Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

1,2-Dichloropropane; Propylene dichloride

EC number: 201-152-2
CAS number: 78-87-5

CLH-O-0000004490-79-03/F

Adopted

4 June 2014

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 1,2-DICHLOROPROPANE; PROPYLENE DICHLORIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: 1,2-dichloropropane (DCP); propylene dichloride (PDC)
EC number: 201-152-2
CAS number: 78-87-5
Dossier submitter: Industry

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2013	Romania	Oltchim S.A	Company-manufacturer	1
Comment received				
A comprehensive document, containing a qualified point of view it could be find in the public attachemend. *ECHA's note: Please see attachment Carcinogenicity of 1,2-Dichloropropane – Review of Information*				
Dossier Submitter's Response				
The submitter appreciates the independent review of the rat inhalation cancer bioassay publication. The submitter also agrees that Umeda et al. (2010) does not give enough detail to fully understand the exact location of tumours within the nasal passage, and cell types involved. Moreover, no historical control data for tumour incidences were reported by the authors. Tumour incidences reach significance at the highest dose levels. However, there is evidence of long-term damage and repair of the nasal mucosa, and chronic inflammation in the bioassay indicative of threshold for tumour development (secondary consequence to toxicological perturbations of inflammation and tissue toxicity). The PDC effects are thus point-of-contract effects from direct interaction of toxicant with the cells that then progress to tumours.				
RAC's response				
Although there are indications of nasal irritation in the repeated dose toxicity studies, in neither of the studies is irritation in the lungs observed. In addition, some of the in vitro genotoxixity studies are positive and although the in vivo genotoxicity studies are negative, there are no such studies with inhalation exposure. RAC considers that, due to lack of data, a genotoxic mode of action cannot be excluded.				

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2013	France		MemberState	2
Comment received				
It would have been useful to have the number of animals used in each study reported.				
Dossier Submitter's Response				
The animal numbers were provided in the IUCLID endpoint summaries for each study. These fields have not transferred to the CLH report. The animal numbers were as follows: In the Umeda et al. (2010) 2-year inhalation study, groups of 50 F344/DuCrj (SPF) rats per sex per dose were exposed to PDC at 0 (clean air control), 80, 200, or 500 ppm for 6 h/day, 5 days/wk for 104 weeks. In the recently published Matsumoto, Umeda et al. (2013) 2-year inhalation study, groups of 50				

BDF1/Crlj (SPF) mice per sex per dose were exposed to PDC at 0 (clean air control), 32, 80, or 200 ppm (v/v) for 6 h/day, 5 days/wk for 104 weeks.

In the mouse gavage study (NTP, 1986), 50 female and 50 male B6C3F1 mice per group were administered PDC in corn oil at doses of 0, 125 or 250 mg/kg body weight (bw) 5 times per week for 103 weeks.

In the rat gavage study (NTP, 1986), PDC was administered in corn oil 5 times per week for 103 weeks to groups of 50 female F344/N rats at 0, 125 or 250 mg/kg bw and to groups of 50 male F344/N rats at doses of 0, 62 or 125 mg/kg bw.

RAC's response

noted

Date	Country	Organisation	Type of Organisation	Comment number
16.12.2013	Germany		MemberState	3

Comment received

The DE CA requests a more detailed description of the available test results serving as a basis for the classification proposal and comparing them strictly with criteria for sufficient evidence of carcinogenicity.
In table 8 of Part B, chapter 1.3 "Physico-chemical properties" the unit of the relative density should be g/cm3 instead of g/cm-3.

Dossier Submitter's Response

The typo in the unit was corrected.

Proposed Classification for 1,2-PDC:
Category 2 Carcinogen. Suspected human carcinogen.

Category 2 Carcinogen classification is proposed for PDC based on limited evidence obtained from both human case reports and animal studies. The strength of evidence, based on inhalation cancer bioassays with rodents and uncertainties associated with human worker reports, is not sufficiently convincing to place PDC in Category 1B. Strength of evidence and additional considerations for carcinogenicity classification are discussed below.

The proposed classification of Category 2 Carcinogen for PDC is based on point-of-contact papillomas in the nasal tissues of F334 rats at the highest concentrations of both sexes and combined bronchiolo-alveolar adenomas/carcinomas in BDF1 mice, reaching significance at the highest dose in females. There was a dose-response increase in preneoplastic nasal lesions in rats, but not in mice exposed to PDC. Furthermore, three cases of rare esthesioneuroepithelioma at low (two) and mid (one) doses were reported in male rats, with no dose-response relationship. No historical control data for tumour incidences were stated by the authors in the rat report.

Although the mouse inhalation PDC cancer bioassay has recently become available (Matsumoto, Umeda et al., 2013), tumour responses were seen in single sexes. In the females, concentration-dependent increase in combined incidence of bronchiolo-alveolar adenomas/carcinomas reached significance at the highest dose level of 200 ppm PDC and exceeded the JBRC historical control data (the incidence ranges not given), whereas in males, no dose-response was seen; at the low and high doses, combined bronchiolo-alveolar adenoma/carcinoma incidences (18/50 at each dose) reached statistical significance in males. For males exposed to 200 ppm PDC, the incidence of benign Harderian gland adenomas of the eye exceeded the maximum incidences of the JBRC historical control data (ranges also not given). There is no Harderian gland in men (Albert et al., 1986) and Harderian gland adenomas have no relevance to humans (Cohen, 2004). The remaining tumor incidences were within the incidences of the JBRC historical control data. The incidence of pre-neoplastic bronchiolo-alveolar hyperplasia was not increased in any exposure group. In the liver, there were no changes, including altered cell foci. Anemia and systemic toxicity in chronically exposed animals is inferred from results of subchronic mouse study.

The PDC-induced tumours seen in rodents are point-of-contract effects from direct interaction of toxicant with the target cells that then progress to cancer. The statistically significant tumour

incidences only at highest doses in rodents indicate threshold mechanism. The evidence indicates non-genotoxic mode of action (MoA) for tumour development from toxicological perturbations of inflammation and tissue toxicity. The human relevance for respiratory tumours seen in rodent studies cannot be excluded.

Results from reliable *in vivo* assays demonstrate that PDC was not genotoxic in mouse micronucleus and rat dominant lethal assay. Sex-linked Recessive Lethal Test in *Drosophila melanogaster* was also negative. Ames assays produced mixed outcomes, but no mutagenicity or cytotoxicity was detected up to 2000 µg PDC/plate pre-incubated with TA 98, TA 1537, TA 100, TA 1535 *Salmonella typhimurium* in the absence or presence of S9 activation in GLP-compliant assay (NTP, 1986). Overall, PDC has returned consistently negative results in *S. typhimurium* tester strains TA1537 and TA98 at up to 5800 µg/ml in the absence or presence of S9, in addition to TA1978 and TA1538 strains, whereas TA100 and TA1535 produced mixed results (IARC, 1999). In CHO and V79 cells, SCE were induced after PDC treatment. However, IARC's 1999 evaluation of PDC potential to cause genotoxicity was concluded as negative.

Toxicokinetic studies in animals indicate rapid absorption and widespread systemic distribution of PCD after oral and inhalation exposure, including bone marrow. PDC is subsequently metabolized and excreted in urine (as conjugated metabolites) or in exhaled air (predominately as carbon dioxide). Only trace amounts remained in tissues 48 hr after treatment. Metabolic analyses identified three N-acetylcysteine conjugates predominating in rat urine: N-acetyl-S-(2-hydroxypropyl)-L-cysteine, N-acetyl-S-(2-oxo-propyl)-L-cysteine, and N-acetyl-S-(1-carboxyethyl)-L-cysteine. Unchanged PDC was not found in urine. Short duration studies demonstrated glutathione depletion in the liver (Trevisan et al., 1898; Nucci et al., 1990; Imberti et at., 1990; Trevisan et al., 1991).

The liver (rats, mice) and red blood cell (rabbit, rat, mice) are the target tissues for systemic toxicity following repeated PDC exposure. Site-of-contact effects, consistent with repeated local irritation, have been described in stomach (after oral gavage) and nasal tissue (from inhalation).

By way of related analogues, chlorinated solvents appear to have similar mechanisms of toxicity with common target organs of liver, lung and kidney. Reactive and oxidative metabolites are commonly implicated in the cancer MoA for many of the one and two carbon long chlorinated alkanes. Factors associated with increasing toxicity include increasing number of chlorine atoms per molecule and shorter carbon chain length. Unsaturation and increased degree of oxidation also increases the hazard profile. Decreasing toxicity is associated with increasing carbon chain length, with the three carbon atom species (including PDC) having lower hazard profiles compared with shorter chlorinated alkanes. Two other chlorinated solvents were co-reported with PDC to be associated with cholangiocarcinoma (CC) cases in Japanese printing workers. Dichloromethane (CAS no. 75-09-2) has Carc. 2 (H351. Suspected of causing cancer) and IARC Group 2B classifications (possibly carcinogenic to humans). 1,1,1-Trichloroethane (CAS no. 71-55-6) has no harmonized cancer classification under C&L Regulation 1217/2008 and IARC Group 3 classification (not classifiable as to its carcinogenicity to humans). One carbon shorter 1,2-dichloroethane (CAS no. 107-06-2) has Carc. 1B (H350. May cause cancer) and IARC Group 2B classifications (possibly carcinogenic to humans).

Limited epidemiological evidence that PDC exposure in humans is correlated with increased incidence of biliary tract cancers is available. Although high incidences of CC were reported in Japan, other geographic cohort studies reported considerably lower risk estimates for printing industry workers. In the Japanese reports, co-exposure with other chlorinated solvents as well as a significant link to smoking were identified for affected workers. Animal studies with PDC do not point to biliary tract as a target organ for tumour development, even though haemolytic anaemia is the common systemic toxicity observed in all tested species. Tumour locations between species in the inhalation cancer bioassays were different, but point to site of contact and threshold mechanism. Based on weight of evidence, non-genotoxic MoA is proposed for PDC-induced rodent tumours of respiratory tract.

RAC's response

Noted.
The data on the human cases of cholangiocarcinomas was also evaluated by RAC and included in the opinion. Although data are only limited and a well performed epidemiological study also analysing confounding factors is not available, RAC considered the evidence supportive for classification as Carc. 1B.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.12.2013	Netherlands		MemberState	4

Comment received

The mechanism by which this substance induced tumours is not clear from the provided data. An overview of the repeated dose toxicity after inhalation and information on the mutagenicity of propylene dichloride would be useful to assess mechanism of action and inform classification as these are important factors to assess the overall level of concern. Therefore, an overview of the available data is requested.

Dossier Submitter's Response

The overview of repeated dose toxicity via inhalation route is provided below. Mutagenicity results have been discussed in Comment No. 8.

10 F344/DuCrj (SPF) rats per sex per dose were exposed to test material at 0, 125, 250, 500, 1000, and 2000 ppm PDC 6 hours/day, 5 days/week for 13 weeks (Umeda et al., 2010). It was not stated whether a specific guideline was followed. No NOAEC was determined from the study. 13-week exposure to PDC induced inflammation, hyperplasia of the respiratory epithelium, and atrophy of the olfactory epithelium at 125 ppm (study LOAEC for males and females, all animals per group affected) and above. In the nasal cavity, hyperplasia of the respiratory epithelium and atrophy of the olfactory epithelium occurred in both male and female rats exposed to 125 ppm and above, and the averaged severity scores of these two lesions increased in a concentration-dependent manner. Hyperplasia of the respiratory epithelium was characterized by an increased number of ciliated columnar epithelial cells and accompanied by goblet cell hyperplasia. The hyperplasia was located diffusely in the dorsal or septum region of Level 1. Atrophy of the olfactory epithelium was characterized by decreases in epithelial thickness and the number of olfactory sensory cells and often accompanied by necrosis of the olfactory sensory cells and respiratory metaplasia of the olfactory epithelium. Atrophy was located in the dorsal region of Levels 2 and 3.

Inflammation of the respiratory epithelium significantly increased in the male rats exposed to 1000 and 2000 ppm. At the higher exposures, haemolytic anaemia and lesions of liver and adrenal gland in females were observed. In the liver, swelling of centrilobular hepatocytes was observed in both male and female rats exposed to 2000 ppm. Increased hematopoietic activity in the spleen and bone marrow, a compensatory response to hematotoxicity, was noted in the 1000 and 2000 ppm-exposed rats of both sexes. Increased hemosiderin deposition resulting from hemolysis of erythrocytes was observed in the spleen of male rats exposed to 1000 and 2000 ppm and female rats exposed to 500 ppm and above. Fatty change in the adrenal gland was significant in the female rats exposed to 2000 ppm. One female mortality was reported for 2000 ppm dose group. No exposure-related lesions were observed in any other organs in the PDC-exposed rats of either sex. Although authors report that the criteria for MTD was achieved in the cancer bioassay based on survival and terminal body weights, doses for the bioassay should have been lower and based on the haemolytic anaemia seen in the 500 ppm dose group and nasal tissue damage at 125 ppm in the 13-week study. The mid and high doses chosen for the follow up cancer bioassay were higher than lowest (LOAEC) dose obtained in this 13-week study. As such, the inhalation cancer bioassay was set up to assess high-dose effects and was confounded by systemic toxicity.

In the newly identified Matsumoto, Umeda et al. (2013) subchronic study, groups of 10 BDF1/Crlj (SPF) mice per sex per dose were exposed to PDC at 0 (clean air control), 50, 100, 200, 300 or 400 ppm (v/v) for 6 h/day, 5 days/wk for 13 weeks. In the study, no NOAEC was also established. Anaemia was seen in all PDC-treated male mice, and in ≥300 ppm treated females. Six males and one female from 400 ppm group and two males from 300 ppm groups died during the study. Growth rates were suppressed dose-dependently reaching statistical significance in males exposed to ≥200 ppm; no growth retardation was reported for females, but significant toxicity was reported for females. The absolute and relative liver weights were significantly increased in both sexes exposed to ≥300 ppm PDC. The relative spleen weights were significantly increased in both sexes at 400 ppm. Platelets increased in males exposed to ≥300 ppm and in high dose females. Total bilirubin, AST, ALT and LDH were increased in 400 ppm dose group animals. ALP was significantly increased in males at

≥300 ppm PDC. Respiratory metaplasia, atrophy and necrosis of the olfactory epithelium occurred in both sexes exposed to ≥300 ppm PDC. Hyperplasia in the forestomach was significantly increased in 400 ppm dose males and ≥300 ppm dose females. Histopathological changes in liver of male and female mice exposed to ≥300 ppm PDC were reported, including swelling of centrilobular hepatocytes and necrosis of centrilobular hepatocytes. Increased extramedullary hematopoiesis was observed in males exposed to 400 ppm and females exposed to ≥300 ppm. Hemosiderin deposition and increases in megakaryocyte numbers were significant in 400 ppm dose group animals.

In another set of subchronic inhalation studies, according to OECD 413 guideline, NZW rabbits (7 animals/sex/dose) were exposed to PDC vapors at 0, 150, 500 and 1000 ppm (0, 0.675, 2.25 and 4.5 mg/l), whereas Fischer 344 rats and B6C3F1 mice (10 animals/sex/dose) were exposed to 0, 15, 50 and 150 ppm PDC vapors for 6 hours/day, 5 days/week for 13 weeks (Dow, 1988).

Dose selections for these sub chronic studies were based on the results of the preceding 2-week inhalation study, where mice were exposed to 0, 30, 100 or 300 ppm PDC, whereas rats and rabbits were exposed to 0, 100, 300 or 1000 ppm PDC for 6 hour/exposure for nine exposures over two weeks. Body weight gains of rats exposed to PDC (all levels) decreased from controls. The olfactory mucosa of the nasal turbinates in rats was affected at all exposure levels. The extent and severity of degeneration of the olfactory mucosa was directly correlated with exposure level. Male and female mice exposed to 300 ppm PDC had increased liver weights and decreased thymus weights. Some mice exposed to 100 and 300 ppm PDC had degenerative changes in the olfactory mucosa, but these changes were not as severe as observed in rats at the same exposure levels. Only minor liver changes were observed histologically in mice exposed to 300 ppm PDC. Male rabbits exposed to 1000 ppm PDC had equivocal degenerative changes in the olfactory mucosa. 2-week NOEC of 30 ppm for mice, 300 ppm for rabbits, and no NOEC for rats were established in the study.

Minimal toxicological effects were recorded in male and female NZW rabbits following whole body exposure to 0, 150, 500 or 1000 ppm PDC for 13 weeks (Dow, 1988). Treatment-related changes were limited to alterations in red cell parameters (decreased RBC, HGB, PCV) in male rabbits exposed to 150-1000 ppm PDC (LOAEC of 150 ppm), and in females exposed to 500 or 1000 ppm (NOAEC of 150 ppm). The overall pattern of changes was consistent with regenerative macrocytic normochromic anaemia. Minimal degeneration of olfactory epithelium in high dose males was also observed, but females were unaffected. Under the conditions of the study, the LOAEC for PDC exposed via inhalation for 13 weeks was 150 ppm.

Minimal toxicological effects were recorded in male and female F344 rats following whole body exposure to 0, 15, 50 or 150 ppm PDC (0, 0.068, 0.225 and 0.675 mg/l) for 13 weeks (Dow, 1988). Body weights for high-dose exposed animals were significantly decreased throughout the study, but not in rats exposed to 15 ppm PDC. There were no toxicologically significant changes in hematological parameters. There were no toxicologically significant changes in urinalysis parameters. Several slight, but statistically significant effects in organ weights were noted in rats exposed to 50 or 150 ppm PDC. A qualitative reduction in the amount of abdominal adipose tissue was also noted in some high dose males. These changes were considered of negligible toxicological relevance. Histopathologic effects attributable to PDC exposure were confined to the upper respiratory tract of rats exposed to PDC. Very sight or slight degeneration of the olfactory mucosa in the anterior portion of the nasal cavity was noted for all rats exposed to 50 or 150 ppm PDC, but not in 15 ppm exposure group. Very slight or slight hyperplasia of the respiratory mucosa was also present in the majority of rats of both sexes exposed to 50 or 150 ppm PDC, with very slight hyperplasia detected in 1/4 of the low dose group animals. This hyperplasia was focally restricted to the anterior portion of the nasal tissues. Chronic inflammation of nasal tissue was present in all groups, including controls, but was slightly more prevalent in high dose rats of both sexes. Treatment related effects were limited to a minor reduction in body weight and very slight hyplasia of the nasal respiratory epithelium, considered to be an adaptive response, with the study NOAEC of 15 ppm.

There were no effects in B6C3F1 male or female mice attributed to 0, 15, 50 or 150 ppm (0, 0.068, 0.225 and 0.675 mg/l) PDC exposure for 6 hours/day, 5 days/week for 13 weeks (Dow, 1988). Exposure to highest concentration of 150 ppm PDC did not result in mortality or overt signs of toxicity. The body weights of animals from the treated groups were comparable to control values. There were no toxicologically significant changes in hematological parameters. There were no dose-dependent changes observed in the treated animals. RBC and HGB were slightly, but significantly

decreased in low and high dose male mice, with no effect at 50 ppm; all treated females were indistinguishable from controls. PCV was statistically significantly increased in low dose animals, but there were no effects in mid and high dose groups. At necropsy, there were no grossly visible lesions attributable to exposure to PDC. There were no dose-dependent histopathological changes observed in the treated animals. The study NOAEC was 150 ppm.

Overall, the recent subchronic F334 rat inhalation study utilized exposure levels that consistently induced significant nasal passage effects at all 125, 250, 500, 1000, 2000 ppm PDC concentrations. Systemic toxicity was reported at the highest two doses with the study LOAEC of 125 ppm (Umeda et al., 2010). No NOAEC was established in mouse subchronic study based on 50, 100, 200, 300 or 400 ppm PDC concentrations; significant mortality and systemic toxicity in exposed mice were seen (Matsumoto et al., 2013). Haemolytic anaemia in PDC-exposed rats and mice reported by Umeda and colleagues indicate common target organ for systemic toxicity. Moreover, doses that induced anaemia in animals in subchronic studies were carried over to rodent inhalation cancer bioassays. Findings from GLP Dow 1988 F334 rat subchronic study, utilizing lower doses, were limited to minor reductions in body weight and very slight hyperplasia of the nasal respiratory epithelium, with study NOAEC of 15 ppm. Histopathologic effects in the upper respiratory tract of mid (50 ppm) and high (150 ppm) dose groups were of very sight or slight degeneration of the olfactory mucosa in the anterior portion of the nasal cavity. Very slight or slight hyperplasia of the respiratory mucosa was also present in the majority of rats of both sexes exposed to mid and high PDC dose. Chronic inflammation of nasal tissue was present in all groups, including controls, but was slightly more prevalent in high dose rats of both sexes.

RAC's response

The additional data on repeated dose toxicity and mutagenesis are included in the Background Document.

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2013	Romania	Oltchim S.A	Company-manufacturer	5

Comment received

Base on Umeda study there is evidence of long-term damage and repair of the nasal mucosa, although the report does not give enough detail to understand fully the exact location within the nasal passage, or all the cell types involved. However, the degree of damage cause by the test item did not cause any ulcers or affect the life span of the test animals.
The carcinogenesis mechanism, induced by 1,2-dichloropropane, appear to be not very clear, based on provided study.
The only way to make a solid argumentation, for or against the proposed classification, would be to make a full review of the histopathological slides by a very experienced histopathologist, then to get a panel of expert pathologists to review relevant slides.

Dossier Submitter's Response

The submitter agrees that there are remaining uncertainties about the key events underlying tumour development. However, systemic toxicity and haemolytic anaemia were reported for PDC-exposed animals and these effects should have been taken under consideration as MTD for setting of doses for inhalation cancer bioassays. Overall, evidence indicates threshold, non-genotoxic mode of action for tumour development from direct interactions of PDC with target cells.

RAC's response

Although there are indications of nasal irritation in the repeated dose toxicity studies, in neither of the studies irritation in the lung is observed. In addition, some of the in vitro genotoxixity studies are positive and although the in vivo genotoxicity studies are negative, there are no such studies with inhalation exposure. RAC considers that, due to lack of data, a genotoxic mode of action cannot be excluded.

Date	Country	Organisation	Type of Organisation	Comment number

20.12.2013	France		MemberState	6

Comment received

These data have not been reported in the CLH report but DCP is reported to be mutagenic and able to induce sister chromatid exchange and chromosome aberrations in cultured Chinese hamster ovary cells and V79 cells. This information has to be included in the CLH report since it could help to assess the mechanism of action of the carcinogenic activity of DCP as discussed in the study by Umeda et al.

Esthesioneuro-epitheliomas observed in the study by Umeda et al. seemed to be very rare variant of olfactory neuroblastoma. Even this type of cancer was not observed at the highest dose, it was observed in two different doses (80 and 200 ppm).
Moreover there was a dose-dependent increase in the number of papillomas in both sex, which occurred in a spontaneous manner according to Schwartz et al. (1994) on extremely rare occasions. In this study the 2-year inhalation exposure to DCP also resulted in significant increases in the incidences of hyperplasia of the transitional epithelium and squamous cell hyperplasia. According to the authors induction of these lesions occurred at lower exposure concentrations than those, which induced nasal
tumours. Therefore DCP is a nasal carcinogen, and based on this assertion and on the hepatocellular neoplasms observed in mice, DCP should rather be classified at least as Carc. 1B.

Additionally, it should be noted that human data exists since in a report of a Panel meeting of the Japanese Ministry of Health, Labour and Welfare described several cases of bile duct cancer among employees of a printing firm, and concluded these cases were likely due to the use of cleaning agents containing 1,2-dichloropropane. Thus, there is reasonable evidence that 1,2-dichloropropane may be a carcinogen for humans. Even if this report is in Japanese, these data have to be included in an updated dossier. These human data are a further justification for a classification of 1,2 DCP as at least Carc. 1B.

Dossier Submitter's Response

The requested mutagenic and genotoxic data are described in detail in Comment 8 and the justification for cancer classification is laid out in Comment 3.

NTP oral cancer bioassay conducted in rats and mice found some evidence of an increased incidence of hepatic adenocarcinomas in male and female mice relative to controls in the presence of liver damage and decreased body weight (females only). However, it was stated that the incidence of liver adenomas was within the historical control range for this strain of mouse suggesting the finding was of marginal toxicological significance. It was considered that other factors (such as spontaneous biological variation) may have contributed to the increased incidence of mouse liver tumors following oral exposure. IARC concluded, based on these oral cancer bioassays, that PDC is *not classifiable as to its carcinogenicity to humans* (Group 3).

The rat nasal tumours and mouse lung tumours reported for PDC are point-of-contact effects with threshold mechanism. Although sites within respiratory tract for tumour development are somewhat different between the two rodent species, it is not unexpected considering the different inhalation reposes and metabolic differences between these species. Although rare, the olfactory neuroblastomas in male rats were not dose-dependent and their significance is unknown, but these were included in the proposed Cat. 2 carcinogen classification. No JBRC historical control data for tumour incidence were given in the rat bioassay publication, whereas no ranges for background historical incidences were given in mouse bioassay report. Results from rodent oral and inhalation cancer bioassays with PDC do not point to biliary tract as being a target organ for tumour development; therefore, no clear link with human cholangiocarcinoma cases can be established for PDC.

Cases of rare cholangiocarcinoma (CC) among Japanese printing industry workers were recently reported (Kumagai et al., 2013; Kubo et al., 2014). The standardized incidence ratio (SIR) of 1226 (95% CI 714-1963) and standardized mortality ratio (SMR) of 724 (95% CI 313-1428) were reported in 2013 by the Japanese Ministry of Health, Labour and Walfare for the observation period of 1985 to 2012 (http://www.mhlw.go.jp/english/policy/employ-labour/labour-standards/Occupational.html) based on evaluation of additional cases of bile duct cancer from different provinces and proof-printing

plants. These ratios are lower than initially reported SMR of 2900 by Kumagai et al. (2013). All cases of CC in the Japanese reports were among relatively young workers, ages 25-45. These workers, however, were exposed to many chemicals including several chlorinated solvents, with all cases of CC being linked by Japanese Expert Panel with exposure to PDC in addition to frequent and significant co-exposures with dichloromethane and 1,1,1-trichloroethane. Importantly to note is that in the Kubo et al. (2014) report, majority of affected workers were smokers (13/17 cases), with no information collected on second hand smoking exposure. The authors of the Japanese worker studies concluded that PDC and/or dichloromethane "may cause cholangiocarcinoma" and "other unidentified chemicals might affect the development of the cholangiocarcinoma." However, a recent review of cancer cases among large cohort of the printing industry workers in the Nordic countries followed over 45 years reported SIRs for CC of 2.34 (95% CI 1.45 to 3.57) (Vlaanderen et al., 2013). The NOCCA-JAM reports exposures to benzene, gasoline, dichloromethane, lead, 1,1,1-trichloroethane and toluene, with no data on PDC exposures.

Overall, there is uncertainty and limited evidence that PDC exposure in humans is correlated with increased incidence of biliary tract cancers. There is clear co-exposure with other chemicals and significant link to smoking for affected Japanese workers. Animal studies with PDC do not point to biliary tract as a target organ for tumour development, even though there are commonalities in systemic toxicity. PDC-induced rodent tumours of respiratory tract are site of contact effects with threshold mechanism. Therefore, the registrant states that the available evidence is limited and does not rise to the level of Cat.1B Carcinogen classification for PDC.

RAC's response

The mutagenic and genotoxic data are evaluated by RAC and included in the WoE analysis in the RAC opinion.
The data on the human cases of cholangiocarcinomas was also evaluated by RAC and included in the opinion. Although data are only limited and a well performed epidemiological study also analysing confounding factors is not available, RAC considered the evidence supportive for classification as Carc. 1B.

Date	Country	Organisation	Type of Organisation	Comment number
16.12.2013	Germany		MemberState	7

Comment received

The CLH proposal aims to classify and label of PDC for carcinogenicity. The proposal on classification of PDC as category 2 carcinogen is based on the test results of a study on carcinogenicity by inhalation in male and female rats. The reported data provide indications of a carcinogenic profile of PDC after inhalation.

However, based on the presented information in the CLH-dossier concerning carcinogenicity a valuable comment could not be given. The justification of this proposal provided by the submitter has been found to be unclear and sometimes contradictory.

In the submitted study on carcinogenicity by inhalation in male and female rats, increased incidences of papilloma were observed in the nasal tissues of both sexes exposed at the highest concentration. In addition, preneoplastic lesions were observed in a concentration-dependent manner. Furthermore, three cases of esthesioneuroepithelioma were reported from the PDC-exposed male rats with no dose-response relationship. None of these tumours were found in female rats. In the medical literature the esthesioneuroepithelioma (olfactory neuroblastoma) remains a rare entity and cases in patients range from 8 to 79 years of age (Berger 1924; Seaman 1951; Mashberg et al.1960; Hassoun et al. 1981).

Therefore, the submitter is requested to describe the available test results which serve as basis for the classification proposal in more detail and to compare them strictly with criteria for sufficient evidence of carcinogenicity. In addition, important factors in the evaluation of the tumour findings described in the guidance document, especially the observed tumour type (in this case the esthesioneuroepithelioma), may be taken into consideration when assessing the overall level of concern. Data from repeated dose toxicity studies and germ cell mutagenicity should be also

considered to complete the CLH dossier for carcinogenicity.

Data from a 13-week exposure study were cited and included in the justification for carcinogenicity of PDC by the submitter, but they were not presented in the CLH-report.

Dossier Submitter's Response

The overview of repeated dose toxicity via inhalation route, including Umeda et al. 13-week study, has been provided in Comment No. 4. Germ-cell mutagenicity data have been detailed in Comment No. 8. Overall, *in vivo* findings indicate that PDC is not a somatic or germ cell genotoxicant. Justification for cancer classification has been laid out in Comment 3.

RAC's response

The additional data are included in the WoE analysis by RAC. It is noted that, although *in vivo* genotoxicity data are negative, no data are available following inhalation exposure. Therefore a genotoxic mechanism cannot be excluded for the nasal and lung tumours that are observed in the rat and mouse inhalation carcinogencity studies.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
16.12.2013	Germany		MemberState	8

Comment received

No data regarding the endpoint 'Germ cell mutagenicity (Mutagenicity)´ are considered in the CLH dossier proposing classification for carcinogenicity. Although classification in respect of specific target organ toxicity and germ cell mutagenicity are not proposed in this CLH dossier, the available data to these endpoints should be considered to complete the CLH dossier for carcinogenicity. Please add the available data.

Dossier Submitter's Response

Summary for the endpoint of 'Germ cell mutagenicity´ has been included below. While *in vitro* assays showed mixed outcomes, results from reliable *in vivo* tests demonstrate that PDC was not genotoxic in a mouse micronucleus test or a rat dominant lethal assay. Sex-linked Recessive Lethal Test in *Drosophila melanogaster* was also negative. These findings indicate that PDC is not an *in vivo* somatic or germ cell genotoxicant. In support, IARC's 1999 evaluation of PDC potential to cause genotoxicity was concluded as negative.

In vitro Studies

Results from *in vitro* genotoxicity tests with and without metabolic S9 activation show both positive and negative results.

The mutagenic potential of PDC has been evaluated in a large number of microbial tests in bacteria and fungi (summarized by IARC, 1999). Overall, results from these tests are mixed. PDC was not mutagenic to *Streptomyces coelicolor*, weakly induced mutations, but not chromosomal effects, in *Aspergillus nidulans*. PDC has returned consistently negative results in *Salmonella typhimurium* tester strains TA1537 and TA98 at up to 5800 µg/ml in the absence or presence of S9, in addition to TA1978 and TA1538 strains, whereas TA100 and TA1535 have returned mixed results under similar conditions (IARC, 1999). In a GLP-compliant study with liquid pre-incubation conducted by the US National Toxicology Program, no mutagenic activity or cytotoxicity was detected when PDC (up to 2000 µg/plate) was incubated with four strains of *Salmonella typhimurium* (TA 98, TA 1537, TA 100, TA 1535) in the absence or presence of S9 activation (NTP, 1986). A satisfactory response was obtained with the positive controls. DPC was also not cytotoxic or mutagenic in these tester strains when evaluated in the absence or presence of S9 using a plate incorporation method (up to 3150 µg/plate, with or without glutathione supplementation; Oesch, 1979). Exposure to PDC vapour also failed to produce a response in the presence or absence of S9 and glutathione supplementation (Oesch, 1979), whereas dichloroethane was positive in TA100 and TA1535 under these same conditions.

When tested in mammalian cells *in vitro*, no increase in mutations was detected at the thymidine kinase locus in mouse L5178Y cells after incubation with up to 1000 nl/ml PDC in the absence of S9 (cytotoxic at >800 nl/ml), while assays in the presence of S9 provided evidence of mutagenicity at or around the threshold for cytotoxicity (80 nl/ml) (Myhr and Caspary, 1991). In an assessment of clastogenic potential, the number of chromosomal aberrations present in CHO cells exhibited a dose-related response (reported as a 5- or >16-fold increase) after incubation with 1370 or 1580 µg/ml PDC in the absence of S9, and an approximate 4-fold increase in the number of aberrant cells exposed to 660 or 950 µg/ml in the presence of S9 (NTP, 1986). In another series of *in vitro* experiments, CHO cells exhibited a dose-related increase in sister chromatid exchanges (SCE) after exposure to PDC, with an approximate doubling in response after incubation with 376 or 1127 µg/ml PDC, both in the presence and absence of S9 (NTP, 1986). In V79 cells, both in the presence and absence of S9 1,2-PDC and 1,3-PDC had lowest potential to increase SCE (Von der Hude et el., 1987). SCE–inducing potency of three carbon compounds increased with degree of oxidation of the third substituted carbon atom and with unsaturation.

In vivo Studies

Reliable and interpretable *in vivo* results demonstrate that PDC is not an *in vivo* somatic or germ cell genotoxicant.

Results from a recent GLP-compliant OECD 474 guideline mouse micronucleus study demonstrated no evidence of cytogenetic damage in CD-1 mice bone marrow up to 600 mg/kg bw by gavage (corn oil vehicle) on 2 consecutive days (Spencer *et al*., 2003). Systemic toxicity (2°C drop in body temperature) was noted in high dose animals, while results from the range-finder investigation indicated that higher treatment levels (1000 mg/kg bw and above) were lethal. A satisfactory response was obtained with the positive control, cyclophosphamide. The results demonstrate no potential for PDC to damage genetic material present in actively replicating bone marrow cells, as toxicokinetic data have demonstrated that PDC is distributed evenly across all tissues, including bone marrow.

Similarly, negative results were reported in a GLP rat dominant lethal guideline study (Hanley *et al*., 1989). Male SD rats (n = 30/group) received PDC in drinking water at 0, 28, 91 or 162 mg/kg bw/day for at least 13 weeks. The high dose was a saturated solution of PDC in water. They were then mated with untreated females for two successive one-week periods. A positive control group (cyclophosphamide, 100 mg/kg bw, 48 hr prior to mating) was included in the study. Mating and fertility indices were comparable between the control and PDC-treated groups (96-100%), but decreased significantly in the positive controls. Slight variations in number of *corpora lutea*, number of implantations, pre-implantation losses and resorption rates were noted in the first or second week of mating in the low and high dose groups (mid-dose group not different from control), but the magnitude of the change was within the normal control ranges. In contrast, the positive control group showed a 2-fold increase in pre-implantation loss and a 10-fold increase in resorption rate. Overall it was concluded that PDC had no capacity to induce heritable mutations in male SD rats following at least 13-week oral treatment with up to 162 mg/kg bw/day.

Klimisch 4, Woodruff *et al*., 1985 did not demonstrate induction of sex-linked recessive lethal mutations in *Drosophila melanogaster after 4 hour* inhalation to 33849 µg PDC/m^3. Limited information, from a non-standard test method of unknown reliability, indicates that the number of polyploid mononuclear and binuclear hepatocytes was increased in rats following 3 days inhalation exposure to 2200 mg/m^3 PDC (Belyaeva *et al*., 1977). The morphological changes arising after exposure to PDC consisted of degenerative changes in the parenchymatous organs, particularly in the liver. After inhalation exposure for 1 day the percentage of mono- and binuclear cells in the population remained unchanged, by the third day there was a tendency toward an increase in the number of polyploid cells (variation in the number of the chromosomes-different event from the chromosome aberration).

RAC's response

The additional data are included in the Background Document.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 1,2-DICHLOROPROPANE; PROPYLENE DICHLORIDE

Date	Country	Organisation	Type of Organisation	Comment number
16.12.2013	Germany		MemberState	9
Comment received				
Please add the available data from repeated dose toxicity studies.				
Dossier Submitter's Response				
The detailed overview of repeated dose toxicity effects via inhalation route has been provided in Comment No. 4.				
RAC's response				
The additional data are included in the Background Document.				

ATTACHMENTS RECEIVED:

1. **Carcinogenicity of 1,2-Dichloropropane – Review of Information**, submitted by Oltchim S.A on 20/12/2013 [Please refer to comment 1]