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-dihydroxybenzene; pyrocatechol

EC Number: 204-427-5
CAS Number: 120-80-9

CLH-O-0000001412-86-122/F

Adopted

16 September 2016

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 1,2-DIHYDROXYBENZENE; PYROCATECHOL

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: 1,2-dihydroxybenzene; pyrocatechol
CAS number: 120-80-9
EC number: 204-427-5
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.11.2015	France	Rhodia Operations (Member of the Solvay Group)	Industry	1
Comment received				
Comments were already provided by Rhodia Operations under the reference number 35437f4e-9ce8-443c-b7cd-6598bb55cf03. Please find below an additional comment on another hazard class to be added to our previous comments.				
Dossier Submitter's Response				
FR (01/2016): Thanks for your comment, see answer below.				
RAC's response				
RAC evaluated the Industry comments. For further details please refer to comments No 3, 4, 6, 8, 9				

Date	Country	Organisation	Type of Organisation	Comment number
05.11.2015	Germany		Member State	2
Comment received				
- The German CA supports the proposed harmonised classification as Acute Tox. 3 ; H301/H311, Muta. 2; H341 and Carc. 2; H351. - Editorial comments: - The physical chemical data are not included in the technical dossier - In IUCLID section 1.1 the CAS and IUPAC name for 1,2-benzenediol as well as the composition are missing. Please add this information. - In IUCLID section 1.2 the CAS and IUPAC name for 1,2-benzenediol as well as a concentration range are missing. Please add this information.				

- Concerning the reference substance information: The EC name is 1,2 benzenediol and not pyrocatechol (this is only a synonym for the substance). Next to this the CAS and IUPAC name for 1,2-benzenediol as well as almost all molecular and structural information are missing. Please add this information, if available. - Assessment report part B, table 5: Please correct the EC name and the IUPAC name.
Dossier Submitter's Response
Thanks for your comment. The CLH report will not be revised at this stage. However, we agree that EC and IUPAC name shall be 1,2-benzenediol instead of pyrocatechol.
RAC's response
RAC evaluated comments from the German CA regarding the endpoints for classification. The discussion on carcinogenicity is elaborated by RAC in ther response to comment 4.

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2015	France	Rhodia Operations (Member of the Solvay Group)	Industry	3
Comment received				
Catechol has been registered under REACH by 2 different companies. These companies are RHODIA OPERATIONS (lead company) and CFS Europe SpA. Please find below the opinion of these 2 companies on the proposed harmonized classification and labelling of catechol which was prepared by France. Catechol is mainly used as an intermediate for chemical synthesis (>10,000 tonnes per year). The manufacture and all the intermediate uses of catechol meet the strictly controlled conditions as defined in Article 18(4) of the REACH regulation. The non-intermediate uses of catechol concern a very limited tonnage (see the REACH registration dossier of each registrant). The substance is only used for industrial and professional uses and we are not aware of any consumer uses.				
Dossier Submitter's Response				
Thanks for your comment.				
RAC's response				
RAC appreciates Industry information on the industrial uses of pyrocatechol under REACH.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2015	France	Rhodia Operations (Member of the Solvay Group)	Industry	4
Comment received				
Many different carcinogenicity studies with catechol have been reported. Different species, doses and study durations were used and also many initiation/promotion studies were published. Nearly all studies were done in Japan. Carcinogenicity studies with several strains of rats revealed that a dose of 0.8 % catechol in the diet resulted in a significant increase of adenocarcinomas in the glandular stomach for both sexes. At this dose a decrease of the body weight was found, while the liver weight increased. During a carcinogenicity study with a duration of 104 weeks adenocarcinomas of the glandular stomach were only observed at a dietary dose of 0.8 % catechol but not at				

lower doses. At lower doses submucosal hyperplasia, ulceration and adenomas of the glandular stomach were found. This shows that catechol has a local toxic effect on the glandular stomach at low doses while at the high dose of 0.8 % in the diet this results in adenocarcinomas. For this reason there is clearly a threshold for the carcinogenic effects of catechol. Hyperplasia was not only found in the glandular stomach but also in the forestomach of the rats.
A carcinogenicity study with mice (B6C3F1) at a dietary dose level of 0.8 % resulted also in submucosal hyperplasia and adenomas of the glandular stomach but no carcinomas were found (applicable for both sexes) during this 96-week study. Also for mice the body weight decreased, while the liver weight increased at this dose of 0.8 % in the diet.
During studies with Syrian hamsters and catechol no carcinomas of the glandular stomach were found although the study duration was 30 weeks or 20 weeks.
The carcinogenicity studies with rodents have only been performed using an oral exposure route. However an oral exposure route is not relevant for human exposure. During professional and industrial use of catechol workers may be exposed to catechol via inhalation but oral exposure of workers of catechol should not occur. Because an extrapolation from the oral route to the inhalation route is normally not possible for local effects it is questionable if the carcinogenicity data are relevant for humans.
Based on the information from the Ariel regulatory database, the national occupational exposure limit (time weighted average) of catechol in most of the EU countries is 20-23 mg/m3. These values are similar to the ACGIH Threshold Limit Value (TLV) of 5 ppm which is equivalent with 23 mg/m3. Within the REACH dossier a DNEL of 1 mg/m3 has been derived for long term inhalation exposure of workers. This limit is much lower than the current occupational exposure limits which are applicable in the EU.
It could be useful to compare the occupational exposure concentration of workers with the dose level of the carcinogenicity study which results in adenocarcinomas of the glandular stomach. For example if workers would be exposed at 50 % of the DNEL level then the exposure would be 0.5 mg/m3. This would be equivalent with a daily dose of about 0.06 mg/kg bw for workers. The carcinogenicity studies with rats showed adenocarcinomas of the glandular stomach at a dietary dose of 0.8 % which is equivalent with about 480 mg/kg bw. This shows that the daily dietary dose level of the rats is 8000 times (480/0.06) higher than the daily dose level when workers would be exposed to 50 % of the DNEL value. This shows that the dose level which results in adenocarcinomas for rats is much higher than the potential worker exposure level.
In addition to the animal studies with catechol alone, initiation and promotion studies with exposure of rodents to nitrosamines and catechol have been reported. Different nitrosamines, doses, treatment designs and species have been studied. In certain cases catechol decreased the carcinogenicity of the nitrosamines. For example a study reported by Hasagawa *et al*. (1990) revealed that treatment with DHPN (N-bis(2-hydroxypropyl)nitrosamine) and catechol seemed to decrease slightly the incidence of carcinogenic effects (thyroid and lung) observed with DHPN alone. This study was done with rats. In addition a study of Maruyama *et al*. (1991) with hamsters showed that the numbers of atypical pancreatic hyperplasias and adenocarcinomas were significantly decreased if the animals were exposed to BOP (N-nitroso-bis(2-oxopropyl)amine) and catechol when compared to BOP alone. Maruyama *et al*. (1994) reported a similar effect of catechol with hamsters when the initiation was done with BHP (N-nitrosobis-(2-hydroxypropyl)amine). The decrease of the carcinogenic effect of nitrosamines due to exposure to catechol might be due to the antioxidant effect of catechol.

Conclusion for carcinogenicity classification
Based on the available mutagenicity data (studies showed in vivo mutagenicity) and carcinogenicity data (several studies with rats showing adenocarcinomas of the glandular stomach) there are arguments to classify catechol as a category 2 carcinogen.

On the other hand the adenocarcinomas of the glandular stomach have been found only for one species (rats but not for mice or hamsters), for one organ (glandular stomach) and only for one very high dietary dose level of 0.8 %. The adenocarcinomas are due to local effects and there is a clear threshold because lower doses than 0.8 % do not show adenocarcinomas of the glandular stomach. Furthermore the exposure route (oral) is not relevant for human exposure. Finally there are also indications that catechol could reduce the incidence of cancer which might be due to the antioxidant effect of catechol. Overall there are sufficient arguments for not classifying catechol for carcinogenicity. This explains why the REACH registrants have not classified catechol for carcinogenicity in the REACH dossier.
Based on the considerations above the classification of catechol seems to be a borderline case. Certain data could warrant a carcinogen category 2 classification while other elements of the available data indicate that a classification for carcinogenicity is not needed.

Dossier Submitter's Response

Thanks for your comment. The CLP regulation criteria for carcinogenicity are based on hazard only without consideration about risks. Malign and benign tumors were observed in the glandular stomach in rats and only benign tumors in mice after oral exposure. Even if inhalation exposure would be the most relevant for pyrocatechol, all experiments with oral exposure showed benign and/or malign tumors. Adenocarcinomas are observed at dose level of 0.8% in glandular stomach. Gandular stomach was not the only target: increase of incidence of carcinoma were observed in esophagus (Yamagushi *et al*. 1989) and adenoma in pancreas (Hagiwara *et al*. 2001) were noted at 0.8%. Based on the available carcinogenicity data and mutagenicity data (in vivo mutagenicity), a classification as carc. 2 for pyrocatechol is considered warranted.

RAC's response

RAC would like to point out the following:

- ✓ Data collected from all the studies on carcinogenic and co-carcinogenic effect of pyrocatechol that are available in the registration dossier on rodents were consistent.
- ✓ Two species, rats (several strains) and mice (B6C3F1), were susceptible to tumorigenesis. Both sexes were found with adenomas and adenocarcinomas in rats and adenomas in mice.
- ✓ The stomach is the main target organ, with benign tumours observed at doses ≥ 0.2% (in the majority of cases where 0.8% was given) and malignant tumours were observed at doses of 0.4% and 0.8%, with a dose-response relationship evident in the Hagiwara *et al*. (2001) study, where the incidence of adenocarcinomas was not statistically significant.
- ✓ Survival of rodents was not affected by pyrocatechol exposure. The decrease in body weight observed ranged from -10% to -41% at the end of exposure at a dose of 0.8%. RAC notes that the 41% decrease in body weight refers to female mice in the Hirose study (1993a) , where the incidence of adenocarcinomas in females was found to be 43%. Average loss of body weight observed at 0.8% of pyrocatechol in male mice was calculated from all available studies in the CLH dossier to be 17.7 ± 4.73 %. At doses of 0.16% and 0.2% (Hirose *et al*. 1997 and Hirose *et al*. 1991, respectively) the observed % weight loss was 13 and 7%, respectively. No adverse effects on survival rates were observed. 1995 study, where statistically similar mortality with the control groups was observed. Slight reduction in the food consumption was also observed (Hagiwara *et al*. 2001: essentially similar to control group; Hirose *et al*. 1990: reduction 6%; Kawabe *et al*. 1994: reduction 8.6%; Hirose *et al*. 1993b: reduction 4.6%; Wada *et al*. 1998: reduction 15.3%), which was as anticipated, since the target affected organ is the stomach. These results suggest that **it is unlikely** that tumours may have been induced at a dose higher than the Maximum Tolerated Dose (MTD).
- ✓ The potential reversibility of glandular stomach lesions induced by catechol was studied by Hirose *et al*. (1992). Incidences of submucosal hyperplasia, adenomas and adenocarcinomas, average number of tumours per rat, and the size of tumours in the glandular stomach of rats treated with 0.8% of catechol from 12 to 96 weeks increased in a time dependent manner. After cessation of catechol treatment, the average number of tumours per rat tended to

slightly decrease, although the size of tumours tended to increase. Labelling indices in both adenomas and non-tumorous areas decreased significantly after cessation of catechol treatment.

- ✓ Other sites of tumourigenesis were also found: the pancreas (acinar cell adenomas: Hirose *et al*., 1993a: at 0.8% w/w in 1/29 male rats; Hagiwara *et al*., 2001: at 0.2% and 0.4% w/w in 1/25 male rats; at 0.8% w/w in 6/25 male rats) and esophagus (Hirose *et al*., 1993b: papillomas 3/15 male rats). Neoplastic lesions (papillomas, hyperplasia) were found in the tongue, oesophagus and lungs in tumour promotion studies (Hirose *et al*. 1993b, 1990; Yamagushi *et al*., 1989).
- ✓ The mechanism through which pyrocatechol may express its carcinogenic potential is still not fully understood. Both stochastic genotoxic as well as non-genotoxic mechanisms are likely to play a role. A generally accepted hypothesis is that pyrocatechol induces oxidative DNA damage. It is for instance assumed that in aqueous environment (pH around or above neutrality) pyrocatechol undergoes Cu2+ -mediated autoxidation to generate Cu+ and semiquinone radicals (Oikawa *et al*., 2001). Binding of Cu+ to oxygen generates reactive oxygen species, but also reduction of semiquinone radicals into 1,2-benzoquinone may have the same effect (IARC Monogr. Eval. Carcinog. Risks. Hum., 1999). These reactive oxygen species may ultimately lead to DNA damage, and thus to the risk of cancer development. The presence of antioxidant enzymes, such as superoxide dismutase and catalase, should remove reactive oxygen species resulting in reduced DNA damage, but so far these enzymes did not clearly influence pyrocatechol-induced DNA damage *in vitro* (Oikawa *et al*., 2001). Further research is needed to clarify these findings. At the same time, DNA methylation may play an important role in the early stage of stomach carcinogenesis. Tatematsu *et al*. (1993) has exposed male rats to catechol (0.8%) for 60 weeks. The aim of the study was to assess the methylation patterns of the rat pepsinogen1 (Pg1) gene. Catechol induced adenomatous hyperplasia but no adenocarcinomas in the glandular stomach. An increase of specific methylation of CCGG sites of Pg1 gene was noted in the pyloric mucosa. The alteration of methylation of the Pg1 gene is considered an early effect in the carcinogenic process and progressive methylation changes occur with tumour development. Furthermore, DNA labelling methods showed a slight induction of submucosal growth in the glandular stomach and an elevation of DNA synthesis in the pyloric gland cells. Since cell proliferation is well correlated with tumour promotion, these results suggest that catechol may have promoting potential for rat stomach carcinogenesis (Shibata *et al*., 1990a and 1990b). In addition, pyrocatechol was found to be locally genotoxic with regards to duodenum cells (significant increase in DNA strand breaks using the Comet assay) (Study report No 18255, 2008) and to oesophageal epithelial cells. Another mechanism of induction of tumours in the glandular stomach by pyrocatechol could be associated with the "gastrin hypothesis" (Chandra *et al*., 2010; Larsson *et al*., 1988; Håkanson and Sundler 1990), which applies to antisecretory drugs, such as omeprazole. In the Hagiwara *et al*. (2001) study, serum gastrin levels were found elevated at a dose of 0.1% w/w (NS) and from 0.2% w/w the increase in gastrin levels reached up to 50% both at 34 and 104 weeks, with a clear dose-response relationship and statistically significant correlation with the proliferative lesions of the pyloric gland. The gastrin hypothesis may be outlined as follows: (1) Inhibition of gastric acid secretion leads to elevated antral pH and, secondarily, to the release of gastrin from the antral gastrin cells into the blood stream. (2) Gastrin causes both general hypertrophy of the oxyntic mucosa and hyperplasia of the ECL cells in the oxyntic mucosa. Hypergastrinemia secondary to inhibition of gastric acid secretion by drugs such as omeprazole is generally associated with a topical effect on the fundic mucosa resulting in increased stomach weight and increased mucosal thickness (hypertrophy) (White *et al*., 1998; Rohr and Tuch 1992; Creutzfeldt *et al*., 1986). Such histopathological findings are consistently observed in all studies with pyrocatechol. Because no endocrine cell hyperplasia or tumours were found in the fundic region in Hagiwara *et al*. (2001), the study authors supported the hypothesis that tumorigenesis in the glandular stomach caused by pyrocatechol could be a secondary proliferative response of the gastrin secreting G-cells in the pylorus. Despite the possibility that the "gastrin hypothesis" MoA operates, the possibility that pyrocatechol may exert its carcinogenic effect by its irritating properties, also a nongenotoxic mechanism, cannot be entirely excluded. Chronic exposure to irritants may induce continuous cell proliferation, making the cells prone to DNA damage. The fact that the vast majority of the observed effects are focused on the glandular stomach, which represents local application of the irritant may contribute to this theory. Nevertheless, in all studies the administration of

pyrocatechol was made via the diet and not by gavage, rendering the mode of administration less extreme. In addition, the carcinogenic effects observed in the forestomach were less severe than those observed in the glandular stomach. In contrast, significant ulceration was observed in the glandular stomach (at 104 weeks) at the same or higher doses than adenomas (0.4% vs 0.2%) which were also observed within 34 weeks (Hagiwara *et al*., 2001). Ulcerations were observed to a lesser extent than adenomas for a given dose (e.g. Wistar rats 43% vs 97%, Lewis rats 70% vs 97%, at dose 0.8% w/w) (Tanaka *et al*., 1995), thus the mode of action of irritancy is considered less predominant for carcinogenicity. Therefore, bearing in mind all the above, a consideration to downgrade from a Category 1 to Category 2 classification due to chronic stimulation of cell proliferation, as suggested in the CLP Guidance (p. 380), is not applicable for pyrocatechol.

- ✓ In conclusion, according to 3.6.1.1 and 3.6.2.2.3 of Annex I of the Regulation 1272/2008/EC and the CLP Guidance, since pyrocatechol can induce benign and malignant tumours in two species in both sexes, pyrocatechol should be classified as a Carcinogen, Category 1B.
- ✓ It is true that recent findings suggest that pyrocatechol possesses potential as a novel therapeutic agent against lung carcinogenesis, for example, in future clinical approaches. On the other hand pyrocatechol is metabolized to its quinone, which reacts with DNA to form depurinating adducts at the N-7 of guanine and N-3 of adenine. The catecholamine dopamine and the metabolite of pyrocatechol (1,2-dihydroxybenzene) of the leukemogen benzene can also be oxidized to their quinones, which react with DNA to form predominantly analogous depurinating adducts. Apurinic sites formed by depurinating adducts are converted into tumour-initiating mutations by error-prone repair. The fact that pyrocatechol behaves as an antioxidant and also carcinogenic is not contentious. Probably in high doses pyrocatechol behaves as a pro-oxidant, as many antioxidants do.

Date	Country	Organisation	Type of Organisation	Comment number
23.11.2015	Sweden		Member State	5
Comment received				
In the CLH report it is proposed that pyrocathecol should be classified as Carc. 2. However, we think that classification as Carc. 1B could be considered, since (i) six of the seven dedicated carcinogenicity studies available in rats are positive, altogether demonstrating that malign and benign tumours are induced in the glandular stomach in both males and females, (ii) the only one dedicated carcinogenicity study in mice is positive, demonstrating that benign tumours are induced in the glandular stomach, and (iii) pyrocathecol is mutagenic.				
Dossier Submitter's Response				
Thanks for your comment. Indeed, pyrocatechol induced malign and benign tumors in the glandular stomach in male rats, mainly. No malign tumors were observed in other species. As a consequence, a classification as carc. 2 for pyrocatechol is considered appropriate.				
RAC's response				
RAC appreciates the comment from the Swedish CA. For more detail see the response to comment 4.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2015	France	Rhodia Operations (Member of the Solvay Group)	Industry	6

Comment received
The REACH registrants support the proposed classification of catechol for mutagenicity category 2. This classification has already been implemented by the REACH registrants.
Dossier Submitter's Response
Thanks for your comment.
RAC's response
RAC appreciates the Industry position on classification of pyrocatechol for mutagenicity.

Date	Country	Organisation	Type of Organisation	Comment number
23.11.2015	Sweden		Member State	7
Comment received				
We agree with the proposal to classify pyrocathecol as Muta. 2. All studies on mutagenic effects in mammalian cells in vitro were positive (i.e. 3 gene-mutation studies, 3 chromosome-aberration studies, 2 micronucleus studies). Of the mutagenicity studies in vitro performed in bacteria (i.e. bacterial reverse mutation test), 2 were positive and 3 were negative. A number of in vivo studies are available, which are used to assess if the mutagenic potential observed in vitro can be expressed in vivo. Of the 2 in vivo micronucleus studies with the highest reliability (reliability 2), 1 was positive and 1 was negative. Of the 3 in vivo micronucleus studies with lower reliability (reliability 3), 2 were positive and 1 was negative. The available in vivo comet assay was positive in duodenum cells after oral administration. Overall, these results support that pyrocathecol has the potential to induce chromosome aberrations in vivo. It can be argued that the positive in vivo comet assay also supports that pyrocathecol has the potential to induce gene mutations in vivo, since the comet assay recognises DNA damage that could lead to gene mutations. A negative in vivo study on gene mutations is available (mouse spot test), but it is unclear if the single dose used was high enough to induce a detectable increase in gene mutations.				
Dossier Submitter's Response				
Thanks for your comment.				
RAC's response				
RAc appreciates the comment from the Swedish on the classification of pyrocatechol for mutagenicity.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2015	France	Rhodia Operations (Member of the Solvay Group)	Industry	8
Comment received				
The REACH registrants support the proposed classification of catechol for acute toxicity category 3 for oral and dermal. This classification has already been implemented by the REACH registrants.				
Dossier Submitter's Response				
Thanks for your comment.				

RAC's response
RAC appreciates Industry position on classification of pyrocatechol for acute toxicity, category 3, via the oral and dermal routes.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
23.11.2015	France	Rhodia Operations (Member of the Solvay Group)	Industry	9
Comment received				
In the REACH dossier of Catechol submitted in 2010 by the Lead Registrant Rhodia Operations, the classification « Eye Dam. Cat. 1 H318 » has been proposed instead of « Eye Irrit Cat. 2, H319 » (official classification). This proposal is based on the key study. Could you please consider this proposal during this Harmonised Classification and Labelling review of Catechol done by France.				
Dossier Submitter's Response				
Thanks for your comment. Based on the published key study of Flickinger, 1976, we agree that Eye Dam. Cat. 1 H318 would be relevant for 1,2-dihydroxybenzene. Indeed, in this study, irreversible effets in eyes were observed at the end of the observation period of 14 days in the 6 rabbits. Nevertheless, as no new data became available after the current harmonised classification "Eye Irrit cat. 2" was agreed, action on this endpoint was not considered justified.				
RAC's response				
RAC could not comment on an endpoint that was not part of the CLH dossier submitted by the DS.				