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1,2-Dichloropropane

MAK Value Documentation, supplement – Translation of the German version from 2021

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Keywords

1,2-dichloropropane; carcinogenicity; skin absorption; cholangiocarcinoma; toxicity; human carcinogen; bile duct; cluster

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated 1,2-dichloropropane [78-87-5] considering all toxicological end points. Available publications and unpublished study reports are described in detail. A cluster of cholangiocarcinoma cases among workers in Japanese printing plants was attributed to 1,2-dichloropropane. Although the workers were exposed to many different chemicals, 1,2-dichloropropane was, with the exception of 3 of the 39 cases, the only known chemical common to all cases. A unique mutational signature found in the cholangiocarcinoma tissue of occupationally exposed printing workers indicates a genotoxic mode of action and/or the involvement of a perturbed immune response for the aetiology of these tumours. 1,2-Dichloropropane caused an increased incidence of lung adenomas in mice at 32 ml/m^3 and tumours in the nasal cavity in rats at 500 ml/m^3 with a statistically significant trend. Thus, 1,2-dichloropropane has been classified in Category 1 for human carcinogens. In vivo studies in animals did not find any significant clastogenic or mutagenic effects after prolonged inhalation or oral administration. Therefore, the substance is not considered to be a germ cell mutagen. 1,2-Dichloropropane is absorbed via the skin in toxicologically relevant amounts and remains designated with "H". A sensitizing potential is not expected from the data available.

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MAK value	–
Peak limitation	–
Absorption through the skin (2020)	**H**
Sensitization	–
Carcinogenicity (2020)	**Category 1**
Prenatal toxicity	–
Germ cell mutagenicity	–
BAT value	–

CAS number	78-87-5
Density at 20 °C	1.16 g/cm^3 (IFA 2019)
Vapour pressure at 25 °C	66.2 hPa (ECHA 2019)
log K_{OW}	2.02 (IFA 2019)
Solubility at 20 °C	2700 mg/l water (IFA 2019)
1 ml/m^3 (ppm) ≙ 4.688 mg/m^3	**1 mg/m^3 ≙ 0.213 ml/m^3 (ppm)**

Documentation for 1,2-dichloropropane was published in 1993 (Greim 1998).

1,2-Dichloropropane is used primarily in the production of other organic substances such as propylene, carbon tetrachloride and tetrachloroethylene. The use of 1,2-dichloropropane as a cleaning agent became widespread in the printing industry in Japan after the implementation of a ban on 1,1,1-trichloroethane (1,1,1-trichloroethane was classified as an ozone-depleting substance and its use has been prohibited since 1996) (IARC 2017).

Numerous cleaning products contain 1,2-dichloropropane as a degreaser. In addition, it is used as a paint and stain remover as well as in adhesives (IARC 2017).

The substance is common in the environment and is found in the groundwater. It is biodegradable only to a very small extent (Tang et al. 2017).

1 Toxic Effects and Mode of Action

A cluster of bile duct carcinoma (cholangiocarcinoma) cases was reported in workers of printing companies in Japan who handled 1,2-dichloropropane during cleaning activities. Compared with the general population, the standardized incidence ratio (SIR) for bile duct carcinomas was found to be increased by a factor of 1000 in this cluster. Tissue samples from the cholangiocarcinomas of the printing workers yielded a higher frequency of mutations with a unique mutational pattern and an increased incidence of strand breaks in comparison with tissue samples from the biliary tract tumours of persons without exposure.

In rats exposed by inhalation for 13 weeks, irritation in the nose was observed at concentrations of 71 ml/m^3 and above. After 2 years, tumours of the nose were found in the group of rats exposed to 500 ml/m^3. After exposure of mice for 2 years, adenomas were observed in the lungs at concentrations as low as 32 ml/m^3. Liver toxicity was detected in mice after 13-week exposure to concentrations of 300 ml/m^3 and above.

In vitro studies with 1,2-dichloropropane reported mutagenic effects; however, these were not confirmed by the findings in animal studies.

In teratogenicity studies, delays in skeletal development with concurrent maternal toxicity were observed in Sprague Dawley rats at a dose level of 125 mg/kg body weight and day and in New Zealand White rabbits at 150 mg/kg body weight and day. Teratogenic effects were not detected in either species.

There are still no reliable data available relating to possible sensitizing effects induced by 1,2-dichloropropane.

2 Mechanism of Action

The mechanistic factors involved in the development of cholangiocarcinomas in humans are discussed below.

2.1 Genotoxicity

In tissue samples collected from the biliary tract tumours of printing workers with exposure to 1,2-dichloropropane (and dichloromethane), the incidence of somatic mutations was markedly increased in comparison with the incidence determined in tissue samples collected from biliary tract tumours in the control group. In addition, a specific mutational pattern was observed in the cholangiocarcinomas induced by 1,2-dichloropropane (Section 4.6; Mimaki et al. 2016, 2020). The unique mutational signature that was found in the printing workers involved preferential trinucleotide mutational changes of GpCpY to GpTpY (Y = pyrimidine base); to date, this has been found in only 13 of more than 23 000 sequenced human tumours (five of these biliary adenocarcinomas) and has since been included in the COSMIC database (Version v93, Catalogue of Somatic Mutations in Cancer of the Sanger Institute as the mutational signature SBS42 "Single Base Substitution"). The tumours observed in the 4 (late onset) control cases primarily harboured NpCpG to NpTpG changes that were found to correspond with the mutational signature SBS1. This signature is found in most tumour types and often correlates with patient age. These mutations are considered to be the product of an endogenous process initiated by the deamination of 5-methylcytosine to form thymine, thereby leading via G:T mismatches to C > T transitions (Sanger Institute 2019). Although in vitro bacterial mutagenicity tests with 1,2-dichloropropane yielded positive findings, in vivo, the substance did not induce mutations in the liver tissue of rats and mice in the GPT test and in the erythrocytes of mice in the Pig-a assay. Additionally, micronucleus tests in the bone marrow cells and in the peripheral blood of mice yielded negative results (Section 5.6.1 and 5.6.2).

Double-strand breaks in the DNA lead to the phosphorylation of the histone protein H2AX by the ATM serine/threonine protein kinase. The modified form is known as γ-H2AX and is identified with antibodies. The incidence of double-strand breaks determined by means of γ-H2AX antibodies in the tumour tissue from printing workers was increased in comparison with the incidence determined in tissue samples taken from biliary tract tumours in the control group. In addition, the incidence of double-strand breaks was found to be increased in the non-neoplastic biliary epithelial cells of the printing workers (Section 4.6; Sato et al. 2014). Following incubation with 1,2-dichloropropane, increased binding of γ-H2AX antibodies was observed in the human hepatocyte cell line WRL-68 and in the human cholangiocyte cell line MMNK-1 (Section 5.6.1; Toyooka et al. 2017). An increased binding of γ-H2AX antibodies is, however, not only evidence of substance-induced double-strand breaks, but can also be caused by apoptotic DNA degeneration (Luczak and Zhitkovich 2018).

An episulfonium ion (thiiranium ion) may develop as a reactive intermediate during the metabolism of 1,2-dichloropropane, leading to the formation of DNA adducts. Evidence of this was found in studies investigating the structurally similar 1,2-dichloroethane. A potential product of this pathway is 1-(glutathione-*S*-yl)thiiranium or, following enzymatic hydrolysis, 1-(2-amino-2-carboxyethyl)thiiranium via *S*-(2-chloroethyl)-L-cysteine (Anders 2008; Greim 1998; IARC 2017).

In Escherichia coli, the expression of the DNA repair enzyme O6-alkylguanine-DNA alkyltransferase unexpectedly increased the cytotoxicity and mutagenicity induced by other dihalogenated alkanes. This indirect genotoxic mechanism may be relevant also for 1,2-dichloropropane (Guengerich 2003; Liu et al. 2002).

Conclusion: It is likely that the genotoxic effects of 1,2-dichloropropane, or its metabolites, contribute to tumour formation. This is supported by the unique mutational patterns found in the cholangiocarcinoma tissue of exposed printing workers.

2.2 Inflammatory responses and the immune system as contributing factors

Precancerous and preinvasive lesions were detected in samples of cholangiocarcinoma tissue taken from printing workers who had been exposed to 1,2-dichloropropane. The lesions included biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasms of the bile duct (IPNB). Chronic inflammation may lead to the development of these precancerous lesions. The formation of tumours may therefore involve a multi-stage process (Sato et al. 2014).

Tumour cells produce and release immunoinhibitory signalling proteins. One of these proteins is programmed death-ligand 1 (PD-L1), which binds to the T cell receptor programmed cell death-1 (PD-1) and leads to the degradation of T cells by apoptosis. The interaction of PD-L1/PD-1 limits peripheral inflammation during an inflammatory response. PD-L1 was found to be expressed in all 10 analysed samples of tumour tissue collected from 9 patients with occupational cholangiocarcinomas. In addition, tumour-associated macrophages expressed PD-L1 and tumour-infiltrating T cells expressed PD-1. Positive immunohistochemical signals were not detected in more than 90% of the biliary tract tumour tissue of the control cases. In comparison, the number of PD-L1-positive mononuclear cells, PD-1-positive lymphocytes and CD8-positive lymphocytes infiltrating the tumour was significantly higher in the cases with occupational biliary tract tumours. PD-L1 was occasionally expressed also in the foci of BilIN and IPNB in patients with occupational cholangiocarcinomas, but not in the control cases and not in the non-neoplastic tissue of either group. Therefore, in the case of occupational cholangiocarcinomas, the immune escape mechanism occurs via PD-L1 as the negative immunoregulator of T cell activity. The authors assume that the tumours that develop in occupational cholangiocarcinoma cases are able to do so because the tumour cells evade immunosurveillance (immune escape) (Sato et al. 2017).

In another cholangiocarcinoma patient, PD-L1 was expressed in the tumour tissue, in BilIN and IPNB, but not in the non-neoplastic tissue of the bile duct. However, at a level of less than 5%, the expression of PD-L1 was not particularly pronounced (Kinoshita et al. 2019).

Activation-induced cytidine deaminase (AID) binds single-strand DNA and deaminates cytosine to uracil. As uracil pairs with adenine during replication, this leads to a C : G to T : A transition. B-lymphocytes use AID to modify antibodies. However, this is a highly regulated and controlled process (somatic hypermutation) in this cell type. As cytokines activate the expression of AID also in other cell types, AID is involved in inflammation-associated neoplastic transformations. In addition, in tumour tissues, the expression of AID correlated with the occurrence of mutations in proto-oncogenes. It is assumed that the enzyme AID is involved in the development of cholangiocarcinomas because it was found to be expressed in the samples of cholangiocarcinoma tissue collected from printing workers. Not only the findings that C : G to T : A transitions are the predominant mutational pattern determined in the tissues from printing workers, but also the increased occurrence of mutations on the non-coding DNA strand (strand bias) point toward an involvement of AID (Section 4.6; Haradhvala et al. 2016; Mimaki et al. 2016; Pilzecker and Jacobs 2019).

The COSMIC database includes 4 mutational signatures that are attributed to the activation of AID (SBS84, SBS85) or AID/APOBEC (APOBEC: Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) (SBS2, SBS13). A visual comparison revealed that the C > T substitutions found in the SBS84 mutational signature exhibit a base substitution pattern of GpCpY to GpTpY very similar to that of the SBS42 mutational signature observed in the tumour tissue of the printing workers. By contrast, SBS2 and SBS13, which are characterized by the mutations TpCpA to TpGpA or TpTpA and TpCpT to TpTpT or TpGpT, and SBS85, which involves predominantly T > A and T > C substitutions, did not reveal any specific agreement with the mutational patterns found in the tumour tissue of the printing workers (Section 4.6; Mimaki et al. 2016, 2020; Pilzecker and Jacobs 2019; Sanger Institute 2019). Mimaki et al. (2020) did not examine the

agreement between the specific GpCpY to GpTpY base substitutions and SBS84. This may be because the authors used an earlier version of the COSMIC database (Sanger Institute 2015), which did not include the SBS84 signature.

Additionally, substitutions from GpCpY to GpTpY and to GpTpA were found in spontaneously immortalized human *TP53* knock-in mouse embryo fibroblasts transgenic for AID (Olivier et al. 2014). The role played by AID/APOBEC in carcinogenesis and the relevant mutation spectra have yet to be fully deciphered and are currently the focus of intensive research (Haradhvala et al. 2016; Rogozin et al. 2016).

In vitro studies using the MMNK-1 cholangiocyte cell line, differentiated THP-1 macrophages and co-cultures of both reported a statistically significant increase in AID expression only in the co-cultured cells after 9-hour incubation with 50 µM of 1,2-dichloropropane (dissolved in DMSO). After incubation with 1,2-dichloropropane, differentiated THP-1 macrophages exhibited considerably higher levels of TNF-alpha. Treatment of MMNK-1 cells with TNF-alpha led, in turn, to increased AID expression. In the co-cultured cells, therefore, the release of TNF-alpha from macrophages induced by 1,2-dichloropropane led to an increase in AID expression in the cholangiocyte cell line. In the comet assay, incubation with 1,2-dichloropropane led to higher levels of DNA damage in the co-cultured cells than in cholangiocyte monocultures. The viability of the cells was 100% or above after incubation with up to 5000 µM of 1,2-dichloropropane (Zong et al. 2019).

Conclusion: This demonstrates that the immune system contributes to tumour formation.

2.3 The contribution of cytochrome P450 and glutathione

A number of different studies were carried out to identify the metabolite or metabolites responsible for the formation of tumours in the bile duct. The studies investigated oxidation by cytochrome P450 2E1 (CYP2E1) followed by glutathione (GSH) conjugation or vice versa (Section 3.2).

In an in vitro study using the liver homogenate of *Cyp2e1*$^{+/+}$ mice (wild type) and *Cyp2e1*$^{-/-}$ mice (CYP2E1 deficient), 1,2-dichloropropane was metabolized in the liver tissue only of wild-type mice. Therefore, the first step of the metabolic pathway involves the metabolic activation of 1,2-dichloropropane by CYP2E1. As liver damage was hardly noticeable in CYP2E1-deficient mice following intraperitoneal injection of 1,2-dichloropropane doses of up to 300 mg/kg body weight and because the 1,2-dichloropropane concentration in the liver remained unchanged, indicating that 1,2-dichloropropane was not metabolized, the authors assume that 1,2-dichloropropane is not directly conjugated with GSH in the liver. In the wild-type mice, intraperitoneal injection of a 1,2-dichloropropane dose of 300 mg/kg body weight increased the activities of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the blood, which was interpreted as a marker for liver damage. Other forms of liver damage were observed at doses of 200 mg/kg body weight and above (necrosis, enlarged hepatocytes around the portal vein, nucleolar hypertrophy); these effects were dependent on the dose (Section 3.2; Yanagiba et al. 2016 a).

Studies with human liver microsomes demonstrated that the CYP2E1 inhibitor diethyldithiocarbamate suppressed the metabolic conversion of 1,2-dichloropropane, while the inhibitors 7,8-benzoflavone (CYP1A2), quinidine (CYP2D6), sulfaphenazole (CYP2C10) and gestodene (CYP3A4 and CYP3A5) did not suppress the metabolic conversion of 1,2-dichloropropane (Guengerich et al. 1991).

CYP2E1 inhibitors reduced the binding of γ-H2AX antibodies after incubation with 1,2-dichloropropane in the human hepatocyte cell line (WRL-68) and in the human cholangiocyte cell line (MMNK-1). This is further evidence that CYP2E1 is involved in the formation of reactive metabolites. The addition of 1,2-dichloropropane increased the levels of reactive oxygen species in the human hepatocyte cell line (WRL-68). This effect is likewise significantly suppressed by CYP2E1 inhibitors (Section 5.6.1; Toyooka et al. 2017).

The non-isoform selective CYP inhibitor 1-aminobenzotriazole inhibits the proliferative effects of 1,2-dichloropropane; this was determined by staining for BrdU and Ki-67. Additionally, the number of apoptotic nuclei (labelled using the TUNEL method) that was induced by exposure to 1,2-dichloropropane was not increased. Both effects were observed

in the biliary epithelial cells of male mice after exposure. An inhibition of proliferation was the only effect observed in the hepatocytes (Section 5.2.1; Zhang et al. 2018).

Other CYP enzymes may oxidize 1,2-dichloropropane, but only to a small extent. This was demonstrated in vitro for bacterial CYP101 (Lefever and Wackett 1994). CYP2A4 mRNA and CYP2A4 protein levels were increased in the liver of hamsters and mice after exposure to 1,2-dichloropropane; this suggests that they are involved in the metabolism of 1,2-dichloropropane. The slight, but statistically significant induction of CYP1A1 mRNA was not observed at the protein level. Also CYP4A14 mRNA was induced, but not investigated at the protein level (Gi et al. 2015 b). As the treatment of CYP2E1-deficient mice with 1,2-dichloropropane had hardly any effect on the activities of the enzymes ALT and AST and caused no additional liver damage, this suggests that CYP2E1-mediated metabolism of 1,2-dichloropropane is decisive for the observed effects (Yanagiba et al. 2016 a).

1,2-Dichloropropane may lead to more severe renal toxicity in male rats because of the higher levels of CYP activity in the kidneys induced by testosterone. This was demonstrated in vitro with metabolically active tissue sections (Section 5.8; Odinecs et al. 1995).

Long-term ethanol consumption increases CYP2E1 expression in the liver. To date, the expression of CYP2E1 has not been observed in the kidneys in humans (IARC 2017; Overton et al. 2008).

A number of findings revealed that CYP2E1 is catalytically active mainly in the mitochondria and not in the endoplasmic reticulum (Guengerich and Avadhani 2018).

CYP2E1 occurs mainly in the liver, irrespective of the species. High levels of CYP2E1 activity were found also in the club cells of the lungs of mice; however, this activity was not observed in rats and humans. The involvement of CYP2E1 in the formation of a potentially carcinogenic metabolite is supported by findings of liver and lung carcinomas and adenomas in mice after inhalation exposure to 1,2-dichloropropane for 2 years, but not in rats, and not in humans after high levels of exposure at the workplace. On the other hand, CYP2E1 activity is markedly higher in the olfactory epithelium of rats than in that of mice or humans. This also explains the development of nasal tumours in rats, which were not observed in mice or in humans after high levels of exposure at the workplace (Green et al. 1997, 2001; Matsumoto et al. 2013; Overton et al. 2008; Umeda et al. 2010; Zhang et al. 2015).

GSH was found to spontaneously and directly bind to 1,2-dichloropropane in vitro and under physiological conditions. The addition of glutathione *S*-transferase theta 1 (GSTT1) led to an only slight increase in metabolite concentrations (Section 3.2; Toyoda et al. 2017).

A single 1,2-dichloropropane dose of 2 ml/kg body weight (about 2300 mg/kg body weight) given to rats (5–12 animals, no other details) by gavage severely depleted GSH levels in the liver, kidneys and blood. No GSH disulphide (GSSG) or cysteine disulphide formed. After 96 hours, GSH levels had returned to normal. Pretreatment with buthionine sulfoximine, which leads to GSH depletion, increased the toxicity of 1,2-dichloropropane. The toxicity was documented on the basis of marked increases in ALT and AST activities (Greim 1998; Imberti et al. 1990).

After oral exposure of hamsters to 1,2-dichloropropane doses of 250 mg/kg body weight on 5 days a week for 4 weeks, CYP2E1 levels were increased in the necrotic, centrilobular zone of the liver. GSTT1 expression decreased with the dose in this zone, reaching statistical significance, but increased in the non-necrotic, periportal zone of the liver (Section 5.2.2; Gi et al. 2015 a).

The DNA double-strand breaks induced by 1,2-dichloropropane and identified by γ-H2AX were suppressed to a lesser extent by ethacrynic acid, an inhibitor of all glutathione transferases, than by the inhibitors of CYP2E1. The studies were carried out in vitro in a human hepatocyte cell line (WRL-68) and a human cholangiocyte cell line (MMNK-1) (no other details; Section 5.6.1; Toyooka et al. 2017).

As the mutagenic effects in the GSTT1-overexpressing TA100 Salmonella strain (TA100-GSTT1) did not increase in severity following incubation with 1,2-dichloropropane, the authors consider this to be evidence that GSTT1 is not involved in the mutagenic effects of 1,2-dichloropropane (Akiba et al. 2017). GSTT1 is the enzyme responsible for the

metabolism of dichloromethane, which in this study led to a marked increase in revertants in the GSTT1-overexpressing TA100 Salmonella strain.

All previously identified metabolites are conjugated with GSH (Section 3.2).

CYP1A, CYP2E1 and CYP3A were detected in human biliary epithelial cells by immunochemical examination. The metabolizing enzymes GSTA, GSTM and GSTP, and microsomal epoxide hydrolase were likewise observed. The expression of the CYP enzymes and microsomal epoxide hydrolase in the biliary epithelial cells was lower by a factor of 5 to 20 than the level of expression in human hepatocytes. GSTM transferase was expressed only in the biliary tract tissue (Lakehal et al. 1999).

Therefore, it is unlikely that the chlorinated metabolite (half mustard form) postulated by Toyoda et al. (2016), which would form after direct conjugation with GSH, is responsible for tumour formation (Section 3.2).

GSTT1 was found to be expressed in foci of BilIN and IPNB, in tumour tissue and in non-neoplastic biliary tissue taken from the affected printing workers (Kubo et al. 2018; Sato et al. 2014, 2017).

Stronger expression of GSTT1 and more efficient conjugation of dichloromethane with GSH was observed in the hepatic tissue of B6C3F1 mice than in human liver tissue. GSTT1 was detected in the nuclei of human biliary epithelial tissue (Sherratt et al. 2002).

The immunohistochemical examination revealed GSTT1 in the hepatocytes and the biliary epithelial cells of untreated ICR mice, F344 rats and human tissue (Sato et al. 2014).

The glutathione *S*-transferases GSTT1, GSTM1 and GSTPi showed similar levels of expression in the hepatocytes and the biliary epithelial cells of C57BL/6J mice, Balb/cA mice, F344 rats, Syrian hamsters and guinea pigs after exposure to 1,2-dichloropropane by inhalation for 7 or 14 days. According to the authors, varying levels of expression do not explain the differences in humans (cholangiocarcinomas after inhalation exposure) and rodents (no cholangiocarcinomas) (Zhang et al. 2015).

Summary: For the metabolites responsible for liver and bile duct toxicity to form, the CYP2E1-catalysed metabolism of 1,2-dichloropropane must occur as the first step. The study findings indicate that GSH is involved in the metabolism, but that GSTT1 transferase plays only a subordinate role in the development of potentially carcinogenic metabolites. As GSTT1 was detected in the biliary epithelial cells of rodents in 2 studies (Sato et al. 2014; Zhang et al. 2015) and in human tissue in the studies of Sato et al. (2014) and Sherratt et al. (2002), and GSTT1 does not appear to be significantly involved in the metabolism of 1,2-dichloropropane, it is not possible to explain the absence of bile duct tumours in rodents on the basis of varying levels of GSTT1 expression or activity.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

The respiratory tract and skin are the primary routes of absorption for 1,2-dichloropropane at the workplace. Systemic toxic effects that developed in humans following ingestion demonstrated that 1,2-dichloropropane is absorbed also through the gastrointestinal tract (IARC 2017).

In 22 workers employed in the plastics and paint industry or in shoe factories who were exposed to 1,2-dichloropropane, the concentrations in the air correlated with the urinary concentrations of 1,2-dichloropropane (no other details; Ghittori et al. 1987).

After exposure of 30 workers to a 1,2-dichloropropane concentration of 7.1 ml/m^3 (time-weighted average (TWA), geometric mean, maximum 23.1 ml/m^3), the mean 1,2-dichloropropane concentration in the urine was found to be 77 µg/l urine. 1,2-Dichloropropane was not detected in the urine of 5 control persons without exposure (Kawai et al. 2015).

1,2-Dichloropropane is rapidly absorbed by rats after inhalation or oral exposure and then found primarily in the liver, kidneys, lungs and blood (Greim 1998).

Groups of 42 male F344/DuCrlCrlj rats were exposed whole-body for 360 minutes to 1,2-dichloropropane concentrations of 80 or 500 ml/m^3. The 1,2-dichloropropane had a purity of 99.5%. Blood samples were taken from the tail vein and necropsy was performed 0, 60, 180 or 360 minutes after the beginning of exposure or 60, 180 or 1080 minutes after the end of exposure. The 1,2-dichloropropane concentrations in the blood and in the tissues increased steadily during exposure to 500 ml/m^3; this means that a steady state was not reached. Three hours after exposure, the levels in the blood had decreased by half, and after 18 hours, it was no longer possible to detect 1,2-dichloropropane in the blood. After 6 hours, the 1,2-dichloropropane concentrations were determined to be about 6 µg/ml in the blood, about 6.2 µg/g in the lungs, about 19 µg/g in the liver, about 12 µg/g in the kidneys and about 270 µg/g in the abdominal fat. In rats, the amounts absorbed in the blood and tissues were disproportionately higher in the animals exposed to a concentration of 500 ml/m^3 than to those exposed to 80 ml/m^3. The AUC (area under the curve) in the blood and tissues was at least 13 times as high in the group exposed to 500 ml/m^3 as in the group exposed to 80 ml/m^3. The half-lives determined in the 80 and 500 ml/m^3 groups were, respectively, 182 and 168 minutes in the blood, 39 and 61 minutes in the lungs, 57 and 125 minutes in the liver, 59 and 127 minutes in the kidneys and 154 and 186 minutes in the abdominal fat. The substance was found to accumulate in the abdominal fat. In the animals exposed to a 1,2-dichloropropane concentration of 80 ml/m^3, the levels in the lungs, liver and kidneys reached a maximum after 60 minutes and then remained at the same level until the end of exposure (360 minutes). The amounts absorbed by these tissues were therefore in equilibrium with metabolic degradation and elimination or exhalation. However, at 80 ml/m^3, the 1,2-dichloropropane concentration in the abdominal fat steadily increased until the end of exposure. In comparison with the concentration levels found in the other tissues, the levels were much higher at about 20 µg/g and decreased more slowly. The high concentration in the abdominal fat is attributed to the ready solubility in lipids and the reduced metabolism and elimination of the substance in that area of the body. At 500 ml/m^3, a steady state was not reached in any of the tissues. The course corresponded to the uptake in the blood at 500 ml/m^3. The authors assume that the metabolism was saturated at 500 ml/m^3. The substance was excreted via the urine or exhaled air (Take et al. 2014).

Groups of 36 male F344/DuCrlCrlj rats were given a single gavage dose of 1,2-dichloropropane of 62 or 125 mg/kg body weight dissolved in corn oil. In both dose groups, the concentrations in the blood (about 1 and 1.2 µg/ml, respectively) and in the lungs (about 2.8 and 3.1 µg/g), liver (about 8 and 15 µg/g), kidneys (about 5 and 7.5 µg/g) and abdominal fat (about 28 and 92 µg/g) reached their maximum after about 60 minutes. The only exception was the blood. At 125 mg/kg body weight, the maximum value in the blood was reached only after 180 minutes. The half-lives were 193 and 315 minutes, respectively, in the blood, 144 and 187 minutes in the liver, 144 and 193 minutes in the kidneys, 114 and 165 minutes in the lungs and 257 and 330 minutes in the abdominal fat. In the 125 mg/kg group, 1,2-dichloropropane was still detected in all tissues and blood after 24 hours, although in minimal amounts (Take et al. 2017). Therefore, the substance remains in the tissues for longer periods of time after exposure.

More than 50% of the absorbed amount of 1,2-dichloropropane is excreted with the urine in the form of a GSH conjugate. After treatment with radioactively labelled 1,2-dichloropropane, only 5% to 8% of the radioactivity was recovered in the faeces. 1,2-Dichloropropane is exhaled via the lungs primarily in unchanged form (about 20%) or as CO_2 (about 20%). After oral exposure of male Wistar rats (5 animals per dose group) to 1,2-dichloropropane doses of 55 or 110 mg/kg body weight, the 1,2-dichloropropane concentrations in the blood reached their maximum levels after 30 minutes. The half-lives of 1,2-dichloropropane in the blood were 3.1 and 5.0 hours, respectively. After exposure to 120 and 440 mg/kg body weight, the maximum concentration in the blood was reached only after 1 to 2 hours and the half-lives were 4.3 and 13.6 hours, respectively (Di Nucci et al. 1988; Greim 1998).

Substances that have undergone degradation in the liver may be excreted via a phase III system such as ABCC2 and ABCG2 (ATP binding cassette subfamily C/G member). After an oral 1,2-dichloropropane dose of 500 mg/kg body weight in olive oil was given to Abcg2 knockout mice (n = 6) and Abcc2-deficient EHBR rats (n = 7–10), marked decreases in the metabolite levels in the bile were observed only in the EHBR rats. In vitro, membrane vesicles containing ABCC2 functioned as an ATP-dependent transporter of the GSH-conjugated metabolites of 1,2-dichloropropane. Therefore, ABCC2 seems to be involved in the elimination of 1,2-dichloropropane metabolites (Toyoda et al. 2016).

A flux of 474 µg/cm^2 and hour (Fiserova-Bergerova et al. 1990) and, using the IH SkinPerm model (Tibaldi et al. 2014), a flux of 35.5 µg/cm^2 and hour was calculated for a saturated aqueous solution. Assuming standard conditions (2000 cm^2 of skin, 1-hour exposure), the amounts absorbed per working day were calculated to be 948 and 71 mg, respectively.

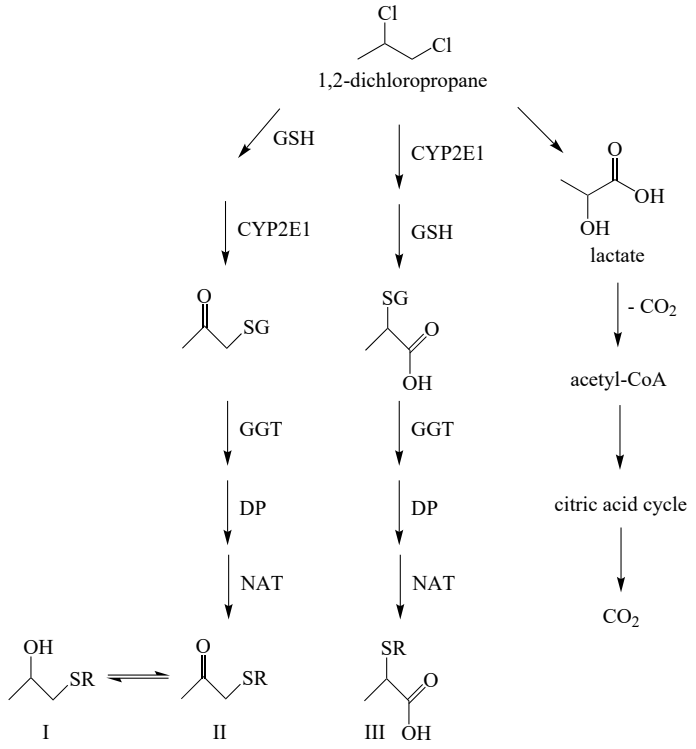
3.2 Metabolism

Like many halogenated alkanes, 1,2-dichloropropane is metabolized by first undergoing oxidative dehalogenation by CYP2E1, followed by GST-mediated conjugation with GSH or vice versa. It is likely that these metabolic steps occur primarily in the liver, allowing for the excretion of the metabolites via the bile (Gonzalez and Gelboin 1994; IARC 2017).

The primary metabolites identified in the urine of F344 rats were *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine, *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine and *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine (Greim 1998).

In Sprague Dawley rats treated with 1,2-dichloropropane, about 25% to 30% of a single oral dose of 100 mg/kg body weight was conjugated with GSH to form the mercapturic acid derivative *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine. In the urine of F344 rats treated with oral doses of 100 mg/kg body weight, 10.2% of the administered dose was recovered as *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine, 14.5% as *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine and 1.8% as *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine (Greim 1998).

The primary metabolites are the products of a combination of CYP oxidation and GSH conjugation (pathways II and III in Figure 1). *N*-Acetyl-*S*-(2-hydroxypropyl)-L-cysteine, the final metabolite described in pathway I, is probably formed via the reduction of *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine from pathway II. The CYP2E1-GST pathway was found to be the primary metabolic pathway (Bartels and Timchalk 1990; IARC 2017).



R = *N*-acetylcysteine
I = *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine
II = *N*-acetyl-*S*-(2-oxoypropyl)-L-cysteine
III = *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine

CYP2E1: cytochrome P450-2E1; DP: dipeptidase; GGT: γ-glutamyltransferase; GSH: glutathione; NAT: *N*-acetyltransferase

Fig. 1 Proposed metabolic pathway of 1,2-dichloropropane (according to ATSDR 2019; IARC 2017)

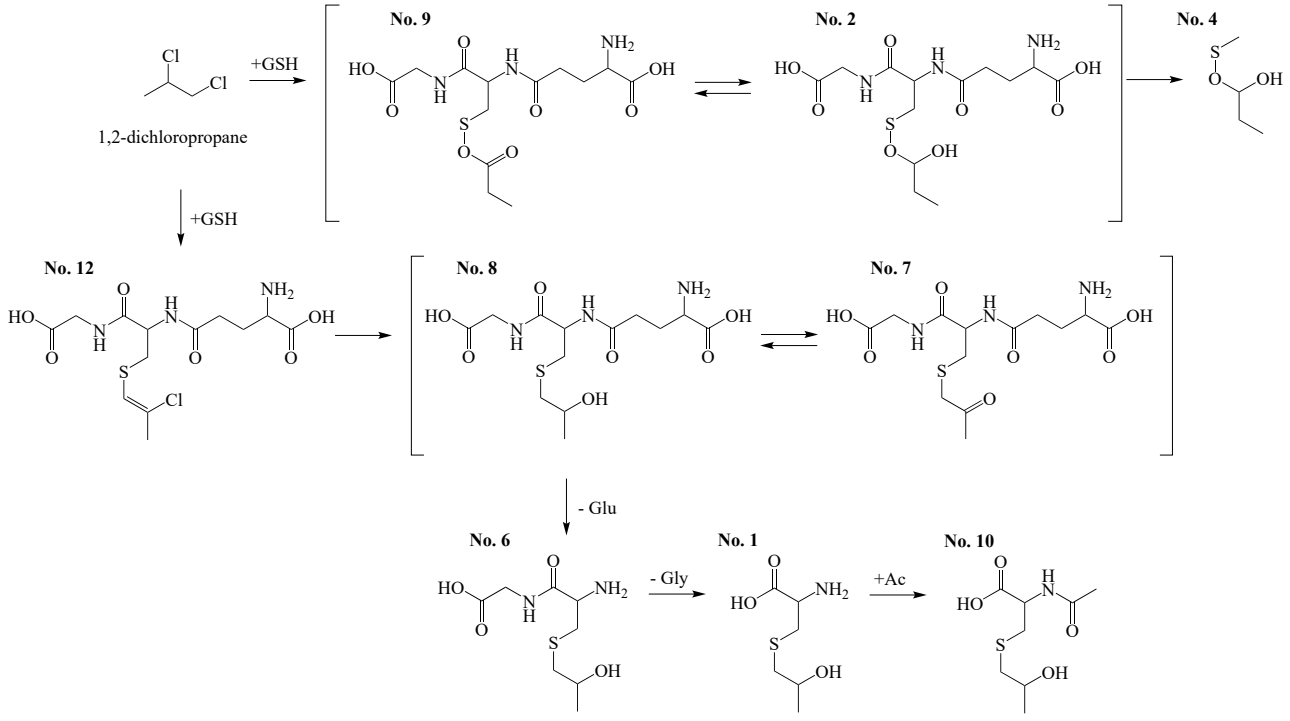
The addition of propylene oxide to this pathway as a reactive metabolic intermediate was suggested in one publication; this publication is available only in Japanese (Yanagiba et al. 2016 b).

Following the addition of 10 mM GSH to CYP2E1 enzymes isolated from human liver tissue, 1,2-dichloropropane was converted to the metabolites 2-oxopropylglutathione and 1-formylmethylglutathione at a rate of 1.1 nmol of product formed per minute and nmol of CYP2E1. In comparison with this, the rate of metabolism in human liver microsomes was 1.2 nmol/min/mg protein; the metabolic rate was markedly reduced by the addition of an inhibitory CYP2E1 antibody. This demonstrates the significance of CYP2E1 for the metabolism (Guengerich et al. 1991).

CYP metabolism is expected to reach saturation in humans at 1,2-dichloropropane concentrations of 150 to 250 ml/m^3; this was estimated in analogy to 1,2-dichloroethane (Kumagai 2014).

In an in vitro study with the liver homogenate of *Cyp2e1*$^{+/+}$ mice (wild type) and *Cyp2e1*$^{-/-}$ mice (CYP2E1-deficient), 1,2-dichloropropane was metabolized in the wild type at a rate of 21.86 nmol/min/mg protein, while the substance was hardly metabolized in the tissues of the *Cyp2e1*$^{-/-}$ mice (0.22 nmol/min/mg protein). A tendency towards this effect was found also in vivo, as the 1,2-dichloropropane concentration in the blood was eliminated more slowly in *Cyp2e1*$^{-/-}$ animals after intraperitoneal injection. However, when compared with the findings in *Cyp2e1*$^{+/+}$ animals, the differences were not statistically significant (Yanagiba et al. 2016 a).

Bile was collected from C57BL/6J mice (number not specified), FVB/NJcJ mice (number not specified) and Sprague Dawley rats (n = 9) 4 hours after treatment with a single oral 1,2-dichloropropane dose of 500 mg/kg body weight in olive oil. The treatment dose of 1,2-dichloropropane of 500 mg/kg body weight was comparable to the estimated intake of the printing workers diagnosed with cholangiocarcinomas. Nine different 1,2-dichloropropane metabolites were identified by UPLC-ESI-MS/MS-based untargeted metabolomics and possible chemical structures were proposed (Figure 2). 1,2-Dichloropropane itself was not detected in the bile samples. The authors described the chlorinated metabolite designated in the figure as metabolite No. 12 as a half mustard (2-chloroethyl alkyl sulfides); however, these do not have a double bond. Following treatment with oral 1,2-dichloropropane doses of 500 mg/kg body weight in olive oil, the metabolites depicted in Figure 2 were determined also in the bile samples from PXB mice (n = 3) with



GSH: glutathione; Glu: glutamic acid; Gly: glycine; Ac: acetyl group

Fig. 2 Proposed pathway for the formation of metabolites in the liver/biliary system (according to Toyoda et al. 2016) with GSH

humanized liver tissue. Following treatment of Sprague Dawley rats with a 1,2-dichloropropane dose of 500 mg/kg body weight in olive oil, the metabolites in the serum were identified by LC-MS/MS analysis. Almost all metabolites were present, with the exception of No. 2 and 6 (Figure 2), but in markedly lower concentrations than those determined in and liver. The authors consider this evidence that metabolites can also pass into the blood (Toyoda et al. 2016). The proposed metabolites in Figure 2 are to be regarded as putative. The binding of GSH via an oxygen molecule from the metabolites No. 2, 4 and 9 in addition to the double bond in metabolite No. 12 is rather unusual from a chemical standpoint. The structures of the proposed metabolites were not verified with reference substances.

In vitro studies likewise demonstrated uncatalysed GSH (6 mM) binding to 1,2-dichloropropane, which increased with the concentration (1%–10% 1,2-dichloropropane solution) and duration of exposure (10 minutes to 6 hours). LC-MS analysis determined the postulated metabolites that are depicted in Figure 2 as No. 12 and 7, but not No. 8. GSTT1 led to an only slight increase in the metabolite concentrations. The authors suggest that bioactivation via GSTT1 appears to play only a subordinate role in the case of 1,2-dichloropropane (Toyoda et al. 2017).

4 Effects in Humans

4.1 Single exposures

Following accidental exposure to 1,2-dichloropropane by inhalation for about 2 hours, acute liver damage, marked changes in blood serum levels and anaemia were observed in a female patient. A liver biopsy carried out 8 days later yielded findings of centrilobular necrosis (ECHA 2019; Lucantoni et al. 1992).

Irritation of the respiratory tract was observed following exposure resulting from the release of 1,2-dichloropropane through a lorry accident (Rubin 1988).

Accidental ingestion led to functional disorders of the liver and kidneys, haemolytic anaemia, metabolic acidosis, heart muscle weakness and shock. Ingestion of large amounts of 1,2-dichloropropane (about 50 ml/70 kg body weight) may be lethal. Post-mortem examinations revealed liver necrosis (Greim 1998).

After the ingestion of unspecified amounts of the cleaning agent Trielina, which contained varying amounts of trichloroethane and 1,2-dichloropropane, effects on the central nervous system (CNS), liver, kidneys and the heart were observed in 56 patients. The incidence of mortality was 6% (Imberti et al. 1987).

Poisoning after accidental ingestion of Trielina (containing 1,2-dichloropropane and 1,2-dichloroethane as degradation products of trichloroethane) caused severe liver damage in 2 cases, leading to death, and severe hepatopathy in 2 other patients. Liver toxicity was not observed if the cleaning agent contained only trichloroethane (Chiappino and Secchi 1968; Secchi et al. 1968).

A case study reported acute intoxication after dermal exposure. A 46-year-old man contaminated his clothing while applying a paint fixative outdoors. The fixative contained 35% to 40% 1,2-dichloropropane and 33% to 38% toluene. He did not remove the clothing and wash himself until 5 hours after the contamination. Within 4 days, he experienced severe kidney and liver damage (kidney failure, rhabdomyolysis, abnormal coagulation and hepatocellular necrosis) and required in-patient treatment. As the patient had been working outdoors and did not describe complaints of the respiratory tract, the authors concluded that the 1,2-dichloropropane had been absorbed primarily through the skin (Fiaccadori et al. 2003).

A 33-year-old with cognitive disabilities drank an unspecified amount of 1,2-dichloropropane and a 66-year-old voluntarily ingested 30 to 40 ml of 1,2-dichloropropane. In both cases, disseminated intravascular coagulation involving an excessive formation of blood clots leading to a loss of clotting factors and thus bleeding was observed in addition to effects on the central nervous system and impaired liver and kidney functions (Perbellini et al. 1985).

4.2 Repeated exposure

4.2.1 Case reports

Severe acute hepatitis developed in a 34-year-old worker at a printing company who was exposed to mixtures containing 1,2-dichloropropane, dichloromethane, trichloroethane as well as other substances in a poorly ventilated room for 5 years and then formulations containing only 1,2-dichloropropane for about 10 years. After undergoing appropriate treatment, normal liver function was restored after several weeks. A number of different tests were performed, yielding negative results for viral hepatitis markers, but positive results for cytomegalovirus IgG and Epstein Barr virus IgG antibodies. Examination of the blood found a marked increase in the activities of AST, ALT and lactate dehydrogenase (LDH) and low prothrombin levels. Alkaline phosphatase and serum γ-glutamyl transpeptidase (γ-GTP) activities were not found to be increased. The patient consumed about 350 ml of beer a day. He smoked about 20 cigarettes a day and his body mass index (BMI) was 18.3 (Kubo et al. 2015).

After handling 1,2-dichloropropane as a cleaning agent for about 5 months, a worker at a company in South Korea complained of diplopia, dizziness and ataxia. Abnormal findings in the bilateral thalamus were detected by brain magnetic resonance imaging, which led to the diagnosis of suspected metabolic encephalopathy. After his health issues became known, readings taken at his workplace determined 1,2-dichloropropane concentrations of 8.4 ml/m^3. However, during the last month of his employment, he may occasionally have been exposed to markedly higher concentrations while removing rust. Two further readings were taken 2 months later, which determined exposure levels of 26.9 and 41.5 ml/m^3 with peaks of 49.8 and 76.6 ml/m^3 during rust removal procedures (Kwak et al. 2018).

4.2.2 Epidemiology

Printing workers in Osaka, Japan, who were exposed to 1,2-dichloropropane and dichloromethane were diagnosed with biliary tract tumours. At the time of the diagnosis, the serum activities of the enzymes AST, ALT, γ-GTP and the serum bilirubin concentrations were found to be increased in most of the patients. Increased γ-GTP activity had already been determined in some of the patients several years prior to their tumour diagnosis (Kubo et al. 2014 a).

Increased activities of AST, ALT and γ-GTP were likewise found in 9 other printing workers with the same diagnosis who were employed at other printing companies (Kubo et al. 2014 a).

The examination of blood samples taken from 10 printing workers with cholangiocarcinomas who had handled 1,2-dichloropropane for periods ranging between 6 and 17 years revealed elevated levels of γ-GTP. This was followed by accompanying increases in AST and ALT concentrations. The erythrocyte counts, haemoglobin levels, haematocrit values, total cholesterol, and triglyceride and fasting plasma glucose levels were within the normal range (Kumagai et al. 2014).

4.3 Local effects on skin and mucous membranes

There are no data available.

4.4 Allergenic effects

There are no new findings available.

4.5 Reproductive and developmental toxicity

There are no data available.

4.6 Genotoxicity

The sequencing of the exomes of intrahepatic cholangiocarcinoma tissue taken from 4 male printing workers in Japan aged 31 to 40 years yielded a mean of 44.8 somatic mutations per million DNA bases (Mb). The mutation frequency and the mutational profile were compared with control samples of cholangiocarcinoma tissue taken from 7 patients with a mean mutation frequency of 1.6/Mb. These 7 control patients who were not exposed to 1,2-dichloropropane were divided into "late onset" (intrahepatic, ♂ 55, ♀ 72, ♂ 73, ♀ 79 years old) and "early onset" (gallbladder tumour ♂ 31, duodenum papilla ♂ 39, extrahepatic ♀ 26 years old) groups. In the printing workers, 1451 ± 1089 (44.6 ± 33.5/Mb) somatic single nucleotide variants (SNV) and 6.8 ± 5.0 (0.2 ± 0.27/Mb) insertions and deletions (INDEL) were identified. By comparison, only 44.8 ± 11.9 (1.4 ± 0.4/Mb) somatic SNV were determined in the exomes of the 4 "late onset" controls. Thus, the mutation frequency in the printing workers was 30 times higher. The predominant mutations in the tumour tissues of the printing workers and the control groups were C : G to T : A transitions and C : G to A : T transversions. However, it was only in the printing workers that the majority of the C : G to T : A transitions were found on the non-coding strand (Mimaki et al. 2016). This so-called "strand bias", which involves mutational asymmetries between the two DNA strands, may occur during the transcription and replication of DNA or through associated DNA repair mechanisms such as transcription-coupled nucleotide excision repair (TC-NER). As TC-NER is responsible for the repair of large DNA-distorting DNA adducts, a "strand bias" is interpreted as a marker for the development of these types of DNA adducts. However, a "strand bias" may also be caused by the disruption of DNA replication processes or the faulty activity of cytidine deaminase enzymes such as AID (Haradhvala et al. 2016; Siriwardena et al. 2016).

By far the most common mutations found in the tumour tissues of the printing workers were base substitutions in trinucleotide sequences of GpCpY to GpTpY (Y = pyrimidine base), followed by NpCpY to NpTpY or NpApY (N = one of the four bases). By comparison, predominantly NpCpG to NpTpG changes were determined in the tumours of the 4 "late onset" controls. After treatment with 1,2-dichloropropane, a whole-genome analysis of the Salmonella strain TA100 revealed an only partial recapitulation of the mutational signature. The printing workers were exposed to 1,2-dichloropropane over a period of 6 to 12 years and 1 printing worker was exposed additionally to dichloromethane for 1 year and 5 months. Two of the printing workers were smokers (20 cigarettes/day) and also regularly consumed alcohol. The unique mutational profile found in the 4 tumour tissues of the printing workers clearly demonstrates that they were exposed to a strong mutagen (Section 5.6.1; Mimaki et al. 2016; Timmermann et al. 2010). The trinucleotide mutational change from GpCpY to GpTpY found in the printing workers is quite unique, while the mutational change from NpCpG to NpTpG predominantly found in the tumours of the 4 controls ("late onset") is found in most types of tumours and often correlates with the age of the patients (Section 2; Sanger Institute 2019).

In a follow-up study of the 4 patients, the exomes of the previously analysed intrahepatic biliary tract tumours were examined in addition to 12 intrahepatic and extrahepatic bile duct lesions. The lesions were 2 invasive carcinomas (patient 1), 5 precancerous lesions (patient 2), an invasive carcinoma (patient 3) and 2 precancerous lesions and 2 recurring invasive carcinomas (patient 4). Cholangiocarcinoma tissue samples from the same 7 patients as in the preceding study were used as the controls. As previously determined in the study of Mimaki et al. (2016), the frequency of somatic mutations was increased with statistical significance in all lesions of the printing workers in comparison with the frequency determined in the control group. No differences were found between the frequency of mutations in the precancerous lesions and of those in the invasive tumours of the printing workers. To decipher the clonal origins, the lesions were analysed for the occurrence of overlapping mutations. In patient 2, there was overlapping between the tumour tissue and 2 precancerous lesions. In patient 3, about 40% of the mutations in both tumours overlapped. Therefore, the overall data suggest that of the 16 investigated lesions, 11 did not share a common clonal origin. The analysis of 125 "driver" genes, which increase the selective growth advantage of tumour cells, identified 5 genes with very high mutation frequencies. These were *ARID1A*, *ARID2*, *MLL2*, *SETBP1*, which code for chromatin-modifying proteins, and *TP53*. The higher mutation frequency of these 5 "driver" genes in invasive carcinogenic lesions in comparison with the frequency in precancerous lesions suggests that accumulation in these genes facilitates their transformation into invasive cholangiocarcinomas. The lesions detected in the printing workers revealed mutation spectra comprising the unique trinucleotide mutational change of GpCpY to GpTpY which were previously identified by Mimaki et al. (2016). By applying non-negative matrix factorization analysis, the authors extracted 3 mutational signatures from the mutation

spectra of each lesion and analysed the contribution of each signature to the mutational spectrum of the respective lesion. The signature with the unique GpCpY to GpTpY changes was predominant in 14 of the lesions. In the other 2 lesions, which demonstrated a lower mutation frequency, the predominant signature was characterized by C to A and C to G transversions. In contrast, the lesions in the control group demonstrated predominantly NpCpG to NpTpG and TpCpW to TpTpW or TpGpW changes. A hierarchical cluster analysis found that the signatures determined in the printing workers did not correspond with the 30 signatures registered in the COSMIC database (Sanger Institute 2015). The prominent GpCpY to GpTpY changes are unique to the SBS42 signature, which has now been included in the latest version of the COSMIC database (Section 2; Alexandrov et al. 2020; Mimaki et al. 2020; Sanger Institute 2019).

The predominance of the unique mutational signature (SBS42) in 14 of the 16 lesions of varying clonal origin identified in the printing workers suggests that their bile ducts were probably exposed to the same strong mutagen which induced multifocal carcinogenesis. The presence of other signatures indicates that several mutagenic processes were probably involved in the development of the lesions, as described for most other types of tumours (Alexandrov et al. 2020).

γ-H2AX antibodies were determined in the tumour tissue, BilIN, IPNB and non-neoplastic biliary epithelial tissue from 4 patients with biliary tract tumours and previous exposure to 1,2-dichloropropane. By binding to phosphorylated histone H2AX, these antibodies serve as markers for an increase in double-strand breaks. Increased binding of the antibody against S100P, which serves as a marker for malignant transformations, was observed only in the tumour tissue and in BilIN and IPNB, but not in the non-neoplastic tissue. Neither γ-H2AX formation nor S100P induction were found in the hepatocytes (Kinoshita et al. 2016, 2019).

The tumour tissue of 8 patients with occupational cholangiocarcinomas (printing workers, Osaka, Japan) was characterized in detail; BilIN and IPNB were detected and compared with the tissue samples from 16 cholangiocarcinomas and BilIN that were associated with hepatolithiasis. Additional comparisons were performed with IPNB from 19 control persons. Using antibodies against γ-H2AX as a marker, a considerable increase in the frequency of DNA double-strand breaks was observed in the foci of invasive carcinomas (7/8), in non-neoplastic tissue (6/8) and in the BilIN (6/8) and IPNB (4/4) of the printing workers. Double-strand breaks were detected in 7 of 16 samples of tumour tissue taken from the control patients, and in the BilIN in 3 of 16 samples. However, no double-strand breaks associated with γ-H2AX antibodies were found in the non-neoplastic tissue samples. γ-H2AX was detected in 6 of 19 IPNB of the control persons. A semi-quantitative analysis revealed a statistically significant increase in γ-H2AX in the tissues of the printing workers in comparison with the tissues of the control group. GSTT1-1 was expressed in all examined tissues, while CYP2E1 was detected only in hepatocytes, but not in biliary epithelial cells, BilIN, IPNB and tumour tissue. Therefore, the expression of GSTT1-1 and CYP2E1 was not different in the tissue samples from patients with occupational cholangiocarcinomas and the control tissue samples. P53 expression was investigated immunohistochemically in 3 tissue samples from printing workers and found to be increased in the invasive foci. *KRAS* and *GNAS* mutations were detected in 1 of the 3 tissue samples (Sato et al. 2014). The authors did not include information pertaining to whether the samples of cholangiocarcinoma tissue were taken from printing workers who were exposed only to 1,2-dichloropropane or only to dichloromethane or to mixtures of 1,2-dichloropropane and dichloromethane.

4.7 Carcinogenicity

A number of publications and a report prepared by the Japanese government investigated a cluster of cholangiocarcinomas found in printing workers in Japan (IARC 2017). In Japan, cholangiocarcinomas are a rare form of tumour with a mean age of onset of 66.5 years for intrahepatic tumours and 68.9 years for extrahepatic tumours. Only about 3% of all cholangiocarcinoma cases are diagnosed in the age group of 25 to 45 years. The incidence of biliary tract tumours worldwide varies with 1 to 2 cases per 100 000 individuals in the United States and 96 cases per 100 000 in northeastern Thailand (Kubo et al. 2014 b). Japan has a cholangiocarcinoma incidence of 5.2 cases per 100 000 and Asia has an incidence of 3.3 cases per 100 000 (Mimaki et al. 2016).

Intrahepatic and extrahepatic biliary tract tumours were found in the printing workers (Hamano et al. 2016). Cholangiocarcinomas are difficult to diagnose, are usually detected at a late stage and are associated with a poor prognosis and high mortality (Khan et al. 2005).

The cholangiocarcinomas that developed in printing workers as a result of exposure to 1,2-dichloropropane (and dichloromethane) exhibited typical characteristics. A comparison of the cholangiocarcinomas that developed in 5 printing workers and in 46 other patients revealed differences in the age of onset and in the findings of liver function tests. The mean age of onset in the printing workers was 35 years (31 to 39 years) and thus much lower than the mean age of onset of 68 years (32 to 83 years) determined in the control group of cholangiocarcinoma patients investigated in this study. Higher levels of γ-GTP activity and thrombocytes were found in the printing workers with occupational cholangiocarcinomas; the difference was statistically significant. While cholangiocarcinomas developed only in male printing workers, 18 of the 46 control cases were females. They had similar BMI values. The printing workers were diagnosed with regionally dilated bile ducts without tumour-induced obstruction (4/5). This finding was determined in only 1 patient of the control group (1/46). Bile ducts with papillary, villous or protruding tumours were observed in 3 of 5 printing workers, but only in 4 of the 46 control cases. Chronic bile duct lesions were detected in all 5 printing workers, but in only 21 of 46 control cases (Hamano et al. 2016). The findings are only of limited validity because the reports included only the values obtained from 5 printing workers with occupational cholangiocarcinomas.

Precancerous lesions (BilIN and IPNB) were observed in all tissue samples from the printing workers, but only in 6 of 46 samples of cholangiocarcinoma tissue taken from the control group (Hamano et al. 2016; Kubo et al. 2014 a, 2016).

Patients with occupational cholangiocarcinomas seemed to suffer a higher incidence of postoperative complications including intra-abdominal infections. Additionally, a higher percentage of bile duct dilations were observed. No cirrhotic changes or other hepatobiliary diseases were determined in the non-cancerous tissue (Kubo et al. 2018).

None of the primary risk factors such as primary sclerosing cholangitis, parasitic diseases such as liver fluke or trematode infections, gallstones, fibropolycystic liver disease, viral hepatitis, exposure to Thorotrast, congenital anomalies of the biliary system, excessive consumption of alcohol or smoking were found to apply to the printing workers with cholangiocarcinomas (Kubo et al. 2014 a; Kumagai et al. 2014).

A number of studies investigating the risk factors for cholangiocarcinomas found that the odds ratio (OR) for cholangiocarcinomas was increased with statistical significance if positive results were obtained in tests detecting antibodies to the liver flukes Opisthorchis viverrini (OR 5.0–27.1) and Clonorchis sinensis (OR 2.7–4.7). Biliary cysts which develop as a result of a pancreaticobiliary maljunction, leading to the presence of pancreatic enzymes in the biliary system, increase the risk of developing cholangiocarcinomas 10 to 50-fold. About 10% of all cholangiocarcinomas are caused by the autoimmune disease primary sclerosing cholangitis. About 2% to 10% of all patients with hepatolithiasis develop cholangiocarcinomas. The development of this gallstone liver disease is often (about 30%) accompanied by a parasitic disease and occurs predominantly in south-eastern Asia. The X-ray contrast agent Thorotrast (alpha-emitter) increased the risk of developing a cholangiocarcinoma 300-fold. Other possible risk factors that harbour a lower risk are chronic enteritis, cholangitis, choledocholithiasis, viral hepatitis and cirrhosis. Additional factors that are suspected of contributing to a slightly higher risk, but for which the findings are not consistent, are diabetes, adiposity, excessive alcohol consumption (> 80 g/day) and smoking (Clements et al. 2020).

The calculated ORs shown in Table 1 were included in a recently published meta-analysis of the risk factors for intrahepatic and extrahepatic cholangiocarcinomas. A comparison of the findings for the Western countries USA, Denmark and Italy and the Asian countries China, Taiwan, Japan and South Korea revealed only a marginal difference for cirrhosis as a risk factor for intrahepatic cholangiocarcinomas (Clements et al. 2020).

Tab. 1 ORs for various risk factors for cholangiocarcinomas (Clements et al. 2020)

Risk factors	OR
bile duct cysts	34.94
choledocholithiasis	18.58
cirrhosis	3.82
gallstones (cholelithiasis)	5.92

Tab. 1 *(continued)*

Risk factors	OR
hepatitis B	2.11
hepatitis C	1.51
alcohol consumption	1.75
cholecystocholithiasis	2.95
chronic enteritis	2.37
type 2 diabetes	1.50
smoking	1.69
high blood pressure	1.21
obesity (BMI > 25 or > 30)	1.20

4.7.1 Studies in printing companies, Japan

4.7.1.1 Printing company in Osaka, Japan

A report published in 2013 described 11 patients with cholangiocarcinomas who worked at two different plants of the same printing company in Osaka, Japan. The company was a small offset proof-printing company that produced only proofs. Only about 10 copies are printed at a time using this printing method, after which the blanket cylinder needs to be cleaned manually and, if necessary, the ink on the press changed to a different colour. Overall, cleaning takes much longer than printing. Up to seven printing presses were operating in the printing rooms at any given time. The workers worked in two shifts (16 hours). The rubber sheet requires cleaning after each new proof, or every few minutes. Different cleaning agents were used at the plants: in some cases 1,2-dichloropropane (1998 to 2006), in some cases mixtures of 1,2-dichloropropane, dichloromethane and 1,1,1-trichloroethane (1987 to 1993) and in some cases mixtures of 1,2-dichloropropane, dichloromethane and petroleum hydrocarbons (1993 to 1998). The ink trains (inking rollers, etc.) were cleaned with kerosine; however, in comparison with other cleaning procedures, this is rarely required. Other petroleum hydrocarbon mixtures were likewise used for cleaning (1993 to 1998). After 2006, 1,2-dichloropropane was replaced by a mixture of glycol ether, alcohols and cycloaliphatic hydrocarbons. The workers wore gloves, but no form of respiratory protective equipment. The ventilation in the rooms was described as inadequate; there was no exhaust system installed near the source. To re-create the exposure situation, the working conditions were reconstructed and a model was prepared. The findings revealed that it is possible to estimate the exposure concentrations from the quantities of chemicals used. The authors estimated the following levels of exposure: 100 to 670 ml/m^3 of 1,2-dichloropropane and 80 to 540 ml/m^3 of dichloromethane in the proof-printing room, 70 to 110 ml/m^3 of 1,2-dichloropropane and 50 to 130 ml/m^3 of dichloromethane in the front room. The age of the printing workers at diagnosis was 25 to 45 years; the age of the workers who died was 27 to 46 years at the time of death. The patients were exposed to 1,2-dichloropropane for a period ranging from 7 to 17 years and the cholangiocarcinoma diagnosis was made 7 to 20 years after the initial exposure. A total of 51 male workers were working in the proof-printing room and 11 male workers in the front room. Of the persons working in the front room, only 1 person was diagnosed with a cholangiocarcinoma. However, this person had sporadically worked in the proof-printing room. Cholangiocarcinomas were not detected in any of the 11 women working at the company at this time. In all patients, tests for hepatitis A and B yielded negative results. From these findings, the authors calculated a standardized mortality ratio (SMR) of 2900 for all male workers employed between 1991 and 2011 (expected deaths 0.00204; 95% confidence interval (CI): 1100–6400), of 5000 for workers working in the proof-printing room (expected deaths 0.001; 95% CI: 1600–12 000) and of 960 for workers in the front room (expected deaths 0.00104; 95% CI: 24–5400). The SIR for male and female employees was 1226. An official investigation found that both the effects of exposure to kerosine and of any incidental exposure to the printing inks were negligible in view of the other exposure (Kumagai et al. 2013; MHLW 2013). A later publication reported shorter periods of exposure to 1,2-dichloropropane and dichloromethane. According to this publication, the printing company used 1,2-dichloropropane as a cleaning agent from 1991 to October 2006 and dichloromethane from 1991 to March 1996

(IARC 2017; Sobue et al. 2015). The printing company in Osaka operated printing plants at four different locations. At one of these plants, 13 workers were already using 1,2-dichloropropane as a cleaning agent in 1987 and continued to do so in the years that followed. These procedures were performed at the other sites beginning in 1991 (90 workers) and in 2002 (16 workers) (Kumagai et al. 2016).

Another study was carried out to supplement the findings of Kumagai et al. (2013) by evaluating other cholangiocarcinoma cases and the exact clinical and pathological findings. This retrospective study of 13 hospitals in Japan identified 17 patients with cholangiocarcinomas who belonged to a group of 111 former or current workers of the offset colour proof-printing department of a printing company in Osaka. This study includes the cases described by Kumagai et al. (2013). At this printing company, the residues of printing ink were removed with cleaning agents including chlorinated hydrocarbons such as 1,1,1-trichloroethane (until 12/1992), 1,2-dichloropropane (until 10/2006) and dichloromethane (until 3/1996). The 22 substances used by this printing company are shown in Table 2. The quantities and length of use were not specified.

Tab. 2 Chemicals handled at the printing company in Osaka, Japan (Kubo et al. 2014 b)

1,1,1-trichloroethane	diethylene glycol monobutyl ether
1,2-dichloropropane	propylene glycol monomethyl ether
dichloromethane	2-methyl-2,4-pentanediol
dichlorofluoroethane	3-methyl-3-methoxybutanol
2-butanol	solvent naphtha (coal)
2-methylpentane	xylene
3-methylpentane	kerosine
n-hexane	mineral oil
cyclohexane	hydrocarbons
isopropyl alcohol	aromatic hydrocarbons
ethanol	printing inks

The age of the patients at the time of diagnosis was 25 to 45 years (mean: 36 years) and the age of the workers who died was 26 to 46 years at the time of death. The patients were between 18 and 28 years old when they began employment at the printing company. A cholangiocarcinoma was diagnosed in 10 currently and 7 previously employed workers of this company. The patients were exposed to 1,2-dichloropropane for periods ranging from 6 years and 1 month to 16 years and 1 month (on average 9 years and 7 months). The period from the beginning of employment to diagnosis ranged from 7 years to 19 years and 9 months (on average 11 years and 4 months). The longest period of time from the end of exposure to diagnosis was 9 years and 7 months. All 17 patients were exposed to 1,2-dichloropropane, 11 patients to dichloromethane and 8 patients to 1,1,1-trichloroethane. Liver values were elevated in 11 of the patients and liver tumours were additionally detected in 2 patients. Thirteen of the patients were smokers. The diagnosis was made using various imaging techniques. Samples taken from 16 patients were examined histologically: resected tissue from 12 persons or biopsies from 4 persons. The diagnoses were re-evaluated at Osaka City Hospital (Japan) and classified according to the WHO criteria as intrahepatic (12 cases) or extrahepatic (5 cases) cholangiocarcinomas. The blood serum levels of the following markers were increased in the patients (number in parentheses): total bilirubin (8), AST (13), ALT (14), γ-GTP (all 17 patients), carcinoembryonic antigen (CEA) (10) and carbohydrate antigen 19-9 (CA 19-9) (10) (Kubo et al. 2014 b). Contrary to the information provided in the text, Figure S1 (Kubo et al. 2014 b) lists an exposure period of 7 years as the shortest period of exposure.

The printing company in Osaka began using 1,2-dichloropropane and dichloromethane for cleaning in 1991 and stopped using dichloromethane in 1996 and 1,2-dichloropropane in 2006. The other 20 chemicals were used in small amounts and for shorter periods of time. By performing a reconstruction experiment, which was based on the assumption that the quantities of 1,2-dichloropropane and dichloromethane used were proportional to the exposure concentration, a

full-shift time-weighted average concentration of 1,2-dichloropropane of 60 to 210 ml/m³ and of dichloromethane of 130 to 360 ml/m³ was determined (IARC 2017).

Another cholangiocarcinoma with extensive lymph node metastasis was diagnosed in a 41-year-old man who had likewise worked at the above-mentioned printing company in Osaka, Japan, for 6 years. He was exposed to 1,2-dichloropropane and dichloromethane during the period of his employment. The condition became manifest 18 years after the patient had stopped working there. The patient regularly consumed large amounts of alcohol. The blood serum levels of the following markers were increased in the patient: total bilirubin, AST, ALT, γ-GTP, CEA and s-pancreas-1-antigen (Kinoshita et al. 2019).

Another study investigated a cluster of cholangiocarcinomas that developed in the printing workers of the printing company in Osaka, Japan. Using the 106 workers (86 men, 20 women) at this printing company as a basis, 1452.4 person-years of exposure to 1,2-dichloropropane and dichloromethane were calculated from the individual periods of employment. The data for 10 other workers of this printing company were not included in the calculation because of missing information. A SIR of 1132.5 (95% CI: 659.7–1813.2) was calculated for the 17 cholangiocarcinoma cases that had occurred up until that point. A SIR of 1319.9 (95% CI: 658.9–2.361) for 560.3 person-years was determined for workers with co-exposure to 1,2-dichloropropane and dichloromethane and a SIR of 1002.8 (95% CI: 368.0–2182.8) for 727.7 person-years for those with exposure to 1,2-dichloropropane alone. As cholangiocarcinomas did not develop in the women, the somewhat higher SIR of 1060.8 (only 1,2-dichloropropane) and 1330.6 (1,2-dichloropropane and dichloromethane) was calculated only for the male workers. Higher SIRs are likewise obtained when a lag time of 3 or 5 years is assumed (Sobue et al. 2015).

The printing company in Osaka, Japan, operated 4 plants. 1,2-Dichloropropane was handled at these sites by 13 workers from 1987 to 1991, by 90 workers from 1991 to 2006 and by 11 or 5 workers from 2002 to 2006, respectively (2 sites). Dichloromethane was used only from 1987 to 1991 and from 1991 to 1996. It was not specified how many of the 13 or 90 employees, respectively, were not exposed to dichloromethane. At all plants of the company, the ink roller was cleaned only with kerosine and after 2006, the blanket cylinder was cleaned using a mixture of glycol ether, alcohols and cyclic hydrocarbons. Several of the 116 employees worked at 2 sites. Excluded from the study were 11 workers who were hired by the printing company after 2006 and 10 workers for whom only incomplete data were available. No respiratory protective equipment was used. The SIR for cholangiocarcinomas was calculated for the years 1987 to 2012 based on the data for 95 workers (78 men and 17 women) who were exposed to 1,2-dichloropropane from 1987 to 2006. The cumulative exposure concentrations were first estimated and the data then divided into four categories. The SIR was calculated for effects occurring immediately at the end of the exposure period and for those occurring after an exposure lag time of 5 years, whereby the exposure in the 5 years leading up to the findings was not included in the calculations. The cumulative exposure to 1,2-dichloropropane was 32 to 3433 ml/m³ × years with a median of 851 ml/m³ × years and a SIR of 1171 (95% CI: 682–1875). The following SIRs were calculated by dividing cumulative exposure to 1,2-dichloropropane into 4 categories: category 1 to 499 ml/m³ × years: SIR 0 (95% CI: 0–537); category 500 to 999 ml/m³ × years: SIR 148 (95% CI: 4–826); category 1000 to 1999 ml/m³ × years: SIR 18 732 (95% CI: 8565 to 35 549) and category 2000 to 3999 ml/m³ × years: SIR 16 817 (95% CI: 6760–34 642). The SIRs calculated for effects occurring in the 4 categories with exposure lagging of 5 years were 0 (95% CI: 0–612), 846 (95% CI: 275–1975), 13 714 (95% CI: 4451–32 007) and 21 894 (95% CI: 8802–45 102). A statistically significant increase in SIRs was determined with increasing exposure, but not in the lowest exposure category of 1 to 499 ml/m³ × years. After adjusting for age, sex, calendar year and exposure to dichloromethane and applying a Poisson regression analysis, the relative risk in the two high exposure categories was 14.9 (95% CI: 4.1–54.3) and 17.1 (95% CI: 3.8–76.2) and with a 5-year lag time 11.4 (95% CI: 3.3–39.6) and 32.4 (95% CI: 6.4–163.9). No association was found between the occurrence of cholangiocarcinomas investigated in this study and exposure to dichloromethane. The exact data are shown in Table 3 (Kumagai et al. 2016).

Tab. 3 Standardized incidence ratios according to sex, exposure and cumulative exposure (Kumagai et al. 2016)

	Person-years	Number of printing workers with CCA	Expected number of CCA	SIR (95% CI)
total number of workers n = 95	1367.3	17	0.01452	1171 (682–1875)
male workers n = 78	1142.0	17	0.01413	1203 (701–1927)
female workers n = 17	225.3	0	0.00039	0 (0–9426)
workers exposed only to 1,2-DCP n = 62	727.5	6	0.00589	1019 (374–2218)
workers exposed to 1,2-DCP and DCM n = 33	639.8	11	0.00863	1275 (636–2280)
cumulative exposure (ml/m³ × years)				
lag time 0 years				
1–499	742.3	0	0.00687	0 (0–537)
500–999	332.3	1	0.00675	48 (4–826)
1000–1999	188.1	9	0.00048	18 732 (8565–35 549)
2000–3999	104.6	7	0.00042	16 817 (6760–34 642)
lag time 5 years				
0	475.0	0	0.00189	0 (0–1949)
1–499	512.3	0	0.00603	0 (0–612)
500–999	222.5	5	0.00591	846 (275–1975)
1000–1999	107.9	5	0.00036	13 714 (4451–32 007)
2000–3999	49.6	7	0.0032	21 894 (8802–45 102)

CCA: cholangiocarcinomas; DCM: dichloromethane; 1,2-DCP: 1,2-dichloropropane; SIR: standardized incidence ratio

The concurrent use of UV curable inks containing photoinitiators may intensify the effects of 1,2-dichloropropane (Section 5.8; Kawasaki et al. 2015). However, to date, this has been demonstrated only in vitro (Section 5.8). Other authors pointed out that UV curable inks began to be used only at a later time and several of the cholangiocarcinomas developed in workers who had no contact with UV curable inks (Kumagai and Kubo 2016).

Summary: The available studies demonstrate that the probability of developing a cholangiocarcinoma increases with statistical significance with cumulative exposure to 1,2-dichloropropane.

4.7.1.2 Other printing companies, Japan

In addition to the 20 cholangiocarcinoma cases that occurred at a printing company in Osaka and were described by Kinoshita et al. (2019), Kubo et al. (2014 b) and Kumagai (2019), another 19 cases of this cancer occurred in Japan that were attributed to exposure at the workplace (Yamada et al. 2014, 2015 a, b).

Two cholangiocarcinoma cases occurred at each of 3 offset proof-printing companies in Miyagi, Fukuoka and Hokkaido, all of which were small companies with less than 50 workers. The 6 patients had been exposed to 1,2-dichloropropane at the workplace for periods ranging between 11 to 17 years. Mathematical models were used to estimate the levels of exposure, yielding 1,2-dichloropropane concentrations of 17 to 180 ml/m³ (79–830 mg/m³) in the proof-printing room and 150 to 620 ml/m³ (690–2900 mg/m³) during cleaning. The time-weighted averages for exposure to 1,2-dichloropropane were in the range from 62 to 240 ml/m³. Four of the printing workers with cholangiocarcinomas were exposed also to dichloromethane concentrations of 0 to 180 ml/m³ (TWA) for 6 to 12 years. While cleaning, the workers

were exposed to dichloromethane concentrations of 0 to 560 ml/m³. None of the workers used respiratory protective equipment and the rooms were not adequately ventilated. The workers were co-exposed to other substances at the different printing companies: in Miyagi to 1,1,1-trichloroethane (3 to 5 years), naphtha and mineral spirit (continuous), in Fukuoka to petrol (15 years, 1 printer), 1,1-dichloro-1-fluoroethane (3 years), mineral spirit (6 years), kerosine and mineral oil (continuous) and in Hokkaido to petrol (5 years), 1,1,1-trichloroethane (8 years), mineral spirit (3 years) and kerosine (continuous). However, the cleaning agents were not handled concurrently. Until 1985, petrol was the only cleaning agent used. Its use was later discontinued. Special, non-routine cleaning procedures were performed only with kerosine and mineral oil. None of the other chlorinated hydrocarbons were handled by all workers with cholangiocarcinomas (Yamada et al. 2014).

In Nagayo, which is located in the prefecture of Aichi, Japan, a worker was diagnosed with a cholangiocarcinoma after working for 11 years at an offset proof-printing company. He was diagnosed 11 years after he stopped working at the printing company. The cleaning agents used by this printing company were dichloromethane (estimated concentrations: 240–6100 ml/m³), 1,1,1-trichloroethane and kerosine. The printing worker consumed minimal amounts of alcohol (three 350 ml beers per week) and smoked for about 10 years (about 4 cigarettes/day). Tests for hepatitis B and C yielded negative results (Kumagai 2014). This publication describes another case of a printing worker with a cholangiocarcinoma. However, this case was already discussed by Yamada et al. (2014) as Case C. This patient did not consume alcohol and smoked about 20 cigarettes/day (IARC 2017).

Another 7 cholangiocarcinoma cases were diagnosed among workers of offset proof-printing companies. Five workers were employed at small printing companies (< 50 employees) and 2 at medium-sized companies (with 50 to 299 employees). The maximum exposure levels were estimated on the basis of the data for the quantities of substance used; the 4 workers with co-exposure to both 1,2-dichloropropane and dichloromethane were estimated to have been exposed to 1,2-dichloropropane concentrations of 230 to 420 ml/m³ (TWA 0–210 ml/m³) and to dichloromethane concentrations of 58 to 720 ml/m³ (TWA 15–270 ml/m³). During this period, they were exposed to 1,2-dichloropropane for 3 to 14 years and to dichloromethane for 5 to 20 years. None of the exposed persons wore respiratory protective equipment. Other substances that were used for cleaning and thus represent sources of co-exposure were nonane (5 years, 1 printing worker), petrol (5 years, 1 employee), mineral spirit (1 to 10 years, 3 employees), 2-butanol (3 years, 1 printing worker), mineral oil (10 years, 1 printing worker), kerosine (6 to 12 years, 2 printing workers), D-limonene (12 years, 1 printing worker), "petroleum solvent" (20 years, 1 printing worker), 1,1-dichloro-1-fluoroethane (13 years, 1 printing worker) and 1,1,1-trichloroethane (5 years, 1 printing worker). Three of the employees were exposed to dichloromethane, but not to 1,2-dichloropropane, for 8 to 12 years at a maximum concentration of 600 to 1300 ml/m³ (TWA 84–440 ml/m³). In addition to dichloromethane, these 3 printing workers were exposed to kerosine (11 to 18 years, 2 printing workers), petrol (12 years, 1 printing worker), methylcyclohexane, ethanol, 2-propanol and 1-propanol (for 8 years in each case, 1 printing worker), mineral spirit (8 years, 1 printing worker), 1,1,1-trichloroethane (11 to 18 years, 2 printing workers) and "petroleum solvent" (8 years, 1 printing worker). The authors assume that dichloromethane promotes the development of cholangiocarcinomas (Yamada et al. 2015 a). The case of 1 printing worker who was diagnosed with a cholangiocarcinoma after exposure to only dichloromethane was described by Kumagai (2014).

A total of 6 cholangiocarcinoma cases occurred at other offset proof-printing companies (4 small companies with < 50 workers and 1 medium-sized company with 50–299 workers), including the case of 1 worker who operated a coating machine. The printing workers were exposed to 1,2-dichloropropane for 2 to 11 years and to dichloromethane for 0 to 22 years. During this 2 to 11-year period, they had co-exposure to maximum 1,2-dichloropropane concentrations of 190 to 560 ml/m³ (TWA 0–230 ml/m³) and to maximum dichloromethane concentrations of 300 to 980 ml/m³ (TWA 20–470 ml/m³); the maximum concentrations were estimated based on the quantities of substance used. The worker who operated the coating machine was exposed to 1,2-dichloropropane for 8 years at concentrations of 5 to 19 ml/m³ (TWA) with peaks of up to 150 ml/m³, but not to dichloromethane. None of the exposed persons used respiratory protective equipment. Other substances that were used for cleaning at the printing companies and thus represent sources of co-exposure were trichloroethylene (8 to 12 years, 2 printing workers), 1,1,1-trichloroethane (5 years, 1 printing worker), 1,1-dichloro-1-fluoroethane (2 to 11 years, 2 printing workers; 8 years, 1 coating machine operator), mineral spirit (4 years, 1 printing worker), kerosine (4 to 12 years, 4 printing workers), mineral oil (7 to 20 years, 3 printing

workers), toluene (4 years, 1 printing worker; 8 years, 1 coating machine operator), "petroleum solvent" (23 years, 1 printing worker), cyclohexane and polyethylene glycol monoethyl ether (2 years, 1 printing worker) and xylene and hexane (4 years, 1 printing worker). Certain cleaning procedures were performed only with kerosine and mineral oil or "petroleum solvent". None of the other chlorinated hydrocarbons were used by all of the workers who were diagnosed with cancer (Yamada et al. 2015 b).

For each of the printing workers (outside of Osaka) who were diagnosed with cholangiocarcinomas, the reports include data for the duration of exposure, the substances used in each case and the calculated levels of exposure to 1,2-dichloropropane and dichloromethane. The studies further describe the methods used to calculate the exposure concentrations (Kumagai 2014; Yamada et al. 2014, 2015 a, b).

Another study includes the clinical data for 9 printing workers who were diagnosed with an occupational cholangiocarcinoma (not employed by the printing companies in Osaka). The age of the patients at the time of diagnosis was 31 to 57 years (average age 44 years); all of the patients were male. Five of the patients were exposed to 1,2-dichloropropane and dichloromethane, 2 to 1,2-dichloropropane, dichloromethane and 1,1,1-trichloroethane and 2 to dichloromethane and 1,1,1-trichloroethane. The exposure periods ranged from 3 years and 10 months to 19 years. None of the workers consumed excessive amounts of alcohol, 6 were smokers. Intrahepatic tumours were observed in 4 patients, extrahepatic tumours in 5 patients. Lymph node metastases were found in 3 patients. Preneoplastic or early preinvasive neoplastic lesions such as BilIN or IPNB in addition to chronic bile duct injury were observed in all patients. In 2 of 3 patients for whom these data were available, the levels of γ-GTP activity had already been found to be increased several years prior to the tumour diagnosis. Serum levels for CEA (2 patients), CA 19-9 (6 patients) and Dupan-2 (3 of 4 patients) were increased (Kubo et al. 2014 a).

4.7.1.3 Cholangiocarcinomas in printers, overview

Of the 37 cases with an occupational cholangiocarcinoma reported up to the year 2016, 22 underwent surgical treatment. As the data available for 1 patient were incomplete and the primary tumour of another patient was suspected to be pancreatic cancer, the clinical findings, the laboratory results and the pathology of the tumour tissue were examined for 20 patients. Five of the patients were exposed only to 1,2-dichloropropane, 8 to 1,2-dichloropropane and dichloromethane and 6 to 1,2-dichloropropane, 1,1,1-trichloroethane and dichloromethane. One patient was exposed to 1,2-dichloropropane and 1,1-dichloro-1-fluoroethane. At the time of diagnosis, the patients were between 29 and 57 years old. One patient consumed > 80 g ethanol/day and 13 patients were smokers. None of the patients had hepatitis B or C. The precancerous changes observed in 15 of the 16 examined patients were BilIN-2/3 lesions in the bile duct and peribiliary glands, while IPNB lesions were determined at different specimen sites in 11 of 15 patients. Chronic bile duct disease was observed in 15 of 16 patients. BilIN, IPNB and chronic bile duct disease were rarely found in the small bile ducts of the liver. Elevated serum levels of CEA (6 patients) and CA 19-9 (12 patients) were detected. Neither cirrhotic changes nor hepatobiliary disease were found in the non-cancerous tissue. A recurrence was observed in 12 of 20 patients and 2 patients developed metastases in the liver or in the lymph nodes. One patient died of biliary and liver cirrhosis without recurrence of the tumour (Kubo et al. 2016; Tomimaru et al. 2015).

When the characteristic findings in the printing workers from Osaka who were diagnosed with cholangiocarcinomas were compared with those in the workers from other printing companies, clear agreement was found in the statistically significant increases in γ-GTP levels, regionally dilated bile ducts, BilIN and IPNB in the surgical specimens and chronic bile duct injury. The mean age of disease onset was 36 years for the printing company in Osaka and 44 years for the printing companies located outside of Osaka. The CA 19-9 antigen was found to be increased in 13 of 17 printing workers from Osaka and in 6 of 9 printing workers from other printing companies. The ratio intrahepatic : extrahepatic : intrahepatic plus extrahepatic tumours differed by location with 10 : 5 : 2 cases in Osaka and 4 : 5 : 0 at the other printing companies (Kubo et al. 2014 a).

The occupational cholangiocarcinoma cases in Japan are summarized in Table 4.

Tab. 4 Representation of the number of cholangiocarcinoma cases by exposure

Activity	Total number	Exposure to			References
		1,2-dichloropropane and dichloromethane	only 1,2-dichloropropane	only dichloromethane	
printing workers in Osaka	20[a)]	14	6	–	Kinoshita et al. 2019; Kubo et al. 2014 b; Kumagai 2019
printing workers, other printing companies	18	13	2	3	Kumagai 2019; Yamada et al. 2014
printing workers, location not specified	5[a)]		3[a)] dominant exposure, no other details	2[a)] dominant exposure, no other details	Kumagai 2019
coating machine operator	1		1		Yamada et al. 2015 b
total	39 (44[a)])	27	9 (12[a)])	3 (5[a)])	

[a)] some cases not yet published (Kumagai 2019)

4.7.2 Other case–control and cohort studies

A prevalence ratio of 1.31 (95% CI: 0.91–1.89) was calculated for male printing workers from different printing companies who were diagnosed with cholangiocarcinomas and all male workers in the printing industry in Japan, with a ratio of 1.78 (95% CI: 0.63–5.00) for the age group of 30 to 40-year-olds. According to the authors, this demonstrates that the marked increase in risk does not apply for the entire printing industry (Okamoto et al. 2013).

On the basis of the findings of a European multicentre case–control study (ECC) of rare tumours with unknown aetiology which was carried out between 1995 and 1997, an OR of 1.88 was calculated for printing workers and an OR of 3.26 for typographers. After adjusting for a history of gallstones, an OR of 2.42 was calculated for printing workers and an OR of 5.78 for typographers (Ahrens et al. 2014).

The data for workers (aged 30 to 64 years) at printing companies were correlated with the data collected by the cancer registries of Finland, Iceland, Norway and Sweden for a period of 45 years. The database of the Nordic Occupational Cancer Study (NOCCA) was used. The database included 74 949 persons in the category printing workers and associated professions. The SIRs were calculated for specific types of tumours that developed in men and women working in the printing industry. In the men, elevated SIRs were found for liver tumours (1.35; 95% CI: 1.14–1.60; 142 cases) and for intrahepatic cholangiocarcinomas (2.34; 95% CI: 1.45–3.57; 21 cases). The SIR of 1.13 determined for the 53 cases of extrahepatic cholangiocarcinomas did not represent a marked increase. The SIRs for women working in the printing industry showed a similar pattern. However, the values were based on only 32 cases of liver tumours and 8 cases of intrahepatic cholangiocarcinomas. The job exposure matrix of the NOCCA database does not yet include data for exposure to 1,2-dichloropropane (Vlaanderen et al. 2013).

An analysis of the data collected for a prospective cohort study carried out from 2003 to 2009 with 50 884 women aged 35 to 74 years (Sister Study) found a weak positive association between the environmental burden of 1,2-dichloropropane (air sampling) and the occurrence of breast cancer with a hazard ratio of 1.1 (95% CI: 0.98–1.23) in the highest quintile of exposure to 1,2-dichloropropane with levels > 1.93×10^{-3} µg/m^3. An analysis including only oestrogen receptor-positive invasive breast cancer cases determined a hazard ratio of 1.19 (95% CI: 1.02–1.38) in the highest quintile (Niehoff et al. 2019; Sandler et al. 2017).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

The LC_{50} values were determined to be 2256 mg/m³ (about 480 ml/m³) and 720 ml/m³ in mice (Greim 1998).

The LC_{50} values for rats were 9400 and 14 000 mg/m³ (Greim 1998).

Exposure of Drosophila larvae for 48 hours yielded an LC_{50} value of 14.4 mg/m³ (Chroust et al. 2007).

After exposure of male Wistar rats (10 per group) to 1,2-dichloropropane concentrations of 0, 15, 50, 100, 250, 450, 1000, 1300, 1800 or 4900 mg/m³ for 4 hours, GSH levels were found to be decreased in the liver directly after exposure to concentrations of 100 mg/m³ and above. However, 20 hours later, GSH levels were found to be increased in the liver at 1800 mg/m³ and above. No effects on the serum levels of AST, ALT and alkaline phosphatase and on hepatic lipid peroxidation (determination of malondialdehyde levels) were observed (Di Nucci et al. 1990).

Groups of 5 male B6C3F1 mice were exposed to 1,2-dichloropropane concentrations of 0 or 300 ml/m³ and/or a dichloromethane concentration of 800 ml/m³ for 6 hours, followed by observation periods of 2, 4, 6, 8 or 24 hours. After exposure to **only 1,2-dichloropropane**, the relative liver weights were decreased (about 10%) and the activities of AST and ALT were increased after 2 hours. Hypertrophy of the liver was detected 8 hours after the end of exposure. Hepatocellular necrosis and the inhibition of mitochondrial glutathione *S*-transferase (MGST1) were observed after 18 hours. Four hours after exposure to a mixture of **1,2-dichloropropane and dichloromethane,** increases in relative liver weights (by 10%–20%), in AST and ALT activities and hypertrophy of the liver were found. Hepatocellular necrosis and granular degeneration were observed after 8 hours. According to the authors, the effects induced by 1,2-dichloropropane were intensified by dichloromethane (Wang et al. 2019).

Groups of 2 or 3 male C57BL/6 mice were exposed to 1,2-dichloropropane for 6 hours on day 1 and for 3 hours on day 2 at concentrations of 0, 100, 200 or 400 ml/m³. AST and ALT activities in the blood were not elevated. The relative liver weights were not increased (no other details; Toyooka et al. 2017).

5.1.2 Oral administration

The LD_{50} values in mice were 860 and 960 mg/kg body weight (Greim 1998).

The LD_{50} value in rats was 1942 mg/kg body weight (Greim 1998).

Groups of 5 to 12 (no other details) male Wistar rats were given a single gavage dose of 1,2-dichloropropane of 2 ml/kg body weight (40% v/v in corn oil). GSH depletion in the liver reached statistical significance; this was accompanied by a significant increase in AST, ALT, 5′-nucleotidase, γ-GTP and alkaline phosphatase levels in the serum. There was a moderate increase in serum urea and creatinine levels, which is regarded as evidence of toxic effects induced by 1,2-dichloropropane in the kidneys. The development of haemolysis correlated with the depletion of GSH in the blood of rats (Greim 1998; Imberti et al. 1990).

Groups of 5 male Syrian hamsters or male B6C3F1 mice were given single gavage doses of 1,2-dichloropropane of 500 mg/kg body weight. After 4 hours, the relative liver weights in the hamsters were decreased with statistical significance. No effects were observed in the mice. In both species, GSH levels were depleted by more than 95% (Gi et al. 2015 a).

5.1.3 Dermal application

The LD_{50} after percutaneous application was 8150 mg/kg body weight (no other details; Torkelson and Rowe 1981).

The LD_{50} value in rabbits was 10 115 mg/kg body weight (Greim 1998).

5.1.4 Intraperitoneal injection

An LD_{50} value of 1100 mg/kg body weight was determined for 1,2-dichloropropane in rats (Greim 1998).

After *Cyp2e1*$^{+/+}$ mice (wild type) and *Cyp2e1*$^{-/-}$ mice (CYP2E1-deficient) were given injections of 1,2-dichloropropane of 300 mg/kg body weight, the body weights and relative liver weights as well as AST and ALT activity levels were found to be increased only in *Cyp2e1*$^{+/+}$ mice. There were no haematological findings. No effects were observed in *Cyp2e1*$^{-/-}$ mice. 1,2-Dichloropropane was given to groups of 6 *Cyp2e1*$^{+/+}$ mice or *Cyp2e1*$^{-/-}$ mice by intraperitoneal injection at dose levels of 0, 100, 200 or 300 mg/kg body weight. After 16 hours, dose-dependent liver changes had been induced only in the wild type at 200 mg/kg body weight and above. These changes were manifest as necrosis, enlarged hepatocytes around the portal vein, nucleolar hypertrophy, macrovesicular steatosis (fatty degeneration) and haemocongestion (Yanagiba et al. 2016 a).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

The documentation for 1,2-dichloropropane published in 1993 included an extensive review of inhalation studies with 1,2-dichloropropane. After exposure of rats for 13 weeks, hyperplasia of the respiratory epithelium was observed with a LOAEC (lowest observed adverse effect concentration) of 71 ml/m^3 (no NOAEC (no observed adverse effect concentration)). Liver damage was induced at higher concentrations. In mice, no substance-related effects were observed up to a concentration of 705 mg/m^3, even after exposure for 13 weeks. In guinea pigs, damage to the liver, kidneys, adrenal glands and heart was found after exposure for 134 days to 1880 mg/m^3 (no NOAEC). In rabbits, exposure to 1,2-dichloropropane for 13 weeks reduced the erythrocyte count and decreased haemoglobin and haematocrit levels at a LOAEC of 705 mg/m^3 (no NOAEC). In dogs, haemosiderosis of the spleen and granulomatous lesions of the kidneys were induced after exposure to 1880 mg/m^3 for 134 days (no NOAEC) (Greim 1998).

In a study that examined the distribution of GSTT in the liver, groups of 3 C57BL/6J mice, 3 Balb/cA mice, 3 F344 rats, 3 Syrian hamsters and 3 guinea pigs were exposed whole-body to 1,2-dichloropropane for 7 or 14 days (8 hours a day, 7 days a week) at concentrations of 0, 300, 1000 or 3000 ml/m^3 or 0, 200, 400 or 800 ml/m^3, respectively, and the livers were examined histopathologically. In the mice that survived, liver damage was observed at concentrations of 400 ml/m^3 and above; however, a few animals died even after exposure to the lowest concentrations of 200 or 300 ml/m^3. In the hamsters, the body weights and relative liver weights were decreased at concentrations of 200 ml/m^3 and above and mortality was observed at concentrations of 800 ml/m^3 and above. In the rats, the accumulation of fat-like droplets and changes to the colour of the liver were observed at concentrations of 3000 ml/m^3 and above. All of the guinea pigs died on day 5 of exposure to 300 ml/m^3; however, no other relevant effects were observed. The proliferation marker Ki-67 was detected in the hepatocytes and in the bile ducts of the rats, hamsters and mice; however, there were no differences between the treated animals and the control animals (Zhang et al. 2015). Effects were induced in the rats only at 3000 ml/m^3 after exposure for 7 days. In this study, the NOAEC for the rats was 1000 ml/m^3. The sex of the animals was not specified. The animals were described as male in a later publication (Zhang et al. 2018).

Groups of 6 male C57BL/6JJcl mice were exposed whole-body to 1,2-dichloropropane for 4 weeks (8 hours a day and 7 days a week) at concentrations of 0, 50 or 250 ml/m^3 or to concentrations of 0, 50, 250 or 1250 ml/m^3 with an additional dose of the non-isoform specific CYP inhibitor 1-aminobenzotriazole (50 mg/kg body weight) administered by subcutaneous injection in the mornings and evenings. The relative liver weights, ALT activity and the total bilirubin levels were increased with statistical significance in the animals exposed to 250 ml/m^3 without the inhibitor. In addition, exposure to 250 ml/m^3 led to a statistically significant increase in CYP2E1 and GST activity in the microsomes of the liver, but not in those of the lungs. The CYP inhibitor suppressed the CYP2E1 and GST activity induced by 1,2-dichloropropane. A statistically significant increase in CYP2E1 activity was observed again only after exposure to 1,2-dichloropropane at 1250 ml/m^3. Immunohistochemical staining for BrdU and Ki-67 revealed the increased proliferation of biliary epithelial cells (cholangiocytes) at 250 ml/m^3. In the hepatocytes, only Ki-67 staining yielded evidence of proliferation. The staining of apoptotic nuclei in the TUNEL assay demonstrated a tendency towards an increase

in apoptotic cholangiocytes caused by 1,2-dichloropropane. However, this effect was not detected in the hepatocytes. The CYP inhibitor clearly reduced both the increase in proliferation induced by exposure to 1,2-dichloropropane at 250 ml/m³ and the apoptotic degradation of cholangiocytes. In the hepatocytes, proliferation was affected by the inhibitory effects. The histopathological examination of the liver revealed focal necrosis (2/6) at 50 ml/m³ and above with concentration-dependent increases at 250 ml/m³ (3/6) and biliary hyperplasia (1/6) at 50 ml/m³ and above that reached statistical significance at 250 ml/m³ (3/6). No changes were observed in the control group. Treatment with the inhibitor reduced the focal necrosis, but not the biliary hyperplasia. Fatty deposits were observed in the liver in all animals only after treatment with the inhibitor, except for the animals that were exposed to the high concentration of 1250 ml/m³. No histopathological changes were determined in the lungs. After exposure to 1,2-dichloropropane, significant increases in H2AX phosphorylation could not be demonstrated in the cholangiocytes by immunohistochemistry nor in isolated liver proteins by Western blot analysis (Zhang et al. 2018). The CYP inhibitor thus suppressed the effects of 1,2-dichloropropane in the liver and in the bile ducts. In this study, the LOAEC was 50 ml/m³ without an inhibitor. As a result of the mechanistic focus of this publication, the group size of 6 animals was small. The statistical significance of the increase in liver necrosis and biliary hyperplasia was demonstrated by the linear chi-square trend test.

After whole-body exposure of groups of 10 male and 10 female F344 rats for 13 weeks (6 hours a day and 5 days a week) to 1,2-dichloropropane concentrations of 0, 125, 250, 500, 1000 or 2000 ml/m³, hyperplasia of the nasal turbinates and atrophy of the olfactory epithelium were observed in all animals at the low concentration of 125 ml/m³ and above. Anaemia was observed at 500 ml/m³ and above. In the females, the relative liver weights were increased with statistical significance at 500 ml/m³ and above and the relative spleen weights were increased in both sexes at 2000 ml/m³ and above. Other histopathological lesions in the liver, spleen and bone marrow were observed only at the high concentration of 1000 ml/m³ and above. Body weights were reduced with statistical significance at 1000 ml/m³ and above and feed consumption was reduced at 2000 ml/m³ and above. Total bilirubin levels and γ-GTP activity were increased with statistical significance in female rats at 1000 ml/m³ and above and in male rats at 2000 ml/m³ and above. The substance had a purity of > 99.5% (Umeda et al. 2010). The LOAEC in this study was 125 ml/m³.

Groups of 10 male and 10 female B6D2C1 mice were exposed whole-body for 13 weeks to 1,2-dichloropropane concentrations of 0, 50, 100, 200, 300 or 400 ml/m³ (6 hours a day, 5 days a week). The purity of 1,2-dichloropropane was > 99.5%. Mortality occurred in the males at 300 ml/m³ and above (2/10) and increased at 400 ml/m³ (6/10). One of the 10 females in the high concentration group died after exposure. In the males, but not in the females, a statistically significant decrease in body weights was observed at 200 ml/m³ and above; the body weights decreased with increasing concentration. In all animals, the relative liver weights were increased with statistical significance at 300 ml/m³ and above and the relative spleen weights at 400 ml/m³. Significant haemolytic effects, which increased with the concentration, were first observed in the males at exposure levels as low as 50 ml/m³ (reduced number of erythrocytes, decreased haemoglobin and haematocrit levels, increased mean corpuscular volume). The number of platelets was likewise increased with statistical significance at concentrations of 300 ml/m³ and above. In the females, haemolytic effects were first observed at concentrations of 300 ml/m³ and above. In both sexes, the phospholipid concentration in the blood was increased at 300 ml/m³. The activities of the enzymes AST, ALT and LDH in addition to the total bilirubin levels were found to be increased with statistical significance only after exposure to the high concentration and the alkaline phosphatase activity was decreased. Atrophy and necrosis in the olfactory epithelium, hyperplasia of the forestomach, swelling in the liver and increased erythropoiesis in the bone marrow were observed at concentrations of 300 ml/m³ and above. Lesions in the heart and the liver were observed only after exposure to the high concentration of 400 ml/m³. The study was carried out according to OECD Test Guideline 413 (Matsumoto et al. 2013). In this study, the LOAEC was determined to be 50 ml/m³.

In a 2-year carcinogenicity study, groups of 50 male and 50 female F344 rats were exposed to 1,2-dichloropropane concentrations of 0, 80, 200 or 500 ml/m³ for 6 hours a day and 5 days a week. Hyperplasia of the transitional epithelium and of the squamous epithelium, squamous metaplasia and inflammation of the respiratory epithelium in addition to atrophy of the olfactory epithelium were observed in the nasal cavity even at the low concentration. In the high concentration groups, anaemia was detected in the female rats and the body weights were reduced in both sexes (Section 5.7.2; Umeda et al. 2010). The LOAEC was 80 ml/m³.

In a 2-year inhalation study carried out according to OECD Test Guideline 451, groups of 50 male and 50 female B6D2C1 mice were exposed to 1,2-dichloropropane concentrations of 0, 32, 80 and 200 ml/m^3 (6 hours a day, 5 days a week). The data for carcinogenicity are described in Section 5.7.2. Basophilic changes and mineralization of the renal cortex were observed in the male mice after exposure to the low concentration. In the males, the relative weights of kidney and spleen were increased with statistical significance in the 200 ml/m^3 group. Haemoglobin levels were decreased in the males at 80 ml/m^3 and above and in the females at 200 ml/m^3. In the females, the mean corpuscular volume of the erythrocytes was increased at 200 ml/m^3 (Section 5.7.2; Matsumoto et al. 2013). The LOAEC was 32 ml/m^3; however, adenomas in the lungs were already detected at this concentration.

5.2.2 Oral administration

1,2-Dichloropropane given to hamsters by gavage at an initial dose of 500 mg/kg body weight, followed by doses of 250 mg/kg body weight and day on the subsequent 2 days, led to a statistically significant reduction in body weights, but only to a slight, not significant increase in the relative liver weights. Effects on the body weights or liver weights were not observed in mice given doses of 500 mg/kg body weight and day on 3 days. However, fatty deposits, centrilobular necrosis in the liver and reduced GSTT1 expression in the hepatocytes were found in all treated animals (Gi et al. 2015 a).

In the range-finding study for a teratogenicity study, groups of 7 female New Zealand White rabbits were given 1,2-dichloropropane by gavage from gestation days 7 to 19 at dose levels of 0, 25, 100 or 250 mg/kg body weight. Anaemia was observed at 100 mg/kg body weight and above. The absolute and relative organ weights remained unchanged (Dow Chemical Co 1988).

The treatment of groups of 5 male Syrian hamsters with 1,2-dichloropropane doses of 0, 125 or 250 mg/kg body weight and day (in corn oil, purity > 98%) by gavage on 5 days a week for 4 weeks induced mortality at 125 mg/kg body weight in 1 animal and at 250 mg/kg body weight in 3 of 5 animals. The relative liver weights were found to be increased in the 2 surviving animals of the 250 mg/kg group. Fatty deposits in the liver were observed in all treated animals. The haematological examination and the biochemical analysis of the serum did not yield any findings. At 250 mg/kg body weight, CYP2E1 expression was increased in the necrotic centrilobular zone of the hepatocytes. CYP2E1 was not detected in the cholangiocytes. A dose-dependent increase in the protein levels of CYP2A in the liver was determined by Western blot. GSTT1 protein expression was reduced in the centrilobular zone of the hepatocytes at 125 mg/kg body weight and was no longer detectable at 250 mg/kg body weight. However, a dose-dependent increase was observed in the non-necrotic periportal zone at 125 mg/kg body weight and above. GSTT1 was not found in the cholangiocytes. GSH depletion was not observed. The proliferation marker Ki-67 was detected in the hepatocytes and bile duct cells. However, no differences were observed between the treated animals and the control animals. According to the authors, these effects demonstrate that CYP2E1 is the enzyme responsible for the hepatotoxic effects of 1,2-dichloropropane (Gi et al. 2015 a). On the basis of the observed effects, the (slight) liver toxicity does not appear to be responsible for the mortality.

The treatment of groups of 5 male B6C3F1 mice with 1,2-dichloropropane doses of 0, 125 or 250 mg/kg body weight and day (in corn oil, purity > 98%) by gavage on 5 days a week for 4 weeks led to an increase in the relative liver weights at 125 mg/kg body weight and above. Mortality did not occur. Fatty deposits in the liver were observed in all treated animals. The biochemical analysis of the serum revealed only a dose-dependent increase in total bilirubin levels. The histological examination did not reveal effects in the bile ducts, lungs, kidneys or spleen of the animals. In the hepatocytes, the protein expression of CYP2E1 was not increased, but that of CYP2A. GSTT1 expression was decreased in the hepatocytes of the centrilobular zone at doses of 125 mg/kg body weight and above. It is noteworthy that GSTT1 was not expressed in the hamsters, but only in the cholangiocytes of mice; however, this was not affected by treatment with 1,2-dichloropropane. Depletion of GSH was not observed. The proliferation marker Ki-67 was detected in the hepatocytes and the bile duct cells, but no differences were found between the treated animals and the control animals. On the basis of the effects that occurred, the authors consider CYP2E1 to be the enzyme responsible for the

hepatotoxic effects induced by 1,2-dichloropropane (Gi et al. 2015 a). The centrilobular necrosis observed after 3 days was no longer noticeable once the dose was reduced 4 weeks after the beginning of treatment.

1,2-Dichloropropane was given by gavage to 9 Syrian hamsters at a dose level of 125 mg/kg body weight and day on 5 days a week for 17 weeks. The substance had a purity of > 99% and was administered dissolved in corn oil. The control group consisted of 6 animals that received only corn oil. The body weights of the treated animals were slightly reduced, but not with statistical significance. No effects on the relative liver weights were observed. The histopathological examination did not reveal any lesions in the bile ducts, in the liver and in the pancreas or tumours in the lungs or kidneys. The protein expression of CYP2E1 and GSTT1 was not observed in the control group or after treatment with 1,2-dichloropropane or nitrosamines. The bile ducts, biliary hyperplasia, liver, pancreatic duct and pancreas were examined (Section 5.7.1; Gi et al. 2015 b).

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

1,2-Dichloropropane was applied non-occlusively to the shaved ventral skin of rabbits in an amount of 0.01 ml. No irritation was observed after the substance was left on the skin for 24 hours (Greim 1998).

Semi-occlusive application of 0.5 ml undiluted 1,2-dichloropropane to the abraded skin of 2 male rabbits and 1 female (White Vienna) for 4 hours led to scaly skin, reversible redness of the skin (irritation index for erythema 1.44) and reversible oedema (irritation index for oedema 0.33) after 24, 48 and 72 hours and after 8 days. The substance was found to be slightly irritating (ECHA 2019).

After dermal application to the shaved dorsal skin and to both ears of groups of 5 C57BL/6J mice for 7 days at dose levels of 0, 2.73, 5.75 or 8.75 ml/kg body weight, the skin thickness of the ears was found to have increased with statistical significance at the low dose and above. Immunohistochemical staining revealed that IL-6 and TNF-α levels in the dorsal skin increased with the dose. In addition, vascular proliferation was observed in the dorsal skin. The authors interpret this as clear evidence that 1,2-dichloropropane induces inflammation in the skin (Jin et al. 2019).

5.3.2 Eyes

1,2-Dichloropropane induced pain and irritation in the eyes of rabbits, but no permanent damage (Torkelson and Rowe 1981).

1,2-Dichloropropane was only slightly irritating to the eyes with an index of 2 (maximum: 10) (Greim 1998).

5.4 Allergenic effects

The ECHA database includes a local lymph node assay that was carried out with 1,2-dichloropropane according to OECD Test Guideline 429. The assay yielded negative results. The test substance was applied as a 5%, 20% or 80% formulation in acetone/olive oil (4:1). At these concentrations, the stimulation indices were 1.0, 1.3 and 0.8, respectively (ECHA 2019).

A maximization test carried out with a mixture that in addition to 65% 1,2-dichloropropane contained also dichloropropene yielded positive results (Shell Oil Co 1983); the results are not included in the assessment because of the exposure to a mixture of substances.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a two-generation study, male and female Sprague Dawley rats of the F0 and F1 generations were given 1,2-dichloropropane in the drinking water continuously in concentrations of 0.024%, 0.10% or 0.24%. The F0 animals were exposed for 10 weeks prior to mating, during the mating period, which did not last longer than 3 weeks, and while rearing the pups. The animals of the F1 generation were treated in the same way. The males of the F0 generation were mated for 2 weeks with untreated females for a dominant lethal test and were not given 1,2-dichloropropane in the drinking water during weeks 15 and 16 of the study. No substance-related effects on reproductive parameters (fertility and litter parameters) were observed. Histopathological examination of the F0 and F1 animals did not reveal any substance-related changes in the examined organs (liver in all concentration groups and additionally the reproductive organs, kidneys and bone marrow in the high concentration group), except for increased hepatocellular granulation in all concentration groups. The NOAEL for parental toxicity induced by treatment with 1,2-dichloropropane in the drinking water was reported to be about 25 mg/kg body weight and day, the NOAEL for perinatal toxicity was a 1,2-dichloropropane dose of about 100 mg/kg body weight and day and the NOAEL for effects on reproductive parameters was a 1,2-dichloropropane dose of about 190 mg/kg body weight and day (Greim 1998).

Female F344 rats were exposed to 1,2-dichloropropane concentrations of 0, 50, 100 or 200 ml/m^3 for 8 hours a day for 20 days. The oestrous cycle was prolonged at the low concentration and above and spontaneous ovulation was inhibited (Sekiguchi et al. 2002).

5.5.2 Developmental toxicity

In a teratogenicity study, groups of 30 female Sprague Dawley rats were given gavage doses of 1,2-dichloropropane of 0, 10, 30 or 125 mg/kg body weight and day from days 6 to 15 of gestation. In the high dose group, maternal toxicity was observed in the form of sedation, reduced body weight gains and reduced feed consumption, and resulted in delayed skeletal maturation in the foetuses (delayed ossification of the skull). Teratogenic effects did not occur (Greim 1998; Kirk et al. 1995). In this study, the NOAEL for maternal and developmental toxicity was 30 mg/kg body weight and day.

In another teratogenicity study, pregnant New Zealand White rabbits (18 dams per dose group) were given 1,2-dichloropropane doses of 0, 15, 50 or 150 mg/kg body weight and day by gavage from days 7 to 19 of gestation. In the dams of the 150 mg/kg dose group, anorexia, delayed body weight gains and anaemia were observed and resulted in slight foetotoxicity in the form of delayed skeletal ossification. Teratogenic effects were not observed (Greim 1998; Kirk et al. 1995). In this study, the NOAEL for maternal and developmental toxicity was 50 mg/kg body weight and day.

In the two-generation study described in Section 5.5.1, male and female Sprague Dawley rats of the F0 and F1 generations were given 1,2-dichloropropane in the drinking water continuously in concentrations of 0.024%, 0.10% or 0.24%. No substance-related effects on reproductive parameters (fertility and litter parameters) were observed. Histopathological examination of the F0 and F1 animals did not reveal any substance-related changes in the examined organs (liver and additionally reproductive organs in all concentration groups, kidneys and bone marrow in the high concentration group), except for increased hepatocellular granulation in all concentration groups. The NOAEL for parental toxicity was about 25 mg/kg body weight and day for 1,2-dichloropropane given in the drinking water and the NOAEL for perinatal toxicity induced by 1,2-dichloropropane was about 100 mg/kg body weight and day (Greim 1998).

5.6 Genotoxicity

5.6.1 In vitro

1,2-Dichloropropane did not induce an SOS response in Salmonella typhimurium TA1535/pSK1002 (umu test) (Yasunaga et al. 2004). Several studies found that 1,2-dichloropropane induced mutagenic effects in vitro in the Salmonella strains TA100 and TA1535, both with and without metabolic activation. Therefore, the findings suggest that 1,2-dichloropropane

is a substance that causes base-pair substitutions. Tests investigating the induction of an SOS response in Salmonella typhimurium TA1535 and Escherichia coli yielded negative results both with and without metabolic activation (ECHA 2019; Greim 1998).

In a bacterial mutagenicity test in a Salmonella TA100 strain, which overexpresses GSTT1 (TA100-GST), the number of revertants found after exposure to 1,2-dichloropropane concentrations of 600 to 3000 ml/m^3 was not higher than in the Salmonella strain TA100-pCTC, which carries an empty vector. On the basis of these findings, the authors concluded that GSTT1 does not contribute to the mutagenic effects of 1,2-dichloropropane. Under the same test conditions, dichloromethane caused a marked increase in the number of revertants in the Salmonella strain TA100-GSTT1 (Akiba et al. 2017).

In another bacterial mutagenicity test carried out in the Salmonella strain TA100, the addition of 1,2-dichloropropane concentrations of 500 to 3000 ml/m^3 (purity 98%) without metabolic activation led to a dose-dependent increase in the mutation frequency. The plates were exposed to gaseous 1,2-dichloropropane in a Tedlar gas sampling bag (Tedlar Bag Vaporization Technique). Whole genome sequencing revealed a mutation frequency of 0.2/Mb after incubation with 3000 ml/m^3 and a frequency of 0.6/Mb after incubation with 6000 ml/m^3. Therefore, the mutation frequency was 1.4 to 5.1 times as high as that determined in the control group. The predominant single nucleotide substitution was a C : G to T : A transition. The examination of the trinucleotide mutational pattern revealed a NpCpC to NpTpC change. This was the second most common trinucleotide pattern in the tumour tissue of printing workers (Section 4.6; Mimaki et al. 2016).

The results of the bacterial mutagenicity tests are shown in Table 5. These demonstrate that 1,2-dichloropropane induces mutations only at concentrations in the mg range.

Tab. 5 Bacterial mutagenicity tests with 1,2-dichloropropane

Test system	Concentration	Effective concentration	Cytotoxicity [µg/plate]	Results		References
				–m. a.	+m. a.	
Salmonella typhimurium TA100, TA1535	10 000–50 000 µg/plate	10 000 µg/plate	–	+	+	De Lorenzo et al. 1977
Salmonella typhimurium TA1978	10 000–50 000 µg/plate		–	–	–	De Lorenzo et al. 1977
Salmonella typhimurium TA100, TA1535	1160–11 600 µg/plate	no data	–	+	+	Principe et al. 1981
Salmonella typhimurium TA100	1000–10 000 µg/plate	10 000 µg/plate	–	+	–	Haworth et al. 1983; NTP 1982 a
Salmonella typhimurium TA100	33–2000 µg/plate	1000 µg/plate	2000	+	–	NTP 1982 b
Salmonella typhimurium TA1535	33–2000 µg/plate	1000 µg/plate	–	(+)	–	NTP 1982 b
Salmonella typhimurium TA1535	100–16 667 µg/plate		10 000 –m. a. 3333 +m. a.	–	–	Haworth et al. 1983; NTP 1982 a
Salmonella typhimurium TA100	113–11 300 µg/plate		no data	–	–	Stolzenberg and Hine 1980
Salmonella typhimurium TA100	no data	no data	no data	(+)	–	Matsumoto et al. 2013
Salmonella typhimurium TA100	500–3000 ml/m^3 (in a closed system)	500 ml/m^3	–	+	n. t.	Mimaki et al. 2016
Salmonella typhimurium TA100-GST, TA100-pCTC (with an empty vector)	600–3000 ml/m^3 (in a closed system)	600 ml/m^3	–	+	n. t.	Akiba et al. 2017

Tab. 5 *(continued)*

Test system	Concentration	Effective concentration	Cytotoxicity [µg/plate]	Results		References
				–m. a.	+m. a.	
Salmonella typhimurium TA98, TA100, TA1535, TA1537	1581, 5000 µg/plate		–	–	–	ECHA 2019
Salmonella typhimurium TA98, TA1537, TA1538	1160–11 600 µg/plate		–	–	–	Principe et al. 1981
Salmonella typhimurium TA98, TA1537	33–2000 µg/plate		–	–	–	NTP 1982 b
Salmonella typhimurium TA98, TA1537	33–10 000 µg/plate		10 000 –m. a. 3333 +m. a.	–	–	NTP 1982 a

(+): ambiguous; m. a.: metabolic activation; n. t.: not tested

A mixture containing in addition to 65% 1,2-dichloropropane also dichloropropene induced mutations in the Salmonella typhimurium strains TA100 and TA1535 and in yeasts, but not in the Salmonella typhimurium strains TA98 and TA1537 (Shell Oil Co 1983). The study is not included in the evaluation because of the co-exposure. 1,3-Dichloropropene, which made up 25% of the mixture, was found to be mutagenic in bacterial mutagenicity tests (Henschler 1986, available in German only).

Tests investigating 1,2-dichloropropane with Aspergillus nidulans yielded evidence of mutagenicity, but not of aneuploidy (Greim 1998).

In the comet assay, the incidence of DNA damage was increased with statistical significance in the cholangiocyte cell line MMNK-1 after incubation with 50 µM 1,2-dichloropropane. This effect was stronger after co-cultivation with differentiated THP-1 macrophages (Section 2; Zong et al. 2019).

A method was applied to identify double-strand breaks by means of the binding of γ-H2AX antibodies to phosphorylated histone after incubation of the human hepatocyte cell line WRL-68 and the cholangiocyte cell line MMNK-1 with 1,2-dichloropropane. This revealed dose-dependent effects at concentrations of 1 to 10 mM and after incubation for 4 hours and time-dependent effects after exposure for 1 to 24 hours (5 mM). These effects were weakened markedly by the specific CYP2E1 inhibitors disulfiram and 4-methylpyrazole as well as the non-specific CYP inhibitor 1-aminobenzotriazole; this suggests that CYP2E1 is involved in the formation of reactive metabolites. Ethacrynic acid, an inhibitor of all glutathione transferases, had little effect on the binding of γ-H2AX antibodies induced by exposure to 1,2-dichloropropane. KU55933, the inhibitor of Ataxia telangiectasia mutated (ATM), one of the enzymes that phosphorylate H2AX following the formation of double-strand breaks, reduced γ-H2AX antibody binding following incubation with 1,2-dichloropropane. The addition of 1,2-dichloropropane increased the amount of reactive oxygen species in the human hepatocyte cell line. This effect was likewise markedly weakened by CYP2E1 inhibitors. In WRL-68 cells, the addition of 5 mM of 1,2-dichloropropane did not lead to changes in the cell cycle (Section 2; Toyooka et al. 2017).

1,2-Dichloropropane increased the incidence of sister chromatid exchange in CHO cells (a cell line derived from Chinese hamster ovary) and V79 cells with and without the addition of metabolic activation. In the UDS test, 1,2-dichloropropane did not induce DNA repair synthesis in human lymphocytes. Chromosomal aberrations were observed in CHO cells at concentrations of 660 µg/ml and above with metabolic activation. However, without activation, only few aberrations were observed at the highest concentration tested of 1580 µg/ml (Galloway et al. 1987; Greim 1998).

In rat liver cells (RL4), chromosomal aberrations were not induced after incubation with a 1,2-dichloropropane mixture at concentrations of 0, 5, 10 or 20 µg/ml (Shell Oil Co 1983). The mixture contained 65% 1,2-dichloropropane and 35% dichloropropene. The study is not included in the evaluation because of the exposure to a mixture of substances.

An HPRT gene mutation test carried out with 1,2-dichloropropane in CHO cells yielded positive results only after metabolic activation. In a $TK^{+/-}$ test carried out in mouse lymphoma cells, mutations were induced with metabolic activation (Greim 1998; Myhr and Caspary 1991; Tennant et al. 1987).

The incidence of mutations in the cholangiocarcinoma cell line NCC-CC1 and the kidney cell line HEK293 (human embryonic kidney) was not increased with statistical significance after single or repeated exposures to 1,2-dichloropropane (Mimaki et al. 2016). The cell lines used for testing were presumably metabolically incompetent. Metabolic activation was not applied. It is therefore not possible to draw conclusions from this study whether mutations are induced by a metabolite of 1,2-dichloropropane.

5.6.2 In vivo

Drosophila larvae were exposed to a 1,2-dichloropropane concentration of 14.4 mg/m³. In a separate experiment, this concentration had been found to be the LC_{50}. In the wing mosaic test, a slight but statistically significant increase in the incidence of wing spots was observed after 48 hours (Chroust et al. 2007).

1,2-Dichloropropane did not induce sex-linked recessive lethal mutations (SLRL test) in Drosophila melanogaster (Greim 1998).

Treatment of B6C3F1 mice and Syrian hamsters with gavage doses of 0, 125 or 250 mg/kg body weight and day for 4 weeks did not lead to oxidative DNA damage, because HPLC analysis did not reveal a statistically significant increase in 8-oxodeoxyguanosine levels (no other details; Gi et al. 2015 a).

After exposure of a female F344 rat once for 7 hours to [1-^{14}C]-1,2-dichloropropane in a closed inhalation chamber, 11% of the radioactivity were found as nucleotide adducts in the isolated liver DNA; this corresponds to a covalent binding index (CBI) of 0.8 µmole adduct per mole nucleotide per mmole test substance per kg body weight. Six hours after a single oral [1-^{14}C]-1,2-dichloropropane dose of 0.94, 7 or 255 mg/kg body weight in corn oil was administered by gavage to 1 female F344 rat each, a covalent binding index of 2.2, 1.7 or 0.3, respectively, was determined for the isolated liver DNA. The covalent binding index of 1,2-dichloropropane is lower than that of the carcinogenic aflatoxin B_1 by a factor of 10 000 (Greim 1998).

In two studies, 1,2-dichloropropane was given to groups of 4 male B6C3F1 mice by intraperitoneal injection at doses levels of 0, 112.5, 225 or 450 mg/kg body weight. The incidence of sister chromatid exchange was not increased in the bone marrow. The specimens were examined for DNA damage after 23 and 42 hours (NTP 1987, 1990).

In a comet assay, B6C3F1 mice were exposed to 1,2-dichloropropane concentrations of 0, 150, 300 or 600 ml/m³ for 6 weeks (6 hours/day, 5 days/week); liver tissue was collected 7 days after the last exposure. The comet assay revealed an increase in DNA damage to the liver cells of the treated animals at concentrations of 300 ml/m³ and above; the increase, which was given as % tail intensity, reached statistical significance and was dependent on the dose. DNA damage was not found to be increased with statistical significance after exposure to dichloromethane at 0, 400, 800 or 1600 ml/m³. A mixture containing 1,2-dichloropropane in a concentration of 400 ml/m³ and dichloromethane in a concentration of 150 ml/m³ caused a statistically significant increase in the incidence of DNA damage compared with the effects induced after exposure to only 1,2-dichloropropane in a concentration of 600 ml/m³. However, the effects were not more severe when the mixture was prepared with higher concentrations of the substances (1,2-dichloropropane: 800 ml/m³, dichloromethane: 300 ml/m³) (Suzuki et al. 2014).

In a comet assay, DNA strand breaks were not found to have been induced in the liver 16 hours after a single injection of 1,2-dichloropropane doses of up to 300 mg/kg body weight. The test animals were *Cyp2e1*$^{+/+}$ and *Cyp2e1*$^{-/-}$ mice. The authors assume that any DNA damage that may have been induced had already been repaired (Yanagiba et al. 2016 a).

Groups of 2 or 3 male C57BL/6 mice were exposed to 1,2-dichloropropane concentrations of 0, 100, 200 or 400 ml/m³ for 6 hours on the first day and 3 hours on the second day. Increased phosphorylation of histone H2AX (γ-H2AX) was detected in the liver. This phosphorylation identified by antibodies is a marker of double-strand breaks (Toyooka et al. 2017).

Continuous, whole-body exposure of albino rats to a 1,2-dichloropropane concentration of 2200 mg/m³ for 3 days disrupted hepatocyte mitosis. The number of mononuclear and binuclear hepatocytes with polyploid nuclei (8-fold or 16-fold chromosome number per cell) was increased in comparison with the levels determined in the controls (no other details). This effect was not observed after exposure of the animals to a 1,2-dichloropropane concentration of 2200 mg/m³ for 20 hours (Belyaeva et al. 1977; Greim 1998).

In a mutation test carried out according to OECD Test Guideline 488 at the *gpt*-delta locus, 5 male gpt-delta-C57BL/6J mice per concentration group were exposed to 1,2-dichloropropane in concentrations of 0 or 300 ml/m³, to dichloromethane in a concentration of 800 ml/m³ or to a mixture containing 1,2-dichloropropane in a concentration of 300 ml/m³ and dichloromethane in a concentration of 800 ml/m³ for 6 hours a day on 5 days a week over a period of 4 weeks. Liver tissue was collected from the animals 7 days after the last exposure. When the effects induced by the individual substances were considered, the frequency of gpt mutations in the liver was not increased by exposure to dichloromethane and only slightly by exposure to 1,2-dichloropropane (by 32%, not statistically significant). However, the frequency of mutations was 2.6-fold as high as the control value after exposure to the mixture. The classification of the gpt mutations revealed a statistically significant increase in the incidence of mutations of the A : T base pair (Suzuki et al. 2014).

After 1,2-dichloropropane was given daily by gavage to groups of 7 male F344-gpt-delta rats for 4 weeks in doses of 0, 100 or 200 mg/kg body weight and day, the incidence of mutations in the liver was not increased in the Gpt and Spi⁻ test. Additionally, there were no changes in the CYP2E1 and GSTT1 gene or protein expression in the liver. Under the same conditions, groups of 7 rats were given dichloromethane doses of 0, 250 or 500 mg/kg body weight and day and other groups of 7 rats were exposed to a mixture containing a 1,2-dichloropropane dose of 100 mg/kg body weight and day and a dichloromethane dose of 250 mg/kg body weight and day or a mixture containing 1,2-dichloropropane at 200 mg/kg body weight and day and dichloromethane at 500 mg/kg body weight and day. Again, no effects were observed in this study (Hirata et al. 2017).

In the Pig-a mutation assay, the incidence of mutations in the erythrocytes was not increased after 8 to 10 male B6C3F1 mice per concentration group were exposed by inhalation to 1,2-dichloropropane concentrations of 0, 150, 300 or 600 ml/m³ for 3 or 6 weeks (6 hours a day, 5 days a week). Exposure to a mixture of 1,2-dichloropropane and dichloromethane likewise did not lead to mutations. On the basis of these findings, the authors concluded that 1,2-dichloropropane does not induce Pig-a mutations in the haematopoietic system (Suzuki et al. 2014).

Negative results were obtained in two tests investigating chromosomal aberrations in the bone marrow cells of male B6C3F1 mice given a single intraperitoneal injection. Groups of 8 animals received 1,2-dichloropropane doses of 0, 112.5, 225 or 450 mg/kg body weight and were then examined for DNA damage after 17 or 36 hours. In another test with the administration of doses of 0, 224, 335 or 450 mg/kg body weight, no effects were observed after 36 hours (NTP 1988, 1992, 1993).

After 1,2-dichloropropane was given to CD-1 mice by gavage at a dose level of 600 mg/kg body weight and day on 2 consecutive days, a micronucleus test carried out according to OECD Test Guideline 474 did not detect damage in the bone marrow cells (no other details; OECD 2003).

After inhalation exposure of male B6C3F1 mice to 1,2-dichloropropane concentrations of 0, 150, 300 or 600 ml/m³ for 6 weeks (6 hours a day, 5 days a week), the incidence of micronuclei in the peripheral blood was not significantly increased 18 hours after the last exposure. Exposure to mixtures containing 1,2-dichloropropane in a concentration of 150 ml/m³ and dichloromethane in a concentration of 400 ml/m³ or 1,2-dichloropropane at 300 ml/m³ and dichloromethane at 800 ml/m³ did not lead to a significant increase in the incidence of micronuclei. Each concentration group consisted of 8 to 10 animals. The ratio of polychromatic to normochromatic erythrocytes was unchanged. At a 1,2-dichloropropane concentration of 600 ml/m³, the number of micronuclei-containing cells was slightly increased, but the increase was not statistically significant (Suzuki et al. 2014).

In the dominant lethal test, no effects were observed in male Sprague Dawley rats given 1,2-dichloropropane doses of about 0, 28, 91 or 162 mg/kg body weight and day with the drinking water for 14 weeks (Greim 1998).

5.6.3 Summary

In vitro, 1,2-dichloropropane was found in a number of studies to induce base substitutions in Salmonella typhimurium both with and without metabolic activation. GSTT1 was not involved in the induction of mutations in Salmonella typhimurium. In the human hepatocyte cell line WRL-68 and the cholangiocyte cell line MMNK-1, CYP2E1 inhibition suppressed the increase in double-strand breaks induced by treatment with 1,2-dichloropropane. Additionally, sister chromatid exchange was increased in CHO and V79 cells and chromosomal aberrations were observed in CHO cells. Mutations were induced at the *tk* locus in mammalian cells.

In vivo, 1,2-dichloropropane bound weakly to the DNA of rats. In Drosophila, the number of wing spots was increased in the wing mosaic test, but no sex-linked recessive lethal mutations were found. In mice, sister chromatid exchange was not detected in the bone marrow. After exposure by inhalation, DNA damage was detected in the livers of mice by comet assay and double-strand breaks were identified by the phosphorylation of histone. After exposure by inhalation for 4 weeks, a statistically significant increase in gpt-delta mutations was detected in mice only after co-exposure to a 1,2-dichloropropane concentration of 300 ml/m^3 and a dichloromethane concentration of 800 ml/m^3. In rats, however, the incidence of gpt-delta mutations was not increased after gavage treatment for 4 weeks with 1,2-dichloropropane at a dose level of 200 mg/kg body weight and day or with a mixture consisting of a 1,2-dichloropropane dose of 200 mg/kg body weight and a dichloromethane dose of 500 mg/kg body weight. Likewise, no mutations occurred at the *Pig-a* locus in mice after exposure to up to 600 ml/m^3 for 3 or 6 weeks. Other tests did not detect chromosomal aberrations after intraperitoneal injection or micronuclei after administration by gavage or inhalation. Dominant lethal mutations were not induced in rats after treatment via the drinking water.

Although positive findings were obtained in vitro in bacterial mutagenicity tests with 1,2-dichloropropane, in vivo, the substance did not induce mutations in the liver tissue of rats and mice in the gpt test and in the erythrocytes of mice in the Pig-a assay.

5.7 Carcinogenicity

5.7.1 Short-term studies

Groups of 24 hamsters were given *N*-nitrosobis(2-oxopropyl)amine 4 times at 2-day intervals by subcutaneous injection of 0 or 10 mg/kg body weight, followed by gavage of 1,2-dichloropropane for 15 or 17 weeks at doses of 0, 62.5 or 125 mg/kg body weight and day (9 or 17 animals per group). The incidence of atypical bile duct hyperplasia was not increased. The proliferative activity in the biliary epithelial cells likewise remained unchanged. When 1,2-dichloropropane was given by gavage without pre-treatment with the nitrosamine, cell proliferation in the bile duct was not increased. Promoting effects were not observed in the lungs, the pancreas and the kidneys. The authors suggest that the combined effects of the various substances may have caused the development of cholangiocarcinomas in humans (Gi et al. 2015 b).

5.7.2 Long-term studies

In a 2-year study with exposure of mice to 1,2-dichloropropane concentrations of 0, 32, 80 or 200 ml/m^3, the incidence of bronchoalveolar adenomas and carcinomas was increased at the lowest concentration tested of 32 ml/m^3 and above. In the liver, the incidence of histiocytic sarcomas was increased with statistical significance in male mice at 80 ml/m^3, but not at 200 ml/m^3. At the high concentration, the number of haemangiosarcomas was likewise increased with statistical significance in male mice. Adenomas were detected in the Harderian gland; their number increased with the concentration. The study was carried out according to OECD Test Guideline 451. The exact data are shown in Table 6 (Section 5.2.1; Matsumoto et al. 2013).

After exposure for 2 years (6 hours a day, 5 days a week) to 1,2-dichloropropane concentrations of 0, 80, 200 or 500 ml/m^3, the incidence of papillomas in the nasal cavity of male and female F344 rats was increased with statistical significance at the highest concentration tested of 500 ml/m^3. Hyperplasia, metaplasia and inflammation were observed only in the upper respiratory tract; no damage was found in any of the other organs. The 3 esthesioneuroepitheliomas

(neuroblastomas in the olfactory epithelium) that developed were a new finding and had not been observed in the historical controls. They were therefore considered substance-related. The exact data are shown in Table 6 (see also Section 5.2.1; Umeda et al. 2010).

In a 2-year gavage study with F344 rats and B6C3F1 mice, the incidences of hepatocellular adenomas and carcinomas were found to be increased in mice. The exact data are shown in Table 6 (Greim 1998; NTP 1986).

Tab. 6 Studies of carcinogenicity induced by 1,2-dichloropropane

Author:	Matsumoto et al. 2013
Substance:	1,2-dichloropropane (purity: 99.5%)
Species:	**mouse, B6D2F1/Crlj**, 50 ♂, 50 ♀
Administration route:	inhalation
Concentration:	0, 32, 80, 200 ml/m³
Duration:	2 years, 5 days/week, 6 hours/day
Toxicity:	32 ml/m³ and above: mineralization and basophilic changes in the renal cortex, decreased haemoglobin levels 80 ml/m³ and above: atrophy of the olfactory epithelium (Section 5.2.1)

		Exposure concentration (ml/m³)			
		0	**32**	**80**	**200**
surviving animals	♂	32/50 (64%)	33/50 (66%)	33/50 (66%)	41/50 (82%)
	♀	29/50 (58%)	28/50 (56%)	26/50 (52%)	30/50 (60%)
tumours					
lungs:					
bronchoalveolar adenomas	♂	5/50 (10%)	14/50 (28%)*	9/50 (18%)	12/50 (24%)
	♀	1/50 (2%)	4/50 (8%)	4/50 (8%)	4/50 (8%)
bronchoalveolar carcinomas	♂	4/50 (8%)	6/50 (12%)	6/50 (12%)	8/50 (16%)*
	♀	1/50 (2%)	1/50 (2%)	1/50 (0%)	4/50 (8%)
bronchoalveolar adenomas and carcinomas	♂	9/50 (18%)	18/50 (36%)*	14/50 (28%)	18/50 (36%)*
	♀	2/50 (4%)	4/50 (8%)	5/50 (10%)	8/50 (16%)*
liver:					
hepatocellular adenomas	♂	13/50 (26%)	10/50 (20%)	16/50 (32%)	12/50 (24%)
	♀	6/50 (12%)	7/50 (14%)	9/50 (18%)	8/50 (16%)
hepatocellular carcinomas	♂	5/50 (10%)	5/50 (10%)	3/50 (6%)	6/50 (12%)
	♀	1/50 (2%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
hepatocellular adenomas and carcinomas	♂	16/50 (32%)	14/50 (28%)	17/50 (34%)	18/50 (36%)
	♀	7/50 (14%)	8/50 (16%)	9/50 (18%)	8/50 (16%)
histiocytic sarcomas	♂	1/50 (2%)	4/50 (8%)	7/50 (14%)*	0/50 (0%)
	♀	0/50 (0%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
spleen:					
haemangiosarcomas	♂	0/50 (0%)	3/50 (6%)	3/50 (6%)	5/50 (10%)*
	♀	2/50 (4%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
mammary glands:					
adenocarcinomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
	♀	0/50 (0%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Harderian glands:					
adenomas	♂	1/50 (2%)	2/50 (4%)	3/50 (6%)	6/50 (12%)

Tab. 6 *(continued)*

Author:		Umeda et al. 2010			
Substance:		1,2-dichloropropane (purity: 99.5%)			
Species:		**rat, F344/DuCrj**, groups of 50 ♂, 50 ♀			
Administration route:		inhalation			
Concentration:		0, 80, 200, 500 ml/m³			
Duration:		2 years, 5 days/week, 6 hours/day			
Toxicity:		80 ml/m³ and above: squamous cell metaplasia and inflammation of the respiratory epithelium (Section 5.2.1)			
		Exposure concentration (ml/m³)			
		0	**80**	**200**	**500**
surviving animals	♂	40/50 (80%)	39/50 (78%)	41/50 (82%)	36/50 (72%)
	♀	37/50 (74%)	41/50 (82%)	38/50 (76%)	32/50 (64%)
tumours and preneoplasms					
nasal cavity:					
papillomas	♂	0/50 (0%)	0/50 (0%)	3/50 (6%)	15/50 (30%)**
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	9/50 (18%)**
esthesioneuroepitheliomas	♂	0/50 (0%)	2/50 (4%)	1/50 (2%)	0/50 (0%)*
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
total nasal tumours	♂	0/50 (0%)	2/50 (4%)	4/50 (8%)	15/50 (30%)**
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	9/50 (18%)**
nasal cavity, precursors:					
hyperplasia, transitional epithelium	♂	0/50 (28%)	31/50 (62%)	39/50 (78%)	48/50 (96%)*
	♀	2/50 (4%)	21/50 (42%)	39/50 (78%)	48/50 (96%)*
squamous cell hyperplasia	♂	0/50 (0%)	2/50 (4%)	6/50 (12%)*	27/50 (54%)**
	♀	0/50 (0%)	0/50 (0%)	3/50 (6%)	20/50 (40%)**
squamous cell metaplasia in the respiratory epithelium	♂	5/50 (10%)	31/50 (62%)	41/50 (82%)	49/50 (98%)**
	♀	3/50 (6%)	15/50 (30%)	37/50 (74%)	46/50 (92%)*
Author:		NTP 1986			
Substance:		1,2-dichloropropane (purity: 99.5%)			
Species:		**mouse, B6C3F1**, groups of 50 ♂, 50 ♀			
Administration route:		gavage			
Dose:		0, 125, 250 mg/kg body weight and day			
Duration:		2 years, 5 days/week			
Toxicity:		♂: 125 mg/kg body weight and above: liver necrosis and hepatocytomegaly			
		Dose (mg/kg body weight)			
		0	**125**	**250**	
surviving animals	♂	35/50 (70%)	33/50 (66%)	35/50 (70%)	
	♀	35/50 (70%)	29/50 (58%)	26/50 (52%)*	
tumours					
liver:					
hepatocellular adenomas	♂	7/50 (14%)	10/50 (20%)	17/50 (34%)*	
	♀	1/50 (2%)	5/50 (10%)	5/50 (10%)	
hepatocellular carcinomas	♂	11/50 (22%)	17/50 (34%)	16/50 (32%)	
	♀	1/50 (2%)	3/50 (6%)	4/50 (8%)	

Tab. 6 *(continued)*

		Dose (mg/kg body weight)		
		0	**125**	**250**
hepatocellular adenomas or carcinomas	♂	18/50 (36%)	26/50 (52%)	33/50 (66%)**
	♀	2/50 (4%)	8/50 (16%)*	9/50 (18%)*
thyroid gland:				
follicular carcinomas	♀	0/50 (0%)	0/50 (0%)	2/50 (4%)
follicular adenomas	♀	0/50 (0%)	0/50 (0%)	3/50 (6%)
forestomach:				
papillomas with keratinization	♂	0/50 (0%)	1/48 (2%)	3/48 (6%)
	♀	0/50 (0%)	2/50 (4%)	2/50 (4%)

Author:	NTP 1986
Substance:	1,2-dichloropropane (purity: 99.5%)
Species:	**rat, F344**, groups of 50 ♂, 50 ♀
Administration route:	gavage
Dose:	♂: 0, 62, 125 mg/kg body weight and day; ♀: 0, 125, 250 mg/kg body weight and day
Duration:	2 years, 5 days/week
Toxicity:	♂: 62 mg/kg body weight and above and ♀ 125 mg/kg body weight and above: body weight gains ↓ ♀: 250 mg/kg body weight and above: liver necrosis and foam cell foci

		Dose (mg/kg body weight)			
	♂	0	62	125	
	♀	0		125	250
surviving animals	♂	39/50 (78%)	42/50 (84%)	41/50 (82%)	
	♀	37/50 (74%)		43/50 (86%)	16/50 (32%)
tumours					
mammary glands:					
adenocarcinomas	♀	1/50 (2%)		2/50 (4%)	5/50 (10%)*

*p < 0.05; **p < 0.01

Summary: 1,2-Dichloropropane induced tumours primarily in the nasal cavity of rats and in the lungs of mice after exposure by inhalation and tumours in the livers of mice after gavage administration. The substance was thus found to cause carcinogenic effects. The occupational cholangiocarcinomas observed in humans did not develop in rodents after inhalation or oral exposure.

5.8 Other effects

In the MTT assay to determine cell viability, the incubation of human embryonic lung fibroblasts (MRC-5 cells) with 10 to 100 mM 1,2-dichloropropane for 24 hours induced cytotoxicity with an IC_{50} value of 48.66 mM. Synergistic effects were observed in combination with 1 to 5 mM photoinitiators. Photoinitiators are found in the UV curable printing inks that are used, among other things, in printing processes (Kawasaki et al. 2015).

Incubation of human dermal fibroblasts with 1,2-dichloropropane concentrations of 2.73 or 5.75 µl/ml for 24 hours increased the concentrations of the inflammatory markers TNF-α and IL-6 as well as of VEGF, p-AKT and p-mTOR as factors of angiogenesis (Jin et al. 2019).

In vitro studies with specimens of renal cortex from male Wistar rats demonstrated that incubation with 50 to 250 mM (50–250 nmol) 1,2-dichloropropane reduced GSH levels, released AST and LDH into the medium and increased lipid

peroxidation (determination of malondialdehyde levels). The effects were most pronounced in young animals aged 3 to 4 months. Specimens taken from female animals were found to be less sensitive to the effects of 1,2-dichloropropane. The induction of renal toxicity by 1,2-dichloropropane appears to be caused by a cysteine conjugate that forms during the metabolism of mercapturic acid (Trevisan et al. 1992, 1993).

In the renal sections of male and female Wistar rats, incubation with 62.5 µM 1,2-dichloropropane increased the release of AST and the accumulation of organic anions in the medium, resulting in toxic effects. The renal sections of male rats showed greater sensitivity. No nephrotoxicity was observed in the renal sections of castrated male rats, while female rats pretreated with testosterone exhibited a stronger response to 1,2-dichloropropane. The authors attribute the varying levels of renal toxicity to the higher expression of CYP enzymes in the kidneys of male rats, which is related to higher testosterone levels (Odinecs et al. 1995).

6 Manifesto (MAK value/classification)

The critical effect of 1,2-dichloropropane is the development of biliary tract tumours in humans. Additionally, irritation was observed after inhalation exposure and liver and kidney functions were impaired in humans and rodents after oral and dermal exposure. Haemolytic effects were found in mice after exposure by inhalation.

In humans, exposure to very high concentrations may lead to CNS depression.

Carcinogenicity. In workers employed in the printing industry in Japan who were co-exposed to 1,2-dichloropropane, a cluster of very rare spontaneous cholangiocarcinomas developed with an SIR of > 1000. The age of onset determined for the printing workers of 25 to 55 years was far lower than the mean age of onset of about 65 years in the general population in Japan. The cholangiocarcinoma incidence in the general population was 5.2/100 000. A total of 44 male workers have been diagnosed to date. Nine of the printing workers were exposed only to 1,2-dichloropropane, 27 to a mixture of 1,2-dichloropropane and dichloromethane and 3 only to dichloromethane. With respect to the 5 remaining printing workers, it is only known that 3 of the workers were exposed solely to 1,2-dichloropropane or to higher levels of 1,2-dichloropropane than to dichloromethane and that 2 of the workers were exposed only to dichloromethane or to higher levels of dichloromethane than to 1,2-dichloropropane. On the basis of the data available at this time, it cannot be determined whether the 5 printing workers were also exposed to other mixtures. At all of the printing companies, the printing workers were exposed to other substances, but these were not used for similarly long periods or were used less often for cleaning purposes. For example, kerosine was used for cleaning the ink roller, which was not carried out very often. Therefore, the level of exposure to kerosine was lower than that to halogenated hydrocarbons, which were used for the more frequently required cleaning of the blanket cylinder. No other substance was handled at all plants of the printing companies at which workers developed cholangiocarcinomas, as was the case for 1,2-dichloropropane and dichloromethane. As far more printing workers with cholangiocarcinomas were exposed only to 1,2-dichloropropane than only to dichloromethane, this suggests that 1,2-dichloropropane plays a key role in the induction of the carcinogenic effects. A typical tumour spectrum has yet to be established for dichloromethane from the findings of epidemiological studies.

Smoking and consuming more than 80 g of alcohol per day are not regarded as possible confounders because the risk of developing cholangiocarcinomas was found to be only slightly elevated by these factors. This slight increase would not have caused the high incidences determined in the printing workers.

The data reviewed suggest that the substance is metabolized by CYP2E1 primarily in the liver; the critical metabolite or metabolites are formed along this pathway. Conjugation with GSH by glutathione transferase does not seem to intensify the toxic or carcinogenic effects of 1,2-dichloropropane.

Mutagenic effects were induced in vitro after exposure to 1,2-dichloropropane; these effects were not confirmed in vivo. However, samples of tumour tissue taken from 4 printing workers with occupational cholangiocarcinomas revealed a markedly higher frequency of mutations and double-strand breaks identified on the basis of γ-H2AX antibodies than samples of tumour tissue taken from 4 persons with non-occupational cholangiocarcinomas. The mutation in

the trinucleotide sequence of GpCpY to GpTpY found in the tumour tissue of the printing workers is unique and was not detected in the tissue samples from biliary tract tumours that developed in the 4 persons without exposure. The NpCpG to NpTpG changes determined in persons without exposure are found in most types of tumours and often correlate with the age of the patients. This demonstrates that 1,2-dichloropropane or its metabolites induce genotoxic effects. In addition, the dysregulation of components of the immune response has been shown to contribute to the development of tumours.

In a carcinogenicity study with exposure to 1,2-dichloropropane by inhalation, tumours were induced in rats primarily in the nasal cavity and in the lungs of mice. Tumours were observed in the liver of mice after treatment with gavage doses. Thus, 1,2-dichloropropane was found to induce carcinogenic effects in animal studies.

In a cluster of workers in the printing industry in Japan, cholangiocarcinomas with an SIR >1000 developed at the very early mean age of 36 to 44 years. The majority of these workers were exposed to both 1,2-dichloropropane and dichloromethane, but at least 9 of the workers with cholangiocarcinomas were exposed only to 1,2-dichloropropane. The Commission considers this evidence of a causal relationship and has classified 1,2-dichloropropane in Carcinogen Category 1.

MAK value and peak limitation. 1,2-Dichloropropane has been found to be carcinogenic in humans and animals. The mechanism of the carcinogenic effects and the responsible metabolites have not been identified to date. A complex mechanism involving genotoxic effects and/or the impairment of an immune response is assumed.

Therefore, a MAK value has not been derived and a peak limitation does not apply.

Prenatal toxicity. Teratogenicity studies revealed delays in skeletal development with concurrent maternal toxicity in Sprague Dawley rats at a dose level of 125 mg/kg body weight and day and in New Zealand White rabbits at 150 mg/kg body weight and day. Teratogenic effects were not found in either species. As no MAK value has been derived, the substance has not been classified in a pregnancy risk group.

Germ cell mutagenicity. Even though positive results were obtained in vitro with 1,2-dichloropropane in bacterial mutagenicity tests, in vivo, the substance did not induce mutations in the liver tissue of rats and mice in the gpt test and in the erythrocytes of mice in the Pig-a test. This demonstrates that 1,2-dichloropropane does not induce direct genotoxic effects. However, samples of tumour tissue taken from printing workers with exposure to 1,2-dichloropropane revealed a marked increase in mutation frequency, an increased incidence of DNA double-strand breaks and unique trinucleotide mutational changes compared with the findings from tissue samples taken from biliary tract tumours of the control group. This suggests that genotoxic effects are induced by 1,2-dichloropropane or its metabolites.

In tests investigating clastogenicity in mice, no chromosomal aberrations were induced by intraperitoneal injection and no micronuclei were observed after administration by gavage and inhalation exposure. No dominant lethal mutations were observed in rats given the substance with the drinking water.

For this reason, 1,2-dichloropropane has not been classified in a category for germ cell mutagens.

Absorption through the skin. The dermal LD_{50} value of 1,2-dichloropropane is 10 times as high as the oral LD_{50}. A case report demonstrated that systemically toxic amounts were probably absorbed via the skin (Fiaccadori et al. 2003). There are no other data available for absorption through the skin. Assuming standard conditions and exposure to a saturated aqueous solution, the maximum amount absorbed through the skin is estimated to be 948 or 71 mg per working day (Section 3.1).

On the basis of the findings from the case report (Fiaccadori et al. 2003) and the induction of carcinogenic effects in humans, 1,2-dichloropropane has been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. The data for possible sensitizing effects induced by 1,2-dichloropropane in humans remain unreliable and positive findings were not obtained in animal studies or in vitro studies. Therefore, 1,2-dichloropropane has not been designated with an "Sh" or an "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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