Committee for Risk Assessment

RAC

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

16 September 2016

CLH-O-0000001412-86-122/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonisedclassification and labelling (CLH) of:

Chemical name: **1,2-dihydroxybenzene; pyrocatechol**

EC Number: **204-427-5**

CAS Number: **120-80-9**

The proposal was submitted by **France** and received by RAC on **4 September 2015.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **7 October 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 November 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christina Tsitsimpikou**

Co-Rapporteur, appointed by RAC: **Nikolaos Spetseris**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2016** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	604-016-00-4	1,2-dihydroxybenzene; pyrocatechol	204-427-5	120-80-9	Acute Tox. 4* Acute Tox. 4* Skin Irrit. 2 Eye Irrit. 2	H302 H312 H315 H319	GHS07 Wng	H302 H312 H315 H319			
Dossier submitters proposal	604-016-00-4	1,2-dihydroxybenzene; pyrocatechol	204-427-5	120-80-9	**Retain** Skin Irrit. 2 Eye Irrit. 2 **Add** Muta. 2 Carc. 2 **Modify** Acute Tox. 3 Acute Tox. 3	**Retain** H315 H319 **Add** H341 H351 **Modify** H301 H311	**Retain** Wng **Add** GHS06 GHS08 **Remove** GHS07	**Retain** H315 H319 **Add** H341 H351 **Modify** H301 H311			
RAC opinion	604-016-00-4	1,2-dihydroxybenzene; pyrocatechol	204-427-5	120-80-9	**Retain** Skin Irrit. 2 Eye Irrit. 2 **Add** Muta. 2 Carc. 1B **Modify** Acute Tox. 3 Acute Tox. 3	**Retain** H315 H319 **Add** H341 H350 **Modify** H301 H311	**Modify** Dgr **Add** GHS06 GHS08 **Remove** GHS07	**Retain** H315 H319 **Add** H341 H350 **Modify** H301 H311			
Resulting Annex VI entry if agreed by COM	604-016-00-4	1,2-dihydroxybenzene; pyrocatechol	204-427-5	120-80-9	Carc. 1B Muta. 2 Acute Tox. 3 Acute Tox. 3 Skin Irrit. 2 Eye Irrit. 2	H350 H341 H311 H301 H315 H319	GHS06 GHS08 Dgr	H350 H341 H311 H301 H315 H319			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

1,2-Dihydroxybenzene (CAS number 120-80-9) or Pyrocatechol is a substance with anti-oxidant properties, which already has an entry in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation) and is currently classified as Acute Tox. 4* (H312), Acute Tox. 4* (H302), Eye Irrit. 2 (H319) and Skin Irrit. 2 (H315).

The current CLH proposal was based on the information available in the registration dossiers for pyrocatechol and on literature data.

No human data on pyrocatechol toxicity were included in the CLH dossier except for a study (Hirosawa, 1976) that reported exposure of 13 workers to vapours of pyrocatechol and phenol, with major reported side-effects being complaints related to the upper respiratory tract. Blood pressure and body temperature remained normal and no signs of hepatic or renal dysfunction were observed. These results were not considered by the DS and RAC to be relevant for classification.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral route

Two studies conducted in rats with reliability Klimisch score of 2 (Registrants' evaluation, DS agreed) were used for classification purposes by the Dossier Submitter (DS) (Flickinger, 1976; Lewis, 1996).

The oral $LD_{50}$for males from the Flickinger (1976) study was established at 300 mg/kg bw (95% confidence level, 200-500 mg/kg bw), while the Lewis (1996) study reported an LD_{50} of 358 mg/kg bw.

Therefore, based on the LD_{50} values obtained, the DS proposed that pyrocatechol be classified for acute toxicity in category 3 (H301: Toxic if swallowed) according to the classification criteria in help Regulation. Currently the harmonised classification in Annex VI is category 4*; H302: Harmful if swallowed.

Dermal route

Two studies were available: Study report nº 16948 (1973) and Flickinger (1976). They were both selected by the DS as key studies with reliability scores of 2.

In Study report nº 16948 (1973), an LD_{50} of 600mg/kg bw (male/female) was reported in rats. In Flickinger (1976) the effects observed after dermal administration in male rabbits were local and an LD_{50}= 800mg/kg bw (95% confidence interval: 500-1400) was reported.

Therefore, based on the LD_{50} values obtained, the DS proposed that pyrocatechol should be classified as Acute Tox.3 (H311: Toxic in contact with skin) according to classification criteria of the CLP Regulation. Currently the harmonised classification in Annex VI is category 4*; H312: Harmful in contact with skin.

Inhalation route

No data were available in the CLH report.

Comments received during public consultation

During public consultation (PC), two comments were received on the acute toxicity endpoint via oral and dermal exposure. One Member State Competent Authority (MSCA) supported the classification proposed by the DS. In the same context, all REACH registrants supported the proposed classification of pyrocatechol for acute toxicity category 3 for oral and dermal route. This classification has already been implemented by the REACH registrants in the registration dossier.

Assessment and comparison with the classification criteria

All studies summarised in the CLH report by the DS were from the registration dossier and the data were retrieved from ECHA dissemination website. The DS included in the CLH report only studies that were published in the literature, either in peer reviewed journals or in books.

Oral route

Four studies were available in the registration dossier but only two of them were included in the CLH report: Flickinger (1976) as the key study and Lewis (1996) as supportive evidence. Both studies are reported as having reliability scores of 2.

Based on the executive summary provided by the lead registrant in the registration dossier, in an acute oral toxicity study (Flickinger, 1976), groups of 5 albino male rats were given a single oral dose of pyrocatechol at doses of 0, 158, 316, 630 and 1260 mg/kg bw and observed for 14 days. The mortality incidences are summarized in the following Table:

Administered dose (mg/kg bw)	Mortality (number of deaths in total of 5 animals per dosage group)
158	0
316	2 (1 on day 1, 1 on day 2)
630	5 (all 5 in less than 1 day)
1260	5 (within 1 hour)

No clinical signs were reported and the autopsy on rats that died during the study revealed hyperaemia of the stomach and intestines. None of the rats that did not die but were sacrificed at the end of the experiment revealed gross pathology after pathological examination.

It is worth noting the time within which deaths were observed at each dose.

The oral LD_{50} for males was calculated by Flickinger (1976) to be 300mg/kg bw (95% confidence level, 200-500mg/kg bw). The author did not state the statistical test used. In addition, the purity of the test substance and the strain of rat used are not known. The administration volume and the use of vehicle were not specified. These deviations lower the reliability of this study to a higher Klimisch score. Probit statistical analysis of the Flickinger (1976) data in the above Table (Cambridge, England, Cambridge University Press, https://www.medcalc.org/manual/probitregression.php), revealed an LD_{50} = 287 mg/kg bw, with confidence levels at 95% = 100-473 mg/kg bw.

The Lewis (1996) study, which in the registration dossier, where no summary is present, has been assessed as reliable with restriction (Klimisch 2), is based on data from a 1972 publication. An LD_{50} of 358 mg/kg bw is reported, but the original data were not available in the CLH report. Furthermore, no test substance information was available, no reference was made to a guideline, sex & strain of the animals were not specified, all of which point to a low reliability study.

Due to lack of other evidence, RAC decided to accept the LD_{50} values reported in the literature.

Based on the Guidance on the Application of the CLP Criteria (version 4.1. 2015, CLP guidance, section 3.1.2.3.2), the lowest ATE value available is considered for classification. Therefore, according to Table 3.1.1 of Annex I of CLP,RAC agrees to the DS proposal to classify pyrocatechol as **Acute Tox 3, H301 (Toxic if swallowed), with an ATE value of 300 mg/kg bw**.

Dermal route

Two studies were available, both of with reliability scores (Klimisch) of 2: Study report n° 16948 (1973) and Flickinger (1976).

In an acute dermal toxicity study (Study n° 16948, 1973), CD male and female rats (5/sex/group) were exposed to pyrocatechol by the dermal route for a maximum of 24 hours, at doses of 125, 875 and 1125 mg/kg bw. Animals were then observed for 15 days. The results are summarized in the following table and an LD_{50} 600 mg/kg bw was reported, without any further information on the statistical method used:

Administered dose (mg/kg bw)	Mortality (number of deaths in total of 10 animals per dosage group)	Observations
125	0	No clinical signs
875	10	Tremors 5 minutes after dermal application. Death within 30 minutes after clonic convulsions.
1125	10	

In the second study (Flickinger, 1976), the skin was abraded and 4 rabbits per dose were used. Abrasion may alter skin permeability. Abraded and intact skin of each group of male albino rabbits (age unknown) was in contact with pyrocatechol for a maximum of 24 h. The number of deaths at each dose and times at which the deaths occurred were as follows:

Administered dose (mg/kg bw)	Mortality (number of deaths in total of 4 animals per dosage group)	Observations
250	0	
500	1	Death on day 2
1000	2	Death on day 2
2000	4	Death on day 1

All the rabbits that died during the observation period revealed subdermal hyperaemia and oedema. An LD_{50}= 800 mg/kg bw (95% C.I.: 500-1400) in male rabbits was reported in the manuscript by the author, without any further information on the statistical method used.

The RAC decided to accept the reported LD_{50} values in the literature.

Based on the CLP guidance (section 3.1.2.3.2), the lowest ATE value available is considered for classification. Therefore, according to Table 3.1.1 of Annex I of CLP, RAC agrees with the DS proposal to classify pyrocatechol for acute toxicity (dermal route), as Acute Tox. 3, **H311 (Toxic in contact with skin), with an ATE of 600 mg/kg bw**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

No human studies on possible mutagenic effects of pyrocatechol were available.

The DS, in order to evaluate the mutagenicity of pyrocatechol, selected *in vivo* and *in vitro* studies mainly from the Chemical Safety Report (CSR) of the registration dossier and also from the literature. A considerable number of papers (more than 65) were included in the registration dossier for pyrocatechol. Most of the studies were reported to be of reliability 2 (Klimisch) according to the CSR and only few studies were of reliability 3 (n=10) because of the lack of details about controls.

In vitro gene mutations assays on bacterial and mammalian cells (Study report n° FSR-IPL 060904-01, 2007; Martinez *et al.*, 2000; Tsutsui *et al.*, 1997; Mc Gregor *et al.*, 1988, etc.), *in vitro* mammalian chromosome aberration tests (Tsutsui *et al.*, 1997; Do Ceu *et al.*, 2003 etc.) and sister chromatid exchange assays (Tsutsui *et al.*, 1997; Morimoto 1983, etc.), along with various DNA damage tests (Fabiani *et al.*, 2001; Pellack-Walker *et al.*, 1985; Lee *et al.*, 1989, etc.) provided positive results, indicating mutagenic effects of pyrocatechol in the different *in vitro* models. Genotoxic effects of pyrocatechol on germinal cells have not been studied.

From the overall studies performed during *in vivo* experiments, 3/5 positive micronucleus studies (Marrazzini *et al.*, 1994; Ciranni *et al.*, 1988a; Ciranni *et al.*, 1988b) and a positive screening comet assay in duodenum cells (Study report n° 18255, 2008) suggested that pyrocatechol had potential genotoxic effects, which is consisted with the *in vitro* positive results summarized above.

Furthermore, there were no Absorption, Distribution, Metabolism & Excretion (ADME) data in the registration dossier showing availability of pyrocatechol in reproductive tissues or other evidence of effects of pyrocatechol on reproductive organs.

Based on all the above, the DS proposed to classify pyrocatechol as a germ cell mutagen in Category 2 (H341: Suspected of causing genetic defects).

Comments received during public consultation

During PC, two comments from MSCAs were received, both supporting classification of pyrocatechol as Muta. 2. In addition, comments from industry, including all REACH registrants, supported the proposed classification.

Assessment and comparison with the classification criteria

Classification of pyrocatechol was based on the *in vivo* data with supporting evidence from *in vitro* data.

In vivo studies

In total10 studies are discussed as follows:

Species	Method	Administration	Target organ/ tissue/ cell	Result	Reference
Mouse spot test					
Mouse embryos	Equivalent or similar to OECD TG 484 (reliability 2)	Intraperitoneal injection 22 mg/kg bw on days 9, 10, 11	Developing embryos' melanoblasts	Negative	Fahrig, 1984
DNA damage assays					
Sprague-Dawley male rat	Equivalent or similar to OECD TG 489 (*in vivo* alkaline Comet assay), GLP study (reliability 2)	Oral gavage 100, 200, 400 mg/kg bw /day	Duodenum cells	**Positive**	Study report n° 18255, 2008
Rat male (344/DuCrj)	Equivalent or similar to OECD TG 486 (unscheduled DNA synthesis or UDS test with Mammalian Liver Cells *in vivo*), non GLP study (reliability 2)	Oral gavage Single dose 0, 10, 20, 37.5, 75, 90 mg/ kg bw for 2, 12, 24 hours	Pyloric mucosa of stomach	Negative	Furihata *et al.*, 1989
Rat Wistar male	Not performed according to standard guideline (DNA damage/ repair, unscheduled	Oral 1, 2, 4, 8 g/L/day	Esophageal epithelial cells	**Positive**	Mirvish *et al.*, 1985

	DNA synthesis, injection of tritium-labelled thymidine), non GLP study (reliability 2)				
Mouse NMRI male	Not performed according to standard guideline (DNA damage/ repair, *E. Coli* K-12 DNA repair host-mediated test), non GLP study (reliability 3)	Oral Single dose: 200 mg/ kg bw	Blood, liver, lungs, kidneys, testes	Negative	Hellmer & Bolcsfoldi, 1992
Micronucleus assay					
Mouse CD-1 male	Equivalent or similar to OECD TG 474 (reliability 2)	Intraperitoneal Single dose 10, 20, 30 mg/kg bw	Bone marrow cells	**Positive**	Marrazzini *et al.*, 1994
Mouse CD-1 male	Equivalent or similar to OECD TG 474 (reliability 2)	Oral gavage Single dose 150 mg/kg (high dose led to convulsive seizures)	Polychromatic erythrocytes	Negative	Gad-El-Karim *et al.*, 1985
Mouse NMRI male	Equivalent or similar to OECD TG 474 (reliability 3)	Subcutaneous injection for 6 days (1 per day) 5-42 mg/kg bw	Polychromatic erythrocytes	Negative	Tunek *et al.*, 1982
Mouse CD-1 pregnant female	Equivalent or similar to OECD TG 474 (reliability 3)	Oral (gastric intubation) 40 mg/kg bw	Polychromatic erythrocytes, foetal liver	**Positive**	Ciranni *et al.*, 1988a
Mouse CD-1 male	Equivalent or similar to OECD TG 474 (reliability 3)	Oral and intraperitoneal 40 mg/kg bw	Polychromatic erythrocytes	**Positive**	Ciranni *et al.*, 1988b

Positive results were observed in two species (rat, mouse) and in both sexes in the mouse, both after oral and intraperitoneal administration. Mutations were only assessed in the mouse, while the positive results in rats where in genotoxicity studies.

Overall, positive results were reported from 3/5 micronucleus studies. Furthermore, a positive screening comet assay in duodenum cells suggested a potential genotoxic effect of pyrocatechol. These results were supported by the observed enhanced uptake of tritium-labelled thymidine into the DNA, indicating unscheduled DNA synthesis and altered DNA damage/repair, which was reported in the Mirvish *et al.* (1985) study on oesophageal cancer.

Results collected from *in vivo* experiments revealed that pyrocatechol is able to induce the production of single strand breaks (DNA damage) in cells of the duodenum and oesophageal epithelial cells of rodents after oral treatment (Study report n° 18255, 2008; Mirvish *et al.*, 1985).

Pyrocatechol induced micronucleus formation in a dose-dependent manner after oral and intraperitoneal administration (Marrazzini *et al.*, 1994; Ciranni *et al.*, 1988a, 1988b).

A significant increase in micronuclei in the PCE was measured on male and female mice exposed to 40 mg/kg bw of pyrocatechol after 24 hours by the oral route (Ciranni *et al.*, 1988a, 1988b). This study was of (Klimisch) reliability 3 because it was performed without any positive control. Nevertheless, positive controls are less important in a positive study. A significant induction of micronuclei was also measured 18h after mice were exposed to 10-30 mg/kg bw pyrocatechol intraperitoneally (Marrazini *et al.*, 1994).

In vitro studies

Mammalian cells

In order to evaluate the mutagenic properties of pyrocatechol, 9 tests exploring the lactogenic effects on mammalian cells were examined, all providing positive results (chromatid breaks, chromatid exchange and micronucleus production) without metabolic activation: 1 micronucleus test (Yager *et al.*, 1990), 4 sister chromatid exchange assays equivalent or similar to OECD TG

479 (Tsutsui *et al.*, 1997, Erexson *et al.*, 1985, Morimoto & Wolff 1980, Morimoto 1983) and4 chromosomal aberration assays equivalent or similar to the OECD TG 473 (Tsutsui *et al.*, 1997, Stich *et al.*, 1981, Do Cey *et al.*, 2003, Study report n° FSR-IPL 060904-01, 2007). The majority of the studies (78%) were of (Klimisch) reliability 2.

The following cell lines were used:

Cell line	**Source**
Human lymphocytes	human
Human T-lymphocytes	human
Syrian Hamster Embryo (SHE) Cells	rodent
Chinese Hamster Ovary (CHO)	rodent
Chinese hamster lung fibroblasts (V79 cells)	rodent
Mouse lymphoma L5178Y cells	rodent

In the micronucleus test, human lymphocytes were treated with a range of catechol concentrations (from 0.5 to 250 µM) without a metabolic activation system (Yager *et al.*, 1990). Statistically significant increases in micronucleated cells were observed starting from 0.5 µM and a decrease in cell viability was measured starting from 100 µM. A significant concentration related increase in kinetochore-positive micronucleated cells was apparent, suggesting that catechol was a likely aneuploidy-inducing agents in human lymphocytes.

Pyrocatechol was tested in the concentration range of 0-1000 µg/mL in the sister chromatid exchange (SCE) assays. The lowest dose causing a significant increase of SCE was 5 µg/mL, while cytotoxicity expressed as inhibition of growth was observed even at 10 µg/mL.

In the chromosomal aberration assays pyrocatechol was tested at doses of 0.11 µg/mL to 156.25 µg/mL. Significant increases in aberrant metaphases starting from 0.33 µg/mL and slight but significant induction of aneuploidy in the near-diploid range at 3.3 µg/mL were observed, hence this concentration was considered the lowest effective dose (Tsutsui *et al.*, 1997). Inhibition of growth was noted at 1.1 µg/mL (Tsutsui *et al.*, 1997). Furthermore, results showed that the clastogenic effect of catechol was pH dependent (Do Ceu *et al.*, 2003), while a clear dose-response relationship was observed in the Study report n° FSR-IPL 060904-01(2007).

Three gene mutation studies of pyrocatechol on SHE cells and L5178Y mouse lymphoma cells, conducted similarly or equivalently to the OECD TG 473 (Tsutsui *et al.*, 1997, Mc Gregor *et al.*, 1988; Wangenheim & Bolcsfoldi, 1988), demonstrated the mutagenic activity of the substance. More specifically, pyrocatechol induced gene mutations at the two loci in SHE cells without metabolic activation, while an increase in the mutation frequency (but in a non-dose dependent manner) in the L5178Y mouse lymphoma cells was also reported.

Supportive data on DNA damage in mammalian cells, including single/double strand break DNA, alkali-labile sites, unscheduled DNA synthesis, inhibition of DNA synthesis or inhibition of the DNA repair system and oxidative base damage and apoptosis were also available. The majority of the tests were not performed according to a standard guideline. Both human cell lines (human peripheral blood mononuclear cells – PBMCs, Fabiani, 2001; human leukemic cell line HL-60, Oikawa *et al.*, 2001) and rodent cell lines were used (mouse lymphoma L5178YS, Pellack-Walker *et al.*, 1985; rat hepatocytes, Solveig Walles, 1992; mouse bone marrow cells, Lee *et al.*, 1989).

Bacteria

Of the *in vitro* gene mutation studies performed in bacteria (i.e. bacterial reverse mutation tests similar to OECD TG 471), 2 were positive (Study report n° FSR-IPL 060904-01, 2007; Martinez *et al.*, 2000). In a screening micro method assay of the Ames test performed without repetition, positive results were observed with *Salmonella typhimurium* TA 102 without S9-mix and with kidney S9-mix, but not with liver S9-mix (Study report n° FSR-IPL 060904-01, 2007). The

positive response with strain TA 102 was probably due to a substitution of AT to GC by an oxidative mechanism. Positive results were also obtained with *Escherichia coli* WP2 uvrA/pKM101 strain IC203 without S9, but not with S9 (Martinez, 2000). Strain IC203, deficient in OxyR (its oxyR+ parent is WP2 *uvr*Ar/pKM101 denoted IC188, which is the common strain used in the guideline Ames study), is more sensitive to mutation induced by oxidative damage. In this study, a negative response was observed with WP2 uvrA/pKM101 strain IC188 (with and without S9-mix).

Mutagenic and cytotoxic effects maybe induced by independent chemical species with probably superoxide anion-mediated mutagenicity. Only one study on bacteria (TA102) showed mutagenicity of pyrocatechol suggesting oxidative properties (Study report n° FSR-IPL 060904-01, 2007). The genotoxic effect of pyrocatechol seems to be dose-dependent and linked to its specific oxidative properties. It had not been clearly demonstrated whether or not this genotoxic effect had a threshold.

In conclusion, there are no human data in the literature, and based on the animal data available, there is no concrete evidence that pyrocatechol is mutagenic to germ cells or that it distributes to the reproductive tissues. It could be argued, that the positive *in vivo* comet assay could support the hypothesis that pyrocathecol has the potential to induce gene mutations *in vivo*, since the comet assay recognises DNA damage that could lead to gene mutations. But the lack of relevant data to assess mutagenicity of germ cells prevails. Therefore, the criteria for classify a substance as a germ cell mutagen in Category 1B according to table 3.5.1 of Annex I of CLP and § 3.5.2.4 of the CLP Guidance, are **not met**.

All *in vitro* studies on mutagenic effects in mammalian cells were positive along with 2 mutagenicity studies performed in bacteria. A number of *in vivo* studies confirmed that the mutagenic potential observed *in vitro* can also be expressed *in vivo*. Three *in vivo* micronucleus studies were positive (one of reliability 2, two of reliability 3). 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*.

On this basis, according to the classification criteria of the CLP Regulation summarized in table 3.5.1 of Annex I of CLP, RAC concludes that pyrocatechol should be classified as **Muta. 2; H341 (Suspected of causing genetic defects)**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Twenty nine studies of (Klimisch) reliability 2 were assessed by the DS. These studies were dedicated carcinogenicity studies (8) or tumour promotion studies (20) with pyrocatechol. RAC noted that one study (Kampa *et al.*, 2000) reported on the inhibition by pyrocatechol of the proliferation of 3 prostate cancer cell lines (LNCaP, PC3, DUI45). Three species were studied: rat, mouse and hamster. It was clearly demonstrated that the rat was the most sensitive species.

The DS stated that all the carcinogenicity and tumour promotion studies demonstrated the carcinogenic effect of pyrocatechol on the glandular stomach of rats with formation of adenomas and in some cases adenocarcinomas (Hagiwara *et al.*, 2001; Hirose *et al.*, 1993a; Hirose *et al.*, 1990; Hirose *et al.*, 1992; Hirose *et al.*, 1987; Tanaka *et al.*, 1995; Wada *et al.*, 1998; Kawabe *et al.*, 1994). However, RAC points out that there is one exception in the Hasegawa *et al.* (1990) study, where, after pre-treatment with DHPN, non-significant mucosal and adenomatous hyperplasia in the pyloric region was observed. It is also important to note that, according to the DS, effects appeared at doses of 0.4% and mainly of 0.8%in the diet. RAC, on the other hand, noticed that in the Hirose *et al.* (1991) study, adenomas (60% vs 0% in the basal diet) were

observed in the glandular stomach of the Fischer 344 male rat at 0.2% (ca 120 mg/kg bw/d).At the lowest doses tested, submucosal hyperplasia was observed at the site of administration (stomach) after repeated administration suggesting a dose-related progression to the carcinomas and adenocarcinomas seen in animals given the high dose. In addition, RAC noted that in two mouse studies, adenomas in both sexes (B6C3F1, Hirose *et al.*, 1900; Hirose *et al.*, 1993) and both adenomas and adenocarcinomas in male Balb/c mice were reported (Kobayashi *et al.*, 1997).

After initiation, tumours were also found in the forestomach. Understanding the mode-of-action leading to forestomach tumours to be either a genotoxic (or mutagenic) or non genotoxic (not promutagenic) mechanism is an important consideration for assessing the relevance of forestomach tumours in animals to humans. Tumorigenesis of the forestomach squamous epithelium generally appears to be a continuum, progressing from hyperplasia and dysplasia to benign tumours and eventually to malignancy. For some chemicals (e.g., dichlorvos) where comparative data exist, the dependence of forestomach tumour development on administration by gavage, as opposed to exposure from food or drinking water, strongly suggests that the local concentration at the forestomach mucosa is more important than the total systemic dose on a mg/kg bw basis. Various toxicodynamic factors may influence the development of forestomach tumours. Cytotoxicity and regenerative cell proliferation in the epithelium are involved in the development of forestomach tumours by many orally administered carcinogens. In the case of carcinogens that act through a genotoxic mechanism, cell proliferation may make an important contribution to tumour development. For some carcinogens not known to be genotoxic in the forestomach, irritation leading to enhanced and sustained cell proliferation may be essential for tumour development (IARC, 1999).

Co-carcinogenicity (tumour promotion) studies confirmed the carcinogenic effect of pyrocatechol on glandular stomach of rats, and indicated that pyrocatechol could inhibit the carcinogenic effect of some substances on specific organs, like the effect of BOP (N-nitroso-bis(2-oxopropyl)amine) on the pancreas of the hamster (Maruyama *et al.*,1991). According to the available data, pyrocatechol did not exert a carcinogenic effect on organs other than the site of application (contact) after oral administration: oesophagus and stomach (glandular andforestomach) of the rat. Nevertheless, RAC noted that in the Hagiwara *et al.* (2001) study, acinar cell adenomas in the pancreas of male Fischer 344 rats at a dose 0.8% pyrocatechol were reported.

The DS argued that the specific carcinogenic effect of pyrocatechol on rat glandular stomach after oral administration at high doses was the result of its progressive aggressive action of the mucous membrane by formation of inflammation, apoptosis, erosion and ulceration and then cell proliferation, hyperplasia, responsible after long term exposure to formation of adenoma and carcinoma. A contribution of the genotoxic properties of pyrocatechol cannot be excluded. As hyperplasia is a pre-neoplastic lesion observed in most cases, cell proliferation appears as a determinant factor in the induction of cancer in rodents by pyrocatechol. Irritating and genotoxic properties of pyrocatechol could also contribute to its ability to generate tumours in rodents.

Pyrocatechol may play a role in human gastric cancer development. IARC (1999) have concluded that pyrocatechol is possibly carcinogenic to humans (Group 2B).

The DS, therefore, proposed that, according to results from all carcinogen studies showing induction of tumours in one organ in one species and IARC classification, pyrocatechol should be classified as carcinogenic in category 2 (suspected human carcinogen).

Comments received during public consultation

During public consultation (PC), two comments from MSCAs were received. One MSCA supported classification of pyrocatechol as carcinogen, category 2.

On the other hand, the other MSCA argued that classification as carcinogen, category 1B should be considered. They pointed out that tumorigenicity (benign and malign tumours) is observed in two species: more specifically,6 out of 7 dedicated carcinogenicity studies available in rats were positive, and the single dedicated carcinogenicity study in mice was positive. Malignant and benign tumours were observed in the glandular stomach of both sexes in rats. Benign tumours were observed in both sexes in the glandular stomach of both sexes in mice. Furthermore, pyrocatechol was shown to be mutagenic.

On a totally different line of argumentation, Industry believed that the classification of pyrocatechol for carcinogenicity represented a borderline case. Some data could warrant a carcinogen category 2 classification, while other elements of the available data indicated that a classification for carcinogenicity is not needed. Furthermore the exposure route (oral) is not relevant for human exposure. Finally there were also indications that pyrocatechol could reduce the incidence of cancer, which might be due to the antioxidant effect of catechol.

More specifically, the Industry representative pointed out that:

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- The dose of 0.8% of pyrocatechol in the diet, which resulted in a significant increase in adenocarcinomas in the glandular stomach of rats for both sexes, is considered to be high enough to cause a decrease in body weight and an increase in liver weight. No malignant tumours were reported at lower doses.
- At lower doses, though, submucosal hyperplasia, ulceration and adenomas of the glandular stomach of rats were found. This shows that pyrocatechol 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.
- In mice (B6C3F1) at a dietary dose level of 0.8%, 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.
- In studies on Syrian hamsters, no carcinomas of the glandular stomach were found but the study duration was only 30 or 20 weeks.
- The carcinogenicity studies with rodents have only been performed using an oral route of exposure. However, this route is not relevant for humans, and extrapolation from the oral route to the inhalation route is normally not possible for local effects. Therefore, it is questionable if the carcinogenicity data are relevant for humans.
- Based on the information from an Industrial regulatory database, the national occupational exposure limit (time weighted average) of pyrocatechol 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. The DNEL in the REACH registration dossier is calculated at 1 mg/m3 for long term inhalation exposure of workers. It could be useful to compare the worker occupational exposure concentration against the oral dose level of 0.8% in the carcinogenicity studies, which resulted in adenocarcinomas of the glandular stomach. The equivalent daily oral dose of workers exposed at 50% of the DNEL level (0.5 mg/m3), would be of about 0.06 mg/kg bw, about 8000 times lower than the daily dose in rats (480 mg/kg bw). This shows that the dose level which results in adenocarcinomas for rats is much higher than the potential worker exposure level.
- In a tumour promotion study in rats reported by Hasagawa et al.(1990,) co-treatment with DHPN (N-bis(2-hydroxypropyl)nitrosamine) and pyrocatechol seemed to decrease slightly the incidence of carcinogenic effects (thyroid and lung) observed with DHPN alone.

- In a tumour promotion study of Maruyama et al. (1991) with hamsters, the numbers of atypical pancreatic hyperplasias and adenocarcinomas were significantly decreased if the animals were co-exposed to BOP and pyrocatechol, when compared to BOP alone.
- Maruyama et al. (1994) reported a similar protective effect of pyrocatechol in hamsters treated with BHP (N-nitrosobis-(2-hydroxypropyl)amine). The decrease in the carcinogenic effect of nitrosamines due to exposure to catechol might be due to the antioxidant effect of catechol.

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 for classifying pyrocatechol as a category 2 carcinogen. On the other hand, the Industry representative argued that the adenocarcinomas of the glandular stomach have been found only in one species (rats but not in mice or hamsters) in one organ (glandular stomach) and only using the very high dietary dose level of 0.8%. Additionally, the adenocarcinomas are due to local effects and there is a clear threshold because doses lower than 0.8% do not show adenocarcinomas of the glandular stomach.

Assessment and comparison with the classification criteria

In the CLH dossier, twenty eight studies with pyrocatechol of reliability 2 (Klimisch) were presented; eight were carcinogenicity studies and twenty were tumour promotion studies. Four of the carcinogenicity studies were conducted according to the equivalent or similar OECD TG 451.

In all studies the oral administration route (via diet) was applied and gavage was not used.

Six strains of rat (Fischer 344/DuCrj, Fischer 344, Wistar, Wistar Kyoto, WKY, Sprague Dawley, Lewis), two strains of mouse (Balb/c, B6C3F1) and Syrian golden hamster were studied. Only two studies, one with B6C3F1 mouse and one with the Fischer 344 rat, tested both sexes.

Among the organs studied (forestomach, glandular stomach, liver, lymph nodes, pancreas, kidney, thyroid, nasal cavity, lung, tongue, oesophagus, urinary bladder, intestine) the forestomach, the glandular stomach, the pancreas and the oesophagus were proven prone to tumorigenesis (malignant and/or benign tumours, namely adenomas, acinar adenomas and adenocarcinomas). Some histopathological findings were observed in the liver and the lymph nodes. Tumours on the forestomach were not discussed by RAC, although they could be relevant to humans, since they are observed after oral administration (not gavage) of a non-corrosive mutagenic substance (CLP Guidance 1 – June 2015, p. 375). Nevertheless, pyrocatechol is irritating (to both the skin and eyes, with existing classifications for these hazards in Annex VI of CLP). According to the DS, a meta-analysis of forestomach carcinogens has shown that a majority of them (84% of the 120 evaluated carcinogens) also induced tumours at other sites, while only 19 chemicals (16%) induced tumours exclusively in the forestomach (Proctor *et al.*, Toxicology Science, 98(2):313-26, 2007).

RAC notes that in all the studies presented in the CLH dossier, no evidence of tumorigenesis was observed in the control group.

In the carcinogenicity studies, the dietary pyrocatechol doses were between 0-1% (0- 600 mg/kg bw/d in rats, ca 960 mg/kg bw/d in mice), while in the tumour promotion studies, a fixed dose of 0.8% (480 mg/kg bw/d) was applied in the majority of experiments. A dose of 0.2% in the rat diet was also administered, along with a dose range between 0.48-960 mg/kg bw/d in two Balb/c male mouse studies with N-methyl-N-nitrosourea (MNU) pre-treatment. In the Syrian golden hamster studies, doses were up to 1.5% in the diet (1800 mg/kg bw/d).The duration of exposure varied from 7 days to 104 weeks. Significant increase in the labelling index and the apoptotic index was noticed as early as 7 days after start of exposure (Hirose *et al.*, 1999).

Adenomas were noticed after 24 weeks of exposure, while adenocarcinomas were seen in rats at 52 weeks of exposure (Kawabe *et al.*, 1994) and in mice at 96 weeks of exposure (Hirose *et al.*, 1990; Hirose *et al.*, 1993a).

RAC considered 4 of the carcinogenicity studies, all performed using a methodology consistent with OECD TG 451 as the key studies. From the other 4 carcinogenicity studies, Hirose *et al.*(1997) tested only one low dose (0.16%, ca 19 mg/kg bw/d), the Hirose *et al.*(1992) and Hirose *et al.*(1999) studies applied a similar protocol and provided similar results with the Hirose *et al.*(1993a) and the Hirose *et al.*(1990) studies, while the Hagiwara *et al.*(1996) study focused only on the liver at a dose of 0.8% (ca 480 mg/kg bw/d). In addition, 3 tumour promotion studies were discussed, in which the effects on the pyrocatechol group without pre-treatment were investigated. A further 3 tumour promotion studies were also considered, where pyrocatechol after pre-treatment with methyl-N-amylnitrosamine (MNAN), $NaNO_2$ and MNU increased the incidence of benign and malignant tumours in the oesophagus of rats and the glandular stomach of mice, respectively.

In the following table the studies used by RAC for classification purposes are summarized:

Study	**Species**	**Dosage range and duration**	**Sex**	**Findings**				
				Target organ	**Dose**	**Benign tumours**	**Malign tumours**	**Other**
Carcinogenicity studies								
Hagiwara *et al.*, 2001	Fischer 344/DuCrj rats	0, 0.1, 0.2, 0.4, 0.8% (0, 33, 65, 141, 318 mg/kg bw/d) 104 weeks	Male	Glandular stomach	0.1%			Significant submucosal hyperplasia and ulceration, significant squamous cell hyperplasia in the forestomach, no papillomas or carcinomas in the forestomach
					0.2%	Adenomas 23/25 rats		
					0.4%	Adenomas 25/25 rats	Adenocarcinomas 1/25 (*NS*)	
					0.8%	Adenomas 25/25 rats	Adenocarcinomas 2/25 (*NS*)	
				Pancreas	0.2%	Acinar cell adenomas 1/25 (NS)		
					0.4%	Acinar cell adenomas 1/25 (NS)		
					0.8%	Acinar cell adenomas 6/25		
Hirose *et al.*, 1993a; Hirose *et al.*, 1990	Fischer 344 rats	0.8% (480 mg/kg bw/d) 104 weeks	Male	Glandular stomach	0.8%	Adenomas 30/30 rats	Adenocarcinomas 16/30 rats	Significant submucosal hyperplasia, significant hyperplasia of the forestomach in both sexes
				Pancreas		Acinar cell adenomas 1/29 (NS)		
			Female	Glandular stomach	0.8%	Adenomas 30/30 rats	Adenocarcinomas 13/30 rats	
Hirose *et al.*, 1993a; Hirose *et al.*, 1990	B6C3F1 Mice	0.8% (960 mg/kg bw/d) 96 weeks	Male	Glandular stomach	0.8%	Adenomas 29/30 mice		Significant submucosal hyperplasia, significant hyperplasia of the forestomach in both sexes
			Female	Glandular stomach	0.8%	Adenomas 22/30 mice		
	Wistar rats		Male		0.8%	Adenomas 29/30 rats	Adenocarcinomas 20/30 rats	Significant submucosal

Tanaka *et al.*, 1995	WKY Wistar rats	0.8% (nominal in diet) 104 weeks		Glandular stomach		Adenomas 30/30 rats	Adenocarcinomas 3/30 rats (*NS*)	hyperplasia 30/30 rats Erosion and ulcer 13-24 rats Significant hyperplasia of the forestomach
	Lewis rats					Adenomas 29/30 rats	Adenocarcinomas 22/30 rats	
	Sprague Dawley rats					Adenomas 30/30 rats	Adenocarcinomas 23/30 rats	
Tumour promotion studies								
Wada *et al.*, 1998	Fischer 344 rats	0.8% (ca 480 mg/kg bw/d) (nominal in diet) 52 weeks	Male	Glandular stomach	0.8%	Adenomas 15/15 rats	Adenocarcinomas 1/15 rats	Significant submucosal hyperplasia Mild to moderate significant hyperplasia in the forestomach
Kawabe *et al.*, 1994	Fischer 344 rats	0.8% (ca 480 mg/kg bw/d) (nominal in diet) 52 weeks	Male	Glandular stomach	0.8%	Adenomas 15/15 rats	Adenocarcinomas 5/15 rats	
Hirose *et al.*, 1991	Fischer 344 rats	0.2% (ca 120 mg/kg bw/ day) (nominal in diet) 36 weeks	Male	Glandular stomach	0.2%	Adenomas 9/15 rats	No adenocarcinomas	Significant submucosal hyperplasia, no significant effect on the findings of the forestomach
Yamaguchi *et al.*, 1989	Fischer 344 rats	0.8% (ca 480 mg/kg bw/d) (nominal in diet) 52 weeks	Male	Oesophagus	MNAN (25 mg/kg bw) + pyrocatechol (ca 480 mg/kg bw/d)		Squamous cell carcinomas 64.3%	
Hirose *et al.*, 1993b; Hirose *et al.*, 1990	Fischer 344 rats	0.8% (ca 480 mg/kg bw/d) (nominal in diet) 28 weeks	Male	Oesophagus	0.8% pyrocatechol	Papillomas 3/15 rats		
					NaNO2 (0.3%) + pyrocatechol (ca 480 mg/kg bw/d)	Papillomas 7/15 rats		
Kobayashi *et al.*, 1997	Balb/c mice	0.05, 0.2, 0.8% 20, 35 weeks	Male	Glandular stomach	MNU (120 ppm) + pyrocatechol 0.05%	Adenomas 6/19 mice	Adenocarcinomas 3/19 mice	Pyrocatechol strongly enhanced pre-neoplastic and neoplasticlesions
					MNU (120 ppm) + pyrocatechol 0.2%	Adenomas 7/19 mice	Adenocarcinomas 3/19 mice	
					MNU (120 ppm) + pyrocatechol 0.8%	Adenomas 4/20 mice	Adenocarcinomas 14/20 mice	

As shown in the table above, survival of rodents was not affected by pyrocatechol exposure. The DS argued that a significant lower body weights (from -10% to -41% at the end of most of the experiments relative to control) was noticed at 0.8% pyrocatechol. RAC notes that the 41% decrease in body weight refers to female mice in the Hirose study (Hirose *et al.*, 1993a), where the incidence of adenocarcinomas in females was found to be 43%. The average reduction in body weight observed at 0.8% 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 decrease in body weight was 13% and 7%, respectively. No adverse effects on survival rates were observed. A slight reduction in food consumption was also observed ranging from essentially no difference relative to the control group to 15.3% in 5 studies (Hagiwara *et al.*, 2001; Hirose *et al.*, 1990; Kawabe *et al.*, 1994; Hirose *et al.*, 1993b; Wada *et al.*, 1998) which is as expected, since the affected organ is the stomach. These results do not support the DS suggestion that tumours may have been induced at a dose higher than the Maximum Tolerated Dose (MTD).

Data collected from all these studies on carcinogenic and co-carcinogenic effects of pyrocatechol on rodents were consistent.

Two species, rats (several strains) and mice (B6C3F1 and Balb/c), 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% (0.8% in the majority of cases) 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.

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 glandular stomach of rats treated with 0.8% of catechol from 12 to 96 weeks increased in a time-dependent manner. After cessation of pyrocatechol 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 tumorigenesis were also found: the pancreas (acinar cell adenomas that are difficult to differentiate from adenocarcinomas) (http://www.eurotoxpath.org/nomenclature/index.php) and oesophagus. Neoplastic lesions (papillomas, hyperplasia) were found in the tongue, oesophagus and lungs in tumour promotion studies (Hirose *et al.*, 1993b, 1990 and 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 an aqueous environment (pH around or above neutrality) pyrocatechol undergoes Cu^{2+}-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-benzoquinonemay 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 in specific methylation of CCGG sites of the Pg1 gene was noted in the pyloric mucosa. The alteration of methylation of the Pg1gene is considered to be an early event 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 rats' 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 N° 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 to be elevated at a dose of 0.1% w/w (*NS*) and from 0.2% w/w the increase in gastrin levels reached even 50% both at 34 and 104 weeks, with a clear dose-response relationship and a correlation with the proliferative lesions of pyloric gland.

The gastrin hypothesis may be outlined as follows:

(1) Inhibition of gastric acid secretion leads to elevated antral pH and, secondarily, to 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 Enterochromaffin-like (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 applies, the possibility that pyrocatechol may exert its carcinogenic effect by its irritating properties, also a non genotoxic 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 provide further support 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 after34 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 a 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, the consideration to downgrade a Category 1 to a 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 CLP Regulation, since pyrocatechol can induce benign and malignant tumours in two species in both sexes (mainly) in the glandular stomach, RAC considers that pyrocatechol should be classified as **Carc. 1B; H350 (May cause cancer).**

Additional references

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion.The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by theDossier Submitter and RAC (excluding confidential information).