of cases is too small to draw any final conclusions. A review of workers exposed to vinyl chloride reported similar findings (see also Section 4.7). This study found that exposure to vinyl chloride interacted additively with viral hepatitis infection in the development of liver cancer (Mastrangelo et al. 2004).

A case–control study in China investigated the relationship between the genetic polymorphism of metabolic enzymes and liver damage in vinyl chloride workers. The vinyl chloride concentrations were determined to be in a range from 0.85 to 48 mg/m$^3$ (0.35–20 ml/m$^3$) (geometric mean 7.11 mg/m$^3$). Cumulative exposure was calculated to be in a range from 1047 to 33 357 mg. Cumulative exposure levels > 15 000 mg were defined as high and exposure levels < 15 000 mg were defined as low. The group of workers exposed to vinyl chloride comprised 238 persons and the control group was made up of 212 persons. The differences in the findings of neurasthenia, pharyngeal irritation, liver anomalies (determined by ultrasonography) and haemoglobin changes between the groups of exposed and control persons were statistically significant. The incidence of liver damage was significantly higher in the high exposure group than in the low exposure group. A univariate analysis demonstrated that the CYP2E1 c1c2/c2c2 genotype was associated with liver damage in all exposed persons (Zhu et al. 2005 b).

In a group of persons exposed to high concentrations of vinyl chloride (1000 ml/m$^3$) who were not overweight, 80% had steatohepatitis, and of these, 55% had liver fibrosis and 4 exposed persons (20%) angiosarcomas. Insulin resistance with decreased adiponectin concentrations, increased levels of tumour necrosis factor α and increased levels of interleukin 1β, 6 and 8 were determined in all of the steatohepatitis cases. The antioxidative activity in the serum was reduced. The authors suggested that steatohepatitis may contribute to the development of liver angiosarcomas induced by exposure to vinyl chloride (Cave et al. 2010).

## 4.3 Local effects on skin and mucous membranes

There are no new data available.

## 4.4 Allergenic effects

There are no data available.

## 4.5 Reproductive and developmental toxicity

There are no data available.

## 4.6 Genotoxicity

The incidences of DNA damage, sister chromatid exchange, micronuclei and chromosomal aberrations were increased in the lymphocytes of persons exposed to vinyl chloride (ATSDR 2006). Recent genotoxicity studies are described below.

### 4.6.1 Mutations

In 468 vinyl chloride workers, the occurence of two biomarkers for altered or “mutant” oncoproteins relevant for the development of cancer, ras-p21 and p53, was examined in relation to the exposure. In comparison with the levels determined in the group of control persons without exposure, the OR for the presence of both biomarkers was 7.3

($p < 0.05$) at an exposure level of 40 ml/m$^3$-years. At exposure levels of 10 to 40 ml/m$^3$-years, the OR was 5.7 ($p < 0.05$) and at < 10 ml/m$^3$-years, the OR was no longer statistically significant at 1.7. The authors concluded that persons with exposure > 40 ml/m$^3$-years (> 1 ml/m$^3$) may have an increased risk of liver tumours, but that exposure to vinyl chloride for 40 years at a concentration of 0.25 ml/m$^3$ (= 10 ml/m$^3$-years) does not significantly increase the risk of liver tumours (Brandt-Rauf et al. 2002). Exposure to 0.25 ml/m$^3$ thus represents somewhat less than a doubling of the background risk (OR 1.7) for the occurrence of both biomarkers. Among the control persons, 16/155, or about 10%, tested positive for one of the biomarkers.

In Germany, liver cancer (primary liver cell carcinoma and cholangiocarcinoma) is a rare tumour disease, but one that frequently leads to death. In 2013, this form of cancer made up a total of 1.8% of all malignant tumour diseases in Germany. A total of 2630 women and 6160 men were diagnosed with this form of cancer (Robert Koch-Institut 2016). The lifetime risk of developing liver cancer is markedly lower (estimated: 47% probability of developing cancer (Robert Koch-Institut 2016) × 1.8% = 0.8%) than the incidence of positive oncoprotein biomarkers (10%, see above); therefore, mutations in p53 and ras-p21 are a biomarker with a higher incidence than the development of liver cancer.

A study with the objective of recognizing "mutant" p53 antigens and their specific antibodies in the blood of vinyl chloride workers prior to the manifestation of angiosarcomas investigated 151 exposed workers from a vinyl chloride factory in Italy (exposure range: 4 to 2823 ml/m$^3$) and 136 group-matched workers from the same factory without exposure. No seropositive cases were determined among the control persons. Among the exposed persons, only 3 (2.0%) were found to have a mutant p53 antigen, while 2 other workers had the corresponding specific antibodies (p53-related mutations in 3.3% of the workers in total). The seropositive workers were exposed to vinyl chloride concentrations above 1000 ml/m$^3$. On the basis of their findings, the authors were critical of the much higher seropositivity figures determined by other laboratories and sceptical about the use of the p53 antigen or p53 antibodies as markers for an early tumour diagnosis (Mocci and Nettuno 2006).

In 251 vinyl chloride workers and 36 control persons in Taiwan, the presence of Asp13-p21-KRAS oncoprotein (method of analysis used: chemiluminescence western blotting) and "mutant" p53 (method of analysis: ELISA), both the antigen itself and its antibodies, were determined in blood plasma. The Asp13-p21-KRAS oncoprotein was found in 25 of 251 vinyl chloride workers, but in none of the control persons. A total of 15 of 95 workers in the high exposure group (> 480 ml/m$^3$-months) and 10 of 156 workers with lower levels of exposure (< 480 ml/m$^3$-months) were seropositive. The 36 control persons were not seropositive. After adjusting for age and alcohol consumption, an OR of 4.8 (95% Cl: 0.8–28) was calculated for the workers with high levels of exposure and an OR of 1.2 (95% CI: 0.1–9.8) for the workers with lower levels of exposure. The results were regarded as evidence of a significant linear trend between exposure and positive findings in the plasma ($p = 0.001$). A total of 33 of 251 vinyl chloride workers (13.2%) yielded positive results for p53 overexpression in the ELISA test; of these, 10% tested positive for antigens and 3% tested positive for antibodies. After adjusting for age, alcohol consumption and smoking, a significant association ($p = 0.032$) was established between p53 expression and cumulative exposure to vinyl chloride. However, the authors were critical of the low sensitivity of the p53 antibody test; at best, it was able to identify 20% to 40% of patients with *TP53* mutations (Luo et al. 2003).

The serum of 225 persons exposed to vinyl chloride (from 1987 to 1992) and 111 control persons was analysed. A dose–response relationship was established between exposure to vinyl chloride and the biomarker for mutant p53 in serum by means of the immunoabsorbent test. However, the exposure data were incomplete; because of the lack of data, confounders such as smoking and alcohol could not adequately be taken into account. In addition, the control persons were patients and may therefore differ from the general population (Smith et al. 1998).

*KRAS-2* mutations in angiosarcomas of the liver were analysed in 15 patients with occupational exposure to vinyl chloride (median cumulative exposure 8260 ml/m$^3$-years; range 3900–21 000 ml/m$^3$-years; average period of exposure 249 months). Heterozygous mutations were found in 8 of 15 patients (53%). Five patients revealed a mutation at codon 12 and three at codon 13. The most common changes were G⟶A transitions in 5 liver angiosarcomas; these lead to the substitution of aspartate by glycine in the p21 protein. In addition, mutations in the *KRAS-2* gene in adjacent, non-neoplastic liver tissues were found in 2 patients (Weihrauch et al. 2002 a).

### 4.6.2 Micronuclei

A total of 317 vinyl chloride workers with an average age of 37 ± 8 years were divided into quartiles on the basis of their cumulative exposure (0–0.47, 0.48–1.12, 1.13–6.35 mg/m$^3$-years). An increased frequency of micronuclei in the peripheral blood lymphocytes was determined in the exposed persons (0.347% ± 0.265%; 30 control persons: 0.160% ± 0.13%; 136 workers from the same company without exposure to vinyl chloride: 0.251% ± 0.196%). In comparison with the number of micronuclei determined in control persons, the higher frequency found in exposed workers was statistically significant. The BMDL (95% confidence limit) for a 10% increase in the micronucleus frequency above the 95$^{th}$ percentile for control persons (0.5%) was 0.54 mg/m$^3$-years (0.2 ml/m$^3$-years) for men and 0.23 mg/m$^3$-years (0.1 ml/m$^3$-years) for women; however, it was 1.08 mg/m$^3$ (0.4 ml/m$^3$-years) for men and women together. The BMDL for men and women together is equivalent to a concentration of 0.01 ml/m$^3$ over an exposure period of 40 years (Wang et al. 2013). Biologically, it is not plausible that the individual BMDLs for men and women are both lower than the BMDL for the two groups together. This is probably attributable to the fact that confidence ranges increase with a decrease in group size, which in turn leads to a decrease in the BMDL. In addition, workers who were exposed at levels between 0 to 0.48 mg/m$^3$-years were investigated in a group together with persons without exposure and treated as persons without exposure. The BMDL was evidently not calculated on the basis of the mean values but using the lower limits of the individual exposure quartiles. Overall, the validity of the calculated BMDL is questionable.

In another study, the genotoxic effects of vinyl chloride were determined in human lymphocytes using the micronucleus test. The study investigated 402 vinyl chloride workers and 141 control persons. Exposure to vinyl chloride was found to increase the micronucleus frequency in lymphocytes by a factor of 3.7. To investigate the progression of chromosomal damage, micronuclei were determined in the lymphocytes of workers in 2004 and again in 2007. A higher number of micronuclei was found in 2007 than in 2004; this is evidence of a correlation between the development of micronuclei and the duration of exposure. A correlation was established also between the number of micronuclei and cumulative exposure. Workers with cumulative exposure levels > 40 000 mg and in the range from 4000 to 40 000 mg had a higher number of micronuclei than workers with cumulative exposure levels < 4000 mg (Ji et al. 2010).

### 4.6.3 Influence of gene and enzyme polymorphisms

In the following study, lymphoblast cell lines were used to investigate the possibility of a relationship between the efficiency of excision repair of ethenoadenosine and a key protein of excision repair, the x-ray cross complementing-1 (XRCC1) protein. This protein frequently has a polymorphism at amino acid 399 (Arg > Gln). It was expected that this relationship would be expressed by the variability of persons with exposure to vinyl chloride for mutagenic changes. To determine the cause of this variability, 7 lymphoblast cell lines taken from vinyl chloride workers were closely examined. Four of these cell lines were homozygous wild-type lines and 3 were homozygous polymorphic variants at amino acid position 399 (Arg > Gln). The cell lines were exposed to vinyl chloride and the excision rate of the ethenoadenosine adduct was determined over a period of 24 hours. XRCC1 does not possess enzymatic activity, but acts as a scaffold protein for the coordination of the base excision complex which includes the enzymes DNA glycosylases, apurinic endonuclease-1, DNA polymerase β, DNA ligase III and the poly(ADP-ribose) polymerases 1 and 2. The above-mentioned polymorphism at position 399 lies in that protein domain (amino acids 315–403) which enables the activity of poly(ADP-ribose) polymerases 1 and 2 and of apurinic endonuclease-1. Structural changes in this domain arising from polymorphism (probably resulting from an A–T transversion) reduce the DNA repair capacity by 50%. This finding not only demonstrates that *XRCC1* is involved in the excision of adducts induced by vinyl chloride, but also that a carrier of this type of (relatively common) polymorphism has an increased risk of developing angiosarcomas when handling vinyl chloride (Li et al. 2009 a).

A follow-up study tested the hypothesis whether common polymorphisms in the DNA repair genes *XRCC1* (see above), *ERCC2/XPD* (gene symbol: *ERCC2*; name of the gene product: "TFII basal transcription factor complex helicase XPD subunit") and *CYP2E1 (c2 allele)* are able to increase the susceptibility for tumour formation of persons exposed to vinyl chloride. The mutation frequency in the biomarkers *TP53* and *ras*-p21 (gene symbol: *RASA1*; gene product: "RAS p21 protein activator" ("GTPase activating protein 1")) was used as a measure of this susceptibility. The function of *XRCC1* was explained in the above paragraph; *ERCC2* codes for an ATP-dependent 5′-3′ DNA helicase, which is 1 of 7

components of the fundamental TFIIH transcription complex and thus plays a role in determining the efficiency of the transcription-coupled nucleotide excision repair of etheno-adducts. *CYP2E1* acts as a catalyst in the formation of the proximal metabolites of vinyl chloride, chloroethylene oxide and chloroacetaldehyde. To test the hypothesis, blood samples were taken from 546 workers exposed to vinyl chloride and 51 control persons without exposure. Lymphocyte DNA was first genotyped for the most common polymorphisms at codons of the above-listed genes using standard polymerase chain reaction techniques based on specifically developed starter sequences, and length polymorphisms were then analysed in the resulting restriction fragments. The codon-bound polymorphisms followed the mutation principle: homozygous wild-type; one variant allele (heterozygous); two variant alleles (homozygous variant), whereby the frequency of wild-type codons and variant codons differed as expected. Mutations in the *ERCC2* gene were used as a typical example because their mutation frequency in the biomarkers had been elaborated as a function of cumulative exposure to vinyl chloride: codon 312: Asp-Asp; Asp > Asn; Asn-Asn. Codon 751: Lys-Lys; Lys > Gln; Gln-Gln. The workers were assigned to 3 subgroups of approximately equal size based on their cumulative exposure to vinyl chloride, which was expressed in ml/m$^3$-years: < 1706, 1706 to 5704, > 5704 ml/m$^3$-years. Codon variations in the DNA repair gene were related to mutation frequencies in the biomarkers. Thus, for the chosen example of *ERCC2* and its homozygous variant codon 751 (Gln-Gln), an OR of 1.4 was calculated for the increase in prevalence of the *RASA1* biomarker at an exposure level of < 1706 ml/m$^3$-years, an OR of 3.8 at 1706 to 5704 ml/m$^3$-years and an OR of 9.7 at > 5704 ml/m$^3$-years. These were then compared with the values determined for the wild-type codon 751 (Lys-Lys), with an OR of 1.0 at an exposure level of < 1706 ml/m$^3$-years, an OR of 0.9 at 1706 to 5704 ml/m$^3$-years and an OR of 3.3 at > 5704 ml/m$^3$-years. This demonstrates that, overall, there was a clear increase in mutations in the *RASA1* gene. A qualitatively similar relationship was found between codon 399 (genotypes: Arg-Arg; Arg > Gln; Gln-Gln) of the *XRCC1* DNA repair gene and the biomarker *TP53*. When the authors did not stratify according to vinyl chloride exposure and placed only the transitions at codons from the homozygous wild-type to the heterozygous variant and homozygous variant in relationship with the prevalence of mutations in the biomarkers, a trend towards increasing allele deviations in the biomarker *TP53* was found for the *XRCC1* DNA repair gene that was statistically significant. The same was found in the case of *ERCC2* and the biomarker *RASA1*. Overall, these findings demonstrate that etheno adducts in the above biomarkers (and probably also in other DNA segments) were removed not only via base excision repair, but also via transcription-coupled nucleotide excision repair and that the susceptibility to oncogenic mutations induced by vinyl chloride increased with an increasing number of alleles deviating from wild-type at common polymorphic codons of the examined DNA repair genes. Gene–gene interactions, for example those between *CYP2E1 c2* and *ERCC2* polymorphisms, even yielded supramultiplicative effects (Li et al. 2009 b).

Another study investigated whether specific polymorphisms in the microsomal epoxide hydrolase gene have an effect on enzyme activity and whether this in turn—if different—influences the detoxification metabolism of vinyl chloride. Thus, the microsomal epoxide hydrolase gene was investigated at codons 113 and 139 in lymphocyte samples from 211 French workers exposed to vinyl chloride. At codon 113, 123 (58%) of the workers were homozygous (Tyr/Tyr) (wild-type), 63 (30%) were heterozygous (Tyr/His) and 25 (12%) were homozygous variant (His/His). At codon 139, 136 (64%) of the 211 workers were homozygous (His/His) (wild-type), 69 (33%) were heterozygous (His/Arg) and 6 (3%) were homozygous variant (Arg/Arg). In vitro analyses of the enzyme variants corresponding to the polymorphisms were essentially assigned to 3 activity groups, one group with low levels of activity, one with moderate levels and one with high levels. However, the varying levels of enzyme activity were not reflected in the mutation frequency of the genes used as biomarkers, *RAS-p21* and *TP53*. In addition, a significant trend was not found. The results demonstrate that polymorphisms of microsomal epoxide hydrolase do not have a decisive influence on the susceptibility to the mutagenic effects induced by vinyl chloride (Li et al. 2005 a).

Another study used the same cohort of 211 persons exposed to vinyl chloride (see above) to investigate the detoxifying activity of the various polymorphic glutathione *S*-transferases GSTM1 (glutathione *S*-transferase Mu 1), GSTT1 (glutathione *S*-transferase theta 1) and GSTP1 (glutathione *S*-transferase P or P1-1). It was expected that chloroethylene oxide would be conjugated (that is, quenched) in phase II metabolism with varying effectivity by the above-listed glutathione *S*-transferases, thus forming mutagenic ethenoadenine adducts in varying amounts in the *TP53* gene, which served as a biomarker. For a better understanding, it is important to know that low glutathione *S*-transferase activity

is frequently caused by GSTM1 null, GSTT1 null and GSTP1b(Ile-Val) alleles. By themselves, these polymorphic GST variants did not have a significant independent effect on the *TP53* biomarker. Therefore, they were not the reason for the interindividual differences in DNA damage induced by vinyl chloride in vinyl chloride workers. However, when the concurrent polymorphism of the XRCC1 protein was taken into consideration (*XRCC1* gene: "x-ray cross complementing-1" gene; it sufficed if only one of the two alleles of the gene had the known Arg-Gln transition at codon 399), a marked effect on mutant *TP53* biomarker status was determined in combination with GSTM1 null (55% of the workers) and GSTT1 null (14% of the workers): the OR increased to 8.4 (95% CI: 1.3–54.0) in comparison with the control value of 1.0, which was assigned to workers who were wild-type for XRCC1+GSTM1+GSTT1. Therefore, workers with the genotype that resulted in the high OR value would have a much higher risk (Li et al. 2005 b).

In a cohort of 597 vinyl chloride workers, cumulative vinyl chloride exposure was found to be positively associated with biomarkers for mutant *RAS-p21* and mutant *RAS-p53*. The CYP2E1 variant with a c2 allele, which is a high activity variant, was found to be significantly associated with the presence of either or both mutant biomarkers. The effects of the c2 allele and those of exposure to vinyl chloride were additive. GSTT1 null status was found to have an increased, but not statistically significant association with the mutant biomarkers (Schindler et al. 2007).

The relationship between polymorphisms of the *XPD* DNA repair gene and DNA damage was investigated in 106 men and 44 women exposed to vinyl chloride. The vinyl chloride concentrations were in a range from 0.18 ml/m$^3$ (0.50 mg/m$^3$) to 108.3 ml/m$^3$ (302.16 mg/m$^3$) with a geometric mean of 2.56 ml/m$^3$ (7.15 mg/m$^3$). The mean length of exposure was 12.1 years. The total amount of vinyl chloride absorbed was calculated for each exposed person, taking into consideration the values for alveolar retention and ventilation, the monthly mean exposure concentrations at the workplace and the individual duration of exposure. DNA damage in the peripheral lymphocytes was determined by single cell gel electrophoresis assay (comet assay). The genomic DNA of the lymphocytes was used to determine the genotypes of the *XPD* alleles. A correlation was found between the *XPD* 751 Lys/Gln and Gln/Gln genotypes and DNA damage. Lower levels of DNA damage were found in persons with the *XPD* Asp/Asn and Asn/Asn genotypes who were exposed to cumulative amounts of vinyl chloride above 10 000 mg in comparison with the level of damage found in other persons with exposure to vinyl chloride (Zhu et al. 2005 a).

The study described below investigated a possible relationship between common polymorphisms of the cell cycle-regulating genes *TP53* (tumour suppressor protein p53), *CDKN1A* ("*p21*"; inhibitor of the cyclin-dependent kinases 2 and 4, which regulate cell cycle progression at the $G_1$ control point) and *CCND1* (cyclin D1, activator of the cell cycle transition from $G_1$ to S) and micronucleus formation induced by vinyl chloride. The genotypes and alleles (WW = wild-type homozygous; WM = wild-type/mutant, heterozygous; and MM = mutant homozygous) were first determined in the blood lymphocytes of 183 workers (116 men and 67 women) employed in a Chinese vinyl chloride polymerization plant who had been exposed to the chemical for a period of at least 1 year (no data for the level of exposure). Micronucleus levels were determined by stimulating the lymphocytes with phytohaemagglutinin and by blocking cytokinesis in the binucleated stage ("cytokinesis-block micronucleus assay"); a higher frequency of micronuclei was found in cases with G > A polymorphisms at intron 6 of the *TP53* gene (with the WM and also WM plus MM genotypes) and also in cases with the Arg72Pro polymorphism (WM genotype) of the same gene. The increase was determined by comparing the findings with those from cases in which the corresponding alleles were wild-type homozygous. According to the authors, a functional deficit in the DNA repair capacity was attributable to mutant alleles at intron 6 of the *TP53* gene. Therefore, they expected a commensurate level of DNA damage after exposure to vinyl chloride. The study investigated also mutant alleles at intron 3 of the *TP53* gene and at exon 3 of the *CDKN1A* gene, mutations of the *CDKN1A* gene that lead to the Arg31Ser transition and mutations of the *CCND1* gene that cause A870G substitution. None of these polymorphisms had an effect on the micronucleus frequency. Irrespective of the G > A polymorphism at intron 6 of the *TP53* gene, the authors found higher levels of micronucleus formation in women exposed to vinyl chloride than in exposed men and higher levels of micronuclei were induced with increasing age. Contrary to expectations, no significant difference in micronucleus frequency was determined by comparing exposure at higher and lower dose levels. Overall, the authors are of the opinion that it is not sufficient to assess the genotoxic effects of vinyl chloride exposure in humans solely on the basis of the cytogenetic marker "micronuclei". Individual demographic factors also need to be considered (Qiu et al. 2008).

A significantly higher micronucleus frequency was found in persons with the homozygous variant *CYP2E1 c1/c2* and the homozygous and heterozygous variants *XRCC1Arg280/His* than in persons who were wild-type homozygous. Also polymorphisms in the *GSTP1* and *ALDH2* genes influenced micronucleus frequency (Ji et al. 2010).

### 4.6.4 Summary

Vinyl chloride leads to an increase in mutations in the *ras-p21* and *ras-p53* oncogenes, in DNA damage in the comet assay and in the micronucleus frequency in the lymphocytes of exposed persons. Polymorphisms in the *CYP2E1* gene, which lead to increased CYP2E1 activity and thus to the formation of chloroethylene oxide, are associated with increased incidences of mutations in both of these oncogenes. Polymorphisms in the glutathione transferases M1 and T1, which reduce enzyme activity and thus the detoxification of the epoxide, lead to an increase in the mutation frequency of *TP53*. Polymorphisms in DNA repair genes lead to increased levels of mutations in the *TP53* and *RASA1* genes. Persons with one or more of these "unfavourable" enzyme polymorphisms may have an increased risk for DNA damage, micronucleus formation, mutations in the oncogenes and thus also for tumours of the liver.

## 4.7 Carcinogenicity

Vinyl chloride is a human carcinogen (Henschler 1993) and induces primarily angiosarcomas of the liver, but also hepatocellular carcinomas. Angiosarcomas are a type of tumour that occurs very rarely in the general population with an incidence of 0.1 per 1 million per year (Ward et al. 2001). Earlier epidemiological studies yielded evidence of a relationship between exposure to vinyl chloride and brain tumours; however, this was not confirmed by the findings of a meta-analysis (Boffetta et al. 2003).

### 4.7.1 Case studies

A liver angiosarcoma was diagnosed in 2008 in a worker involved in the polymerization of vinyl chloride in an autoclave in the years between 1957 and 1965. The latency period of this tumour type was therefore about 50 years (Bolt 2009). This confirms earlier findings of long latency periods in the development of angiosarcomas after exposure to vinyl chloride (Kielhorn et al. 2000).

Two hairdressers were exposed to hair sprays containing vinyl chloride for 4 to 5 years in the period between 1966 and 1973. They were diagnosed with angiosarcomas of the liver in 2003. The peak concentrations of vinyl chloride were estimated to be in the range from 129 to 1234 ml/m$^3$ (Infante et al. 2009).

### 4.7.2 Case–control studies

In a nested case–control study, 38 patients with lung cancer from a cohort of 1658 vinyl chloride workers were compared with 224 control persons without a history of cancer. A 20% increase in the risk of developing lung cancer was determined for each additional year as a PVC worker. However, a dose–response relationship was not determined (Mastrangelo et al. 2003).

Among 691 male and 588 female patients with histologically diagnosed renal cell carcinomas, an increased OR of 2.0 (95% CI: 1.2–3.3) was determined on the basis of data collected by questionnaire for patients with exposure to vinyl chloride (Lewis and Rempala 2003).

A very involved, large case–control study examined whether lung cancer is induced by exposure to vinyl chloride. Fifteen hospitals located in the eastern and central European countries of Romania, Hungary, Poland, Slovakia and the Czech Republic and in the cities of Moscow and Liverpool took part in the study, which was carried out under the auspices of the IARC. All of these countries and regions are known to have particularly high mortality from lung cancer. In the above-listed centres, a total of 2861 new cases of lung cancer were diagnosed for which there was reasonable evidence that they were caused by exposure to vinyl chloride; these were compared with 3118 matched control cases (most of them from the same clinic). During interviews and by completing up to 18 questionnaires the cancer patients

provided detailed information about the chemicals-related work they had performed and the duration of the work. The smoking habits of the cancer and control cases were categorized into 10 levels of smoking intensity. The OR for lung cancer in persons exposed at some time in their lives to vinyl chloride was 1.05 (95% CI: 0.68–1.62). An OR of 1.23 (95% CI: 0.64–2.37) was calculated when only workplaces with a high likelihood of exposure were taken into account. When the data were analysed based on the length of exposure or cumulative exposure, a slight, but not statistically significant, increase in risk was found only for workers who were exposed for a particularly long time to high levels. Taking a latency period of 20 years into consideration had little effect on the results. No upward dose–response trend was found in spite of a slightly increased risk in the highest category of exposure, irrespective of which exposure index was used (Scélo et al. 2004).

### 4.7.3 Cohort studies

In a multicentric study conducted in 4 countries (Italy, Norway, Sweden and the United Kingdom), cancer mortality was investigated in a total of 12 700 workers from 19 vinyl chloride plants. In 11 of the plants, the workers were exposed to a mixture of monomeric and polymeric vinyl chloride, while workers in 2 of the plants were exposed to monomeric vinyl chloride only. Five of the other plants produced polyvinyl chloride (PVC) and 1 plant processed PVC. A total of 22 groups were formed on the basis of a "job exposure matrix". Quantitative exposure data were available for 9775 workers. A total of 2664 deaths (all causes) (SMR 0.85, 95% CI: 0.82–8.88) were registered, including 883 deaths from malignant neoplasms (SMR 0.99, 95% CI: 0.82–0.88). The SMR for liver cancer (53 deaths) was 2.40 (95% CI: 1.80–3.14). In all, 71 liver tumours were found, 37 of these angiosarcomas, 10 hepatocellular carcinomas and 24 other liver tumours. The incidence of liver tumours and angiosarcomas increased with cumulative exposure. The SMR for brain tumours was 0.93 (95% CI: 0.60–1.39), for lung cancer 0.95 (95% CI: 0.84–1.07), for soft tissue sarcomas 1.89 (95% CI: 0.69–4.11), for non-Hodgkin lymphomas 1.19 (95% CI: 0.78–1.75) and for malignant melanomas 1.60 (95% CI: 0.90–2.65) (Ward et al. 2001).

A meta-analysis of 8 independent multicentric studies from the USA, Canada, Europe, the former USSR, China and Taiwan reviewed the cancer mortality data for a total of 43 810 workers from more than 90 vinyl chloride plants. This meta-analysis included the study by Ward et al. (2001). The workers were employed in the production and polymerization of vinyl chloride monomers. However, the studies on which the analysis was based did not report the level of direct exposure to either vinyl chloride or other substances formed during the production process, such as polyvinyl chloride dust or additives. The standardized mortality ratios (meta-SMRs) and their 95% confidence intervals were calculated. Broad inclusion criteria were applied for the selection of individual participants in the analysis; for example, a period of employment lasting 1 year during the specified observation intervals (including 1955–1986; 1942–1972) was included, but also not specifically defined periods of employment over varying periods of time. The years of follow-up also varied from periods of just a few years to several decades; however, all ended in 1997 at the latest. A total of 2079 cases of death from cancer were registered among the 43 810 exposed workers. This number is almost identical to the number of cases that would be expected in a comparable population without exposure (SMR 1.01; 95% CI: 0.95–1.08). A significant increase was reported by all 6 of the 8 studies that determined SMR values for liver cancer. Out of the total 133 deaths that occurred from liver cancer in the two large multicentric studies from the USA and Europe, 65 were known to be cases of angiosarcomas. The remaining 68 deaths from liver cancer included histologically confirmed hepatocellular carcinomas and liver carcinomas of other known histology. When all liver angiosarcomas were excluded, the resulting meta-SMR for the remaining forms of liver cancer was 1.35 (95% CI: 1.04–1.77). In addition, the meta-SMR for soft-tissue sarcomas was significant (2.52; 95% CI: 1.56–4.07). A borderline non-significant meta-SMR was calculated for deaths from tumours of the brain and central nervous system. This increase was mainly due to the large multicentric study from the United States (Mundt et al. 2000), which reported an SMR of 1.42 (95% CI: 1.00–1.97). No increase in deaths from lung cancer was reported. After analysing the data, the authors concluded that vinyl chloride workers not only have the known increased risk of developing angiosarcomas of the liver, but also an increased risk for hepatocellular carcinomas and soft-tissue sarcomas. In addition, the authors suggested that the classification of liver tumours into the (sub-)group of liver angiosarcomas was too restrictive (underdiagnosis). On the basis of the findings of the meta-analysis, the authors assume that increased mortality from cancers of the lungs, brain and the lymphatic and haematopoietic system is possible (Boffetta et al. 2003).

This meta-analysis was followed by the publication of additional cohort studies.

A cohort of chemical workers employed at 3 chemical plants in West Virginia, USA, was observed from 1940 and the cancer mortality was determined for the period from 1940 to the end of 1999. Essentially, the cohort was made up of 30 974 men, including 22 673 (73.2%) part-time workers, 7044 (22.7%) permanent workers and 1258 (4.1%) persons for whom the form of employment could not be determined. These workers were employed mainly in the production of vinyl chloride monomer. Exposure levels were not determined, the sole exposure parameter was the period of employment, added together to person-years with 4 days of full-time employment per week; the specific workplace varied. The 30 974 examined persons were distributed across the following categories according to total length of employment: < 1 year: about 25%; 1 to 4 years: about 22%; 5 to 14 years: about 19%; ≥ 15 years: 34.5%. In spite of these differences in the length of employment, the SMRs were reported for the cohort as a whole. On this basis, SMRs were increased for lymphosarcomas and reticulosarcomas (54 cases; SMR 139, 95% CI: 115–144) and for the category "all other malignant neoplasms" (328 cases; SMR 129; 95% CI: 115–144). Also the SMR for deaths from cancer of the liver and biliary passages was increased; however, the increase was just under the threshold for significance (86 cases; SMR 118; 95% CI: 95–146). According to the authors, a higher than expected number of deaths from kidney and skin cancer was observed, even though the findings were not statistically significant. Overall, the increased SMRs were found primarily among the part-time workers. The authors themselves reported that one limitation of this type of follow-up cohort study of mortality is the absence of data for potentially confounding factors, such as exposure to specific chemicals, and the absence of data for lifestyle factors, such as smoking and alcohol consumption. However, a strength of the study is the nearly complete follow-up of the cohort (95% of the initial participants) (Burns et al. 2006).

A re-analysis used Poisson regression to determine the mortality of 1658 male workers employed in the production of vinyl chloride and polyvinyl chloride during the period from 1972 to 1999. Technicians and employees of the same plant served as the reference group. A significant increase in mortality from all causes of death was observed in all workers (RR 1.55; 95% CI: 1.03–2.35, 229 deaths), in PVC workers employed in packaging (RR 1.72; 95% CI: 1.04–2.83; 49 deaths) and PVC workers employed in production (RR 1.71; 95% CI: 1.09–2.67; 72 deaths). Cases with angiosarcomas of the liver were increased among autoclave workers (RR 9.57; 95% CI: 3.71–24.68, 7 deaths) and cardiovascular diseases among PVC workers employed in packaging (RR 2.25; 95% CI: 1.08–4.70; 12 deaths). Four deaths in the group of workers employed in PVC packaging, 4 deaths in the group of workers employed in PVC production and 6 deaths in the group of other workers (employees in the remaining 24 different departments) were caused by tumours of the haemolymphopoietic system, leukaemia and lymphomas (Gennaro et al. 2008).

A retrospective cohort study established a relationship between exposure to vinyl chloride and hepatocellular carcinomas, leukaemia and tumours of the haemolymphopoietic system. SMR values were determined for 3336 male vinyl chloride workers in Taiwan. All workers were employed in the period before 1994, 36.95% of these prior to 1976, a period when vinyl chloride concentrations were still very high (no quantitative data). The mean employment period was 16.9 years. Mortality from liver cancer was significantly increased in the period from 1989 to 1994 (SMR 1.90, 95% CI: 1.01–3.25), peaked in the period from 1991 to 1996 (SMR 2.31, 95% CI: 1.39–3.61) and was no longer significantly increased in the period from 1994 to 1999 (SMR 1.42, 95% CI: 0.80–2.34). Mortality from leukaemia was significantly increased in the period from 1984 to 1989 (SMR 3.24, 95% CI: 1.24–17.53), peaked from 1985 to 1990 (SMR 7.56, 95% CI: 2.06–1935) and was no longer significantly increased from 1991 to 1996 (SMR 3.24, 95% CI: 0.39–11.69). Mortality from haemolymphopoietic tumours exhibited a similar progression to that for mortality from leukaemia. Mortality from brain or lung cancer was not increased in this study. No information was provided for the level of exposure to vinyl chloride (Hsieh et al. 2011).

In a cohort study with 12 430 workers who were exposed to chloroprene or vinyl chloride, neither an increased risk of mortality from all types of cancers, nor an increased risk of mortality from lung or liver cancer was determined in the workers exposed to vinyl chloride (Marsh et al. 2007). No data were provided for the level of exposure, but the authors assume that the negative findings were obtained because of the relatively low levels of exposure to vinyl chloride.

A cohort from the United States was updated with respect to cancer mortality among persons exposed to vinyl chloride. The incidence of liver angiosarcomas and hepatocellular carcinomas was found to be dependent on cumulative exposure. Among about 10 000 exposed persons, a total of 63 cases of angiosarcomas of the liver and 32 hepatocellular

carcinomas were observed. The exposed persons were assigned to 5 groups on the basis of their cumulative exposure to vinyl chloride (< 63 ml/m$^3$ × years; 63–287 ml/m$^3$ × years, 287–865 ml/m$^3$ × years, 865–2271 ml/m$^3$ × years, ≥ 2271 ml/m × years). In these groups, there were 0, 1, 3, 9 and 50 cases with liver angiosarcomas, respectively, and 3, 3, 1, 4 and 21 cases with hepatocellular carcinomas, respectively. The reference group for angiosarcomas comprised those persons with the three lowest levels of cumulative exposure and was thus made up of 4 cases. For hepatocellular carcinomas, the reference group was the lowest exposure group. In the group with exposure of ≥ 865 ml/m$^3$ × years, the hazard ratio for angiosarcomas was 36.3 and for hepatocellular carcinomas 5.3. The authors are of the opinion that the risk of developing angiosarcomas is not increased at exposure levels below 250 ml/m$^3$ × years, as only one case occurred. The risk for hepatocellular carcinomas was not increased up to 1000 ml/m$^3$ × years (Mundt et al. 2017).

The relationship between exposure to vinyl chloride and the incidence of angiosarcomas of the liver is discussed in more detail in Section 5.8.

In summary, recent studies found a correlation between exposure to vinyl chloride at high concentrations and the development of angiosarcomas and additional evidence for a correlation between exposure to vinyl chloride and hepatocellular carcinomas and soft tissue carcinomas and possibly also tumours of the haemolymphopoietic system.

# 5 Animal Experiments and in vitro Studies

## 5.1 Acute toxicity

There are no new data available.

## 5.2 Subacute, subchronic and chronic toxicity

Groups of 100 female and 100 male Wistar rats were given vinyl chloride at doses of 0, 0.014 or 0.13 mg/kg body weight and day while groups of 50 males and 50 females were given 1.3 mg/kg body weight and day with the feed throughout their lifetime (160 weeks). There was a statistically significant increase in mortality, compared with the incidence in the control animals, only in the high-dose animals after 149 weeks. Body weights and feed consumption were similar in all animals. The glutathione levels in the liver were similar in all animals in weeks 40 and 80. In the female rats, a concentration-dependent and statistically significant increase in basophilic preneoplastic liver foci from 9% to 43% was determined in the animals exposed to the high dose. The authors reported that three earlier experiments carried out at their institute had determined incidences of basophilic liver foci between 12% and 17% in female control animals; therefore, on the basis of these data, the slight increase in basophilic liver foci in the female animals of the low and medium dose groups probably does not have any toxicological relevance. However, 4 studies carried out in parallel to the discussed study determined incidences of basophilic liver foci in the control animals of between 0% and only 8%. A NOAEL (no observed adverse effect level) for the development of basophilic liver foci in female rats cannot be derived on the basis of these data. In addition, the increase in the sum of all preneoplastic foci, including clear cell, mixed cell and eosinophilic foci, was statistically significant in female rats treated at the low dose of 0.014 mg/kg body weight and day ($p = 0.031$). By contrast, the increase in basophilic and clear cell foci in male rats was statistically significant only at the high dose of 1.3 mg/kg body weight and day. In addition, the increase in liver cell polymorphisms was statistically significant in animals of both sexes of the high dose group and there was an increased incidence of liver cysts in the female animals (no other details for the type of cysts) (see Table 3). The increase in the incidence of liver neoplasms was statistically significant only in the male and female animals of the high dose group (Til et al. 1991; see Section 5.7). No NOAEL for changes in the liver was determined in this study.

**Tab. 3** Incidence of liver changes[a)] in Wistar rats after oral exposure to vinyl chloride (Til et al. 1991)

| | | Dose (mg/kg body weight and day) | | | |
|---|---|---|---|---|---|
| | | **0** | **0.014** | **0.13** | **1.3** |
| number of examined rats | ♂: | 99 | 99 | 99 | 49 |
| | ♀: | 98 | 100 | 96 | 49 |
| clear cell liver foci | | | | | |
| one focus or a few foci | ♂: | 12 | 9 | 8 | 16* |
| | ♀: | 4 | 5 | 3 | 13* |
| several foci | ♂: | 0 | 0 | 0 | 3* |
| | ♀: | 0 | 0 | 0 | 6* |
| basophilic liver foci | | | | | |
| one focus or a few foci | ♂: | 4 | 2 | 3 | 8* |
| | ♀: | 9 | 20* | 26* | 19* |
| several foci | ♂: | 0 | 0 | 0 | 0 |
| | ♀: | 0 | 1 | 0 | 2 |
| mixed cell liver foci | | | | | |
| one focus or a few foci | ♂: | 0 | 19 | 2 | 2 |
| | ♀: | 6 | 6 | 4 | 8* |
| eosinophilic liver foci | | | | | |
| one focus or a few foci | ♂: | 1 | 1 | 2 | 1 |
| | ♀: | 0 | 0 | 1 | 10* |
| **all kinds of liver foci (total number)** | ♂: | 17 | 12 | 15 | 30* |
| | ♀: | 19 | 32* | 34* | 58* |
| **number of animals with liver foci** | ♂: | 16 | 12 | 15 | 23* |
| | ♀: | 19 | 27 | 31* | 32* |
| neoplastic nodules (probably adenomas) | ♂: | 0 | 0 | 0 | 3 |
| | ♀: | 0 | 1 | 1 | 10* |
| hepatocellular carcinomas | ♂: | 0 | 0 | 0 | 3* |
| | ♀: | 1 | 0 | 1 | 3 |
| angiosarcomas | ♂: | 0 | 0 | 0 | 1[b)] |
| | ♀: | 0 | 0 | 0 | 2[b)] |
| cysts | | | | | |
| one | ♂: | 1 | 0 | 1 | 0 |
| | ♀: | 3 | 2 | 4 | 1 |
| a few | ♂: | 4 | 4 | 3 | 4 |
| | ♀: | 11 | 11 | 12 | 7 |
| many | ♂: | 0 | 0 | 0 | 0 |
| | ♀: | 3 | 4 | 9 | 24* |
| liver cell polymorphism | | | | | |
| slight | ♂: | 27 | 23 | 26 | 19 |
| | ♀: | 46 | 41 | 49 | 23 |
| moderate | ♂: | 4 | 4 | 7 | 10* |
| | ♀: | 14 | 13 | 8 | 15* |
| marked, severe | ♂: | 1 | 1 | 1 | 3 |
| | ♀: | 2 | 3 | 4 | 9* |

[a)] classified according to Squire and Levitt (1975)
[b)] These tumours are very rare in untreated rats
*$p < 0.05$ (Fisher's exact test)

The values derived from a benchmark calculation for the end point "basophilic liver foci" in female animals were not reliable: the BMD was about 0.0002 mg/kg body weight and the BMDL was $10^{-8}$ mg/kg body weight. The US EPA did not calculate a BMD for this end point either and did not use this effect for the derivation of a NOAEL for non-carcinogenic

end points. Instead, the organization used the end point "liver cell polymorphism" with a NOAEL of 0.13 mg/kg body weight. According to the US EPA, for humans, this is equivalent to 2.5 mg/m$^3$ after lifetime exposure (US EPA 2000) and 14 mg/m$^3$ = 5.5 ml/m$^3$ after working lifetime exposure (× 75/40 years × 52/48 weeks × 7/5 days/week × 20 m$^3$/10 m$^3$).

## 5.3 Local effects on skin and mucous membranes

There are no new data available.

## 5.4 Allergenic effects

There are no new data available.

## 5.5 Reproductive and developmental toxicity

### 5.5.1 Fertility

In a 2-generation study, 30 male and 30 female Sprague Dawley rats per concentration group were exposed whole-body to vinyl chloride concentrations of 0, 10, 100 or 1100 ml/m$^3$. The study was carried out using a method similar to that of OECD Test Guideline 416. Prior to mating for 3 weeks, the animals were exposed for 6 hours a day, on 5 days a week, for 10 weeks, followed by daily exposure for 6 hours (that is, on 7 days a week). Reproductive toxicity was not observed in the parent animals and no effects on the postnatal development of the offspring were observed up to the onset of puberty. In the target organ liver, an increase in the absolute and relative liver weights was found in the F0 and F1 parent animals at concentrations of 10 ml/m$^3$ and above (with the exception of female F1 parent animals) and histological changes in the form of centrilobular hypertrophy at 100 ml/m$^3$ and above (Thornton et al. 2002).

In studies of reproductive toxicity, damage to the seminiferous tubules, disruptions in spermatogenesis and reduced testis weights were observed in rats after inhalation exposure to concentrations of 100 ml/m$^3$ and above for 6 hours a day, on 6 days a week, for 12 months (Bi et al. 1985) and necrosis in the epithelial cells of the testes and disruptions in spermatogenesis after inhalation exposure to 500 ml/m$^3$ for 10 months (Sokal et al. 1980). Both studies are regarded critically because of the procedures used. The temperature and humidity were not kept constant in the inhalation chambers or were not reported. In addition, in the first study, the volume and the air flow were not kept constant between the study groups in the inhalation chambers (ATSDR 2006).

### 5.5.2 Developmental toxicity

Inhalation exposure of groups of 25 Sprague Dawley rats to vinyl chloride concentrations of 0, 10, 100, or 1100 ml/m$^3$ for 6 hours a day from gestation days 6 to 19 did not induce toxic effects on development. Body weight gains were found to be reduced in the dams of all groups in comparison with the body weight gains in the control animals. In addition, increases in the relative kidney weights were observed in the dams at concentrations of 100 ml/m$^3$ and above and in the relative liver weights at 1100 ml/m$^3$ (Thornton et al. 2002).

## 5.6 Genotoxicity

### 5.6.1 In vitro

Vinyl chloride was mutagenic in bacteria and yeasts. Mutagenic effects or maximal effects occurred only with metabolic activation. The most prevalent effects were base-pair substitutions. Vinyl chloride induced clastogenic effects in mammalian cells (ATSDR 2006; Health Council of the Netherlands 2017).

### 5.6.2 In vivo

Vinyl chloride was mutagenic and clastogenic in somatic cells after inhalation exposure. Numerous indicator tests were performed; these found DNA alkylation in rats and mice, DNA damage in mice and the known etheno-DNA adducts in rats (Section 2). Clastogenic effects were determined in the chromosomal aberration test and in the micronucleus test in bone marrow cells. Conversely, no dominant lethal mutations were induced in the germ cells of rats and mice after inhalation exposure to concentrations ranging from 3000 to 30 000 ml/m$^3$ and from 50 to 1000 ml/m$^3$, respectively (5 days to 10 weeks) (ATSDR 2006; Health Council of the Netherlands 2017).

A dose-dependent decrease in methylguanine DNA methyltransferase (MGMT) and XRCC1 and a dose-dependent increase in XRCC3 were determined in isolated hepatocytes after intratracheal instillation of vinyl chloride in rats (Zhu et al. 2004).

$N^2$,3-Ethenoguanine adduct concentrations were determined by LC-MS/MS in the liver, lungs and kidneys of adult and weanling rats after exposure to [$^{13}C_2$]vinyl chloride in a concentration of 1100 ml/m$^3$ for 6 hours a day, on 5 days, or to 1100 ml/m$^3$ on 1 day. In adult animals, 4.1 ± 2.8 adducts/$10^8$ guanine of endogenous and 19.0 ± 4.9 adducts/$10^8$ guanine of exogenous ethenoguanine were determined in the liver, 7.4 ± 0.5 adducts/$10^8$ guanine of endogenous and 8.4 ± 2.8 adducts/$10^8$ guanine of exogenous ethenoguanine in the lungs and 5.9 ± 3.3 adducts/$10^8$ guanine of endogenous and 5.7 ± 2.1 adducts/$10^8$ guanine of exogenous ethenoguanine in the kidneys. In comparison with the levels found in the adult animals, the number of exogenous adducts in weanling rats was 4 times as high in the liver and 2 times as high in the lungs and kidneys (Mutlu et al. 2010).

A study established a relationship between exposure concentrations of vinyl chloride and the formation of $N^2$,3-ethenoguanine, the primary etheno adduct, in the hepatocytes and non-parenchymatic liver cells of exposed Sprague Dawley rats. The study investigated both adult and weanling rats. The adult animals were exposed to vinyl chloride concentrations of 0, 10, 100 or 1100 ml/m$^3$ for 6 hours a day, on 5 days a week, for 1 or 4 weeks. The juvenile rats were similarily exposed, but for only a week. The animals were sacrificed at the end of the exposure period and the hepatocytes and non-parenchymatic liver cells were isolated. $N^2$,3-Ethenoguanine was quantified by acid hydrolysis/IA/GC/ECN/HRMS. The lowest detection limit was 1 to 2 fmol of $N^2$,3-ethenoguanine. The concentration of $N^2$,3-ethenoguanine in unexposed rats was about 0.49 mol/$10^7$ mol guanine. The $N^2$,3-ethenoguanine concentrations both in the hepatocytes and in the non-parenchymatic liver cells increased linearly after exposure in the range from 0 to 100 ml/m$^3$ and reached a plateau at concentrations of 100 ml/m$^3$ and above. After exposure to 10 ml/m$^3$ for 4 weeks, $N^2$,3-ethenoguanine levels increased fivefold in the hepatocytes of adult rats. The authors reported that $N^2$,3-ethenoguanine levels increased by only 50% at a concentration of 1 ml/m$^3$. Adduct concentrations were 2 to 3 times higher in juvenile animals than in adults. No other significant differences were found between the responses determined in hepatocytes and those in non-parenchymatic liver cells. However, the incidence of proliferation in non-parenchymatic liver cells was 2 times greater than that in the hepatocytes (Morinello et al. 2002 a).

$N^2$,3-Ethenoguanine was found also in the brains of weaned rats exposed to vinyl chloride concentrations of 1100 ml/m$^3$ for 5 days, but not in adult rats exposed for up to 4 weeks (Morinello et al. 2002 b).

Angiosarcomas induced in rats after exposure to vinyl chloride were examined for mutations of the connexin 37 (*Cx37*) gene. A total of 22 liver angiosarcomas and 3 hepatic carcinomas were analysed using single strand conformation polymorphism analysis (SSCP) and DNA sequencing. The findings demonstrated that mutations of the *Cx37* gene are very rare (Saito et al. 1997).

## 5.7 Carcinogenicity

After inhalation exposure of Wistar and Sprague Dawley rats to vinyl chloride concentrations of 2.5, 5, 10, 20, 40 or 80 ml/m$^3$ for 3 weeks, a linear relationship was established between the incidence of hepatocellular ATPase-deficient liver foci and the exposure concentration. However, the incidence of hepatocellular ATPase-deficient liver foci was similar to that of spontaneous liver foci in control animals at the two low concentrations of 2.5 and 5 ml/m$^3$ (Bolt 2005).

In a lifetime feeding study in Wistar rats, the increase in the number of liver neoplasms determined in animals of both sexes was statistically significant only in the animals of the high dose group (1.3 mg/kg body weight). The tumour incidence was 1/99, 1/100, 2/99 and 7/49 in male animals and 2/98, 1/100, 3/96 and 8/49 in female animals after exposure to vinyl chloride at doses of 0, 0.014, 0.13 and 1.3 mg/kg body weight and day, respectively. One male and 2 females in the high dose group developed angiosarcomas. Three hepatocellular carcinomas each were determined in the males (3/49) and females (3/49) of the high dose group (Til et al. 1991; see also Section 5.2).

In an inhalation study (Maltoni et al. 1981, 1984) with female Sprague Dawley rats exposed to vinyl chloride concentrations of 1 and 5 ml/m$^3$ (4 hours a day, 5 days a week, 52 weeks), no liver tumours were found after lifelong observation. The incidence of liver tumours (5/40) was increased only after exposure to a vinyl chloride concentration of 25 ml/m$^3$ (US EPA 2000). According to US EPA (2000), the concentration of 5 ml/m$^3$ used for rats in this study is equivalent to a concentration of 0.98 ml/m$^3$ in humans (continuous lifetime exposure concentration necessary for the development of an equivalent amount of metabolites/litre in the liver). Converted to an exposure concentration at the workplace, this is 5.6 ml/m$^3$ (0.98 ml/m$^3$ × 75 years/40 years × 7 days/5 days × 52 weeks/48 weeks × 20 m$^3$/10 m$^3$ = 5.6 ml/m$^3$).

In summary, vinyl chloride induced liver tumours, breast carcinomas, brain tumours, nephroblastomas and tumours in the lungs, forestomach and Zymbal's glands in rats, mice and hamsters given oral doses of 1.3 mg/kg body weight and above and after inhalation exposure at concentrations of 25 ml/m$^3$ and above (Henschler 1993).

## 5.8 Risk assessment

By applying a physiologically-based pharmacokinetic model and taking into consideration findings from carcinogenicity studies performed in animals, a study calculated the risk of developing haemangiosarcomas to be 1.10 to 5.17 per million exposed persons per microlitre of vinyl chloride per cubic metre. The risk calculated on the basis of 3 epidemiological studies was 0.4 to 4.22 per million exposed persons per microlitre of vinyl chloride per cubic metre (Dixit et al. 2003). These risks are valid for lifelong, continuous exposure to vinyl chloride.

On the basis of the findings from a number of animal studies and epidemiological studies, the risk of developing angiosarcomas of the liver was calculated to be $3 \times 10^{-4}$ for workplace exposure at a vinyl chloride concentration of 1 ml/m$^3$ (Bolt 2005; SCOEL 2004).

The following risk assessment is based on the studies of Ward et al. (2001) and Mundt et al. (2017).

It is inferred from the risk assessment of the Health Council of the Netherlands (2017) and the epidemiological study of Mundt et al. (2017) that a risk assessment for angiosarcomas also covers the risk of developing hepatocellular carcinomas in the liver. Therefore, it is not necessary to take tumours other than angiosarcomas into account for the assessment of risk.

An exposure–risk relationship was derived for vinyl chloride (Health Council of the Netherlands 2017). The risk of developing angiosarcomas in the liver was calculated to be 4 in 100 000 after exposure to a workplace concentration of 0.65 mg/m$^3$ for 40 years. This derivation was based on data published by Ward et al. (2001). When all liver tumours are taken into consideration, the risk is about 10% higher. However, as the authors pointed out, unlike angiosarcomas, the other liver tumours were not necessarily induced by vinyl chloride.

In analogy to the publication of Ward et al. (2001), the authors of the study by Mundt et al. (2017) reported the number of angiosarcoma cases and the person-years at risk for the different exposure categories. A dose–response relationship was determined on the basis of the data from both studies (Figure 2).

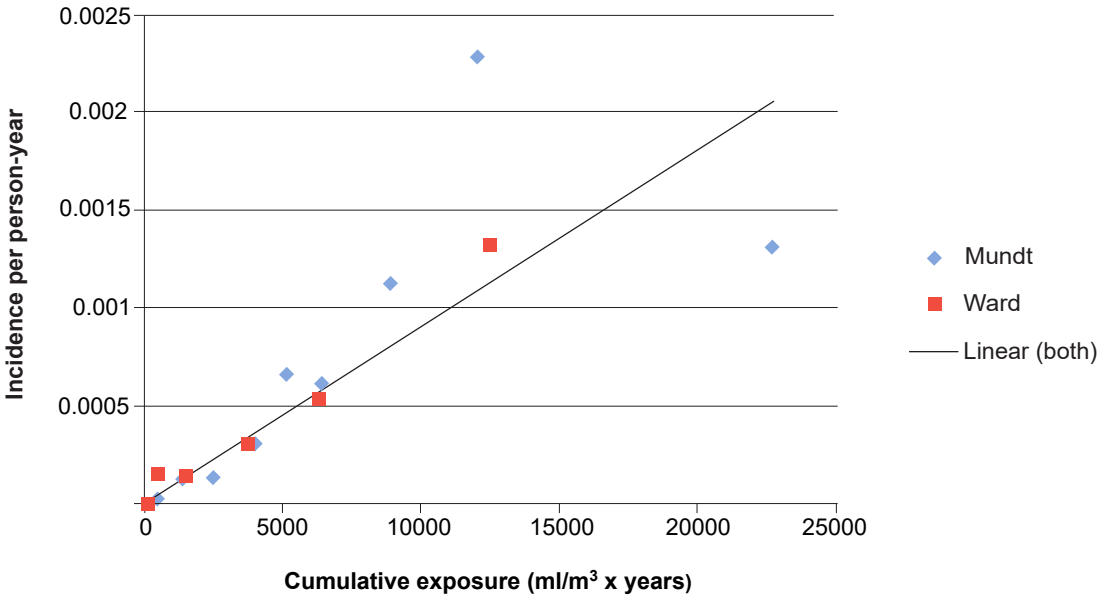


**Fig. 2** Dose–response relationships for liver angiosarcomas from the studies of Ward et al. (2001) and Mundt et al. (2017)

Both cohorts yielded similar dose–response relationships. The following relationship was established between incidence (I; cases per person-years) and cumulative exposure (CE; ml/m³ × years) by analysing the data of the two cohorts together: $I = 9.03 \times 10^{-8} \times CE$

The data were further analysed by applying the so-called life table method, which takes mortality in the population into account. In analogy to official statistics, the calculations were based on a theoretical cohort of 100 000 persons (in this case, exclusively men).

Exposure was determined on the basis of this cohort and resulted in a pre-defined excess risk (for example, 4 in 1000 or 4 in 100 000). In each age group, the number of expected cases was determined by multiplying the number of persons at risk (that is, alive) by the risk arising through exposure. The number of cases were then added together. In the case of angiosarcomas, it was assumed that the risk for the general population was negligible.

In analogy to the derivation of the Health Council of the Netherlands (2017), the risk-based values were derived based on the following assumptions:

1. The workers were exposed for 40 years (between 20 and 60 years of age).
2. The workers were observed until the age of 100.
3. Angiosarcomas occurred only as from 50 years of age.
4. The mortality incidence from Germany for the year 2015 was used.
5. A linear model without intercept was used for the analysis of the dose–response relationship. This means that there is no risk at an exposure concentration of 0 ml/m³.
6. The level of exposure in the highest category proved to be problematical for the determination of the dose–response relationship. Therefore, Ward et al. (2001) assigned a value of > 7532 ml/m³ × years to this category. A value of 12 553 ml/m³ × years was applied in this category for the risk assessment carried out by the Health Council of the Netherlands (2017). With respect to the other values in this category (for example, 10 000 or 15 000 ml/m³ × years), the coefficients obtained for the dose–response relationship varied only slightly. They had only a slight effect on the estimation of risk. Therefore, the factor of 5/3 was used to determine the level of cumulative exposure in the highest category for both cohorts.

For both studies together, a risk of 4 in 1000 exposed persons was determined for exposure for 40 years at a concentration of 40 ml/m³ (95% CI: 32.5–50; 100 mg/m³). At a risk of 4 in 10 000, the concentration is reduced to 4 ml/m³ (95% CI: 3.2–5; 10 mg/m³), at 4 in 100 000 to a concentration of 0.4 ml/m³ (95% CI: 0.33–0.5; 1 mg/m³) (Ulm 2017). Correspondingly, the risk is 1 in 10 000 at an exposure level of 1 ml/m³.

On the basis of the findings from other epidemiological studies, risk levels ranging from 0.5 to 6 angiosarcomas per 10 000 exposed persons were calculated for workplace exposure at a concentration of 1 ml/m³ (SCOEL 2004).

# 6 Manifesto (MAK value/classification)

Vinyl chloride is carcinogenic in humans and induces primarily angiosarcomas of the liver; in addition, it is very probable that the substance leads to the induction of hepatocellular tumours.

**Carcinogenicity.** Vinyl chloride induced tumours of the liver in rats given 1.3 mg/kg body weight or exposed by inhalation to 25 ml/m³ and tumours of the brain, kidneys and Zymbal's gland at higher levels of exposure. Tumours of the liver, breast and lungs were observed in mice (Henschler 1993). Vinyl chloride is mutagenic at high concentrations in vitro and in vivo. Vinyl chloride is metabolized to chloroethylene oxide and chloroacetaldehyde. Both metabolites react with the DNA, forming the 4 etheno adducts $N^2$,3-ethenoguanine, 1,$N^2$-ethenoguanine, 1,$N^6$-ethenoadenine and 3,$N^4$-ethenocytosine. The formation of etheno adducts in the DNA plays an important role in the induction of liver tumours. The metabolism of vinyl chloride exhibits linearity at low concentrations and saturation at concentrations of 100 ml/m³ and above. Cell proliferation plays an important role in the development of carcinogenic effects in the liver also for genotoxic substances; however, this has not been demonstrated for vinyl chloride.

A MAK value at which a very low cancer risk would be expected cannot be derived at this time; therefore, vinyl chloride remains assigned to Carcinogen Category 1.

**Risk assessment.** In a study in rats (Morinello et al. 2002 a), a linear relationship was determined between exogenous $N^2$,3-ethenoguanine adducts of the DNA in hepatocytes and the exposure concentration of vinyl chloride. It was calculated that the amount of exogenous DNA adducts corresponds to 50% of endogenous ethenoguanine adducts after long-term exposure at a vinyl chloride concentration of 1 ml/m³. However, as the contribution of endogenous ethenoguanine adducts to the cancer risk is not known, it is not possible to estimate the contribution of exogenous adducts to the cancer risk. Ever since the level of exposure to vinyl chloride was below 1 ml/m³, no cases with angiosarcomas were reported (Albertini et al. 2003). However, the epidemiological studies do not provide a more exact NOAEC (no observed adverse effect concentration) for the development of liver tumours, which, moreover, depends on the statistical power of the studies. No liver tumours were observed after exposure of rats to 5 ml/m³.

After exposure for 40 years to 0.25 ml/m³, the ORs for "mutations" in the oncoproteins ras-p21 and ras-p53 were doubled. The differences between these findings and those in control persons were not statistically significant (Brandt-Rauf et al. 2002). In control persons, the "prevalence of mutations" in the two proteins was 10% and the risk of developing liver cancer was below 1%. However, the specificity of p53 mutations as a predictor for the induction of angiosarcomas by vinyl chloride and the significance of mutations for the risk assessment with respect to angiosarcomas remains controversial (see Section 2; Sherman 2009; Section 4.6; Mocci and Nettuno 2006).

A NOAEL was not reached in a study in Wistar rats given vinyl chloride with the feed throughout their lifetime (Til et al. 1991). The LOAEL (lowest observed adverse effect level) in females for basophilic liver foci (2 times the value determined in control animals) and for the sum of all preneoplastic foci (1.7 times the value in control animals), including clear cell, mixed cell and eosinophilic foci, was 0.014 mg/kg body weight and day. However, neither the double logarithmic nor the linear plot of basophilic liver foci and all foci in females yielded parallel dose–response relationships with the liver tumor incidence. In the males, the NOAEL for histopathological changes in the liver was 0.13 mg/kg body weight and day (this is equivalent to 5.5 ml/m³ at the workplace). The dose–response relationships determined for basophilic liver foci and liver tumours exhibited a relatively parallel course, that is, the incidence of

foci and liver tumours was not increased up to a dose of 0.13 mg/kg body weight. The increased incidence of liver foci in the females of the lowest dose group did not correspond to an increased incidence of liver tumours. The animals were exposed over their entire lifetime; it would therefore have been possible for tumours to develop from the foci. The dose of 0.014 mg/kg body weight in females is equivalent to a workplace concentration of 0.55 ml/m$^3$.

The exposure–risk relationship below was determined on the basis of epidemiological studies by Ward et al. (2001) and Mundt et al. (2017) which investigated the risk of developing angiosarcomas of the liver and the conversion carried out by Ulm (2017):

**Tab. 4** Exposure–risk relationship for vinyl chloride

| Risk | Vinyl chloride concentration (95% CI) (ml/m$^3$) | Vinyl chloride concentration (mg/m$^3$) |
|---|---|---|
| 4:1000 | 40 (32.5–50) | 100 |
| 4:10 000 | 4 (3.3–5) | 10 |
| 4:100 000 | 0.4 (0.3–0.5) | 1 |

Accordingly, the risk is 1 in 10 000 for exposure to a concentration of 1 ml/m$^3$ for 40 years. As specified in the assumptions listed above, the tumours occur as from 50 years of age and are observed up to 100 years of age. The risk per year is therefore 1/10 000/50 = 2 per 1 million per year. The background risk is 0.1 per 1 million per year. The relative risk is 20 at a concentration of 1 ml/m$^3$.

**Absorption through the skin.** In the past, vinyl chloride was not given the "H" designation (for substances which can be absorbed through the skin in toxicologically relevant amounts). Absorption through the skin from the gas phase is far below 1% of the amount absorbed by inhalation. Therefore, only a negligible amount is absorbed through the skin (Section 3) and the "H" designation has not been given to vinyl chloride.

**Prenatal toxicity.** As it is not possible to derive a MAK value, the substance has not been classified in a Pregnancy Risk Group.

**Germ cell mutagenicity.** Vinyl chloride is genotoxic in bacteria and somatic cells in vitro and in vivo. Vinyl chloride induces gene mutations in bacteria and Drosophila. The mutagenic response in bacteria is amplified by metabolic activation with rat liver preparations. DNA adducts, micronuclei and chromosomal aberrations are induced in rodents. By contrast, vinyl chloride did not cause genotoxic effects in germ cells in dominant lethal tests performed in mice and rats with exposure to concentrations in a range from 3000 to 30 000 ml/m$^3$ or from 50 to 1000 ml/m$^3$ (inhalation, 5 days to 10 weeks). Damage to the testes was observed in reproductive toxicity studies (Bi et al. 1985; Sokal et al. 1980), which demonstrates that vinyl chloride is able to reach the germ cells.

As no effect on the germ cells after sufficient testing in several dominant lethal tests in mice and rats occurred and in view of the findings of testicular damage, it can be concluded that vinyl chloride reaches the germ cells, but not in the amount required to induce dominant lethal mutations. This invalidates the hypothesis that vinyl chloride causes mutagenic effects in the germ cells and the substance is not classified in a category for germ cell mutagens.

**Sensitization.** Data for sensitization in animals are not available. Sensitization in humans is not known and the substance is not designated either with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

# Notes

## Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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