CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Pyrocatechol

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

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

Index Number: **604-016-00-4**

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CONTENTS

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***1,2-dihydroxybenzene; pyrocatechol***
EC number:	***204-427-5***
CAS number:	***120-80-9***
Annex VI Index number:	***604-016-00-4***
Degree of purity:	***≥ 98.5 %***
Impurities:	***Confidential – no information on the impurities is provided in the technical dossier. The substance is classified with a min purity of 98.5% and its unknown impurities.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute toxicity - oral: Acute Tox. 4* - H302 Acute toxicity – dermal: Acute Tox. 4* - H312 Skin corrosion / irritation: Skin Irrit. 2 - H315 Serious damage / eye irritation: Eye Irrit. 2 - H319
Current proposal for consideration by RAC	Acute toxicity - oral: Acute Tox. 3 - H301 Acute toxicity – dermal: Acute Tox. 3 - H311 Mutagen 2 - H341 Carcinogen 2 - H351
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute toxicity - oral: Acute Tox. 3 - H301 Acute toxicity – dermal: Acute Tox. 3 - H311 Skin corrosion / irritation: Skin Irrit. 2 - H315

	Serious damage / eye irritation: Eye Irrit. 2 - H319 Mutagen 2 - H341 Carcinogen 2 - H351

1.3 Proposed harmonised classification and labelling based on CLP Regulation criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none	/	none	Conclusive but not sufficient for classification
2.2.	Flammable gases	none	/	none	Not adequate
2.3.	Flammable aerosols	none	/	none	Not adequate
2.4.	Oxidising gases	none	/	none	Not adequate
2.5.	Gases under pressure	none	/	none	Not adequate
2.6.	Flammable liquids	none	/	none	Not adequate
2.7.	Flammable solids	none	/	none	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	none	/	none	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none	/	none	Not adequate
2.10.	Pyrophoric solids	none	/	none	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	none	/	none	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none	/	none	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	none	/	none	Not adequate
2.14.	Oxidising solids	none	/	none	Conclusive but not sufficient for classification
2.15.	Organic peroxides	none	/	none	Not adequate

2.16.	Substance and mixtures corrosive to metals	none	/	none	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 3 - H301		Acute Tox. 4* - H302	
	Acute toxicity - dermal	Acute Tox. 3 - H311		Acute Tox. 4* - H312	
	Acute toxicity - inhalation				Not evaluated
3.2.	Skin corrosion / irritation			Skin Irrit. 2 - H315	Not evaluated
3.3.	Serious eye damage / eye irritation			Eye Irrit. 2 - H319	Not evaluated
3.4.	Respiratory sensitisation				Not evaluated
3.4.	Skin sensitisation				Not evaluated
3.5.	Germ cell mutagenicity	Mutagen 2 - H341			
3.6.	Carcinogenicity	Carcinogen 2 - H351			
3.7.	Reproductive toxicity				Not evaluated
3.8.	Specific target organ toxicity –single exposure				Not evaluated
3.9.	Specific target organ toxicity – repeated exposure				Not evaluated
3.10.	Aspiration hazard				Not evaluated
4.1.	Hazardous to the aquatic environment				Not evaluated
5.1.	Hazardous to the ozone layer				Not evaluated

1) Including specific concentration limits (SCLs) and M-factors
2) Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:
Signal word: Danger
Hazard pictogram: GHS06, GHS07, GHS08

Hazard statements:
H301: Toxic if swallowed.
H311: Toxic in contact with skin.
H315: Causes skin irritation.
H319: Causes serious eye irritation
H341: Suspected of causing genetic defects
H351: Suspected of causing cancer
Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Pyrocatechol is a chemical substance that has previously been assessed for harmonized classification and labelling. Pyrocatechol was included in directive 91/325/EEC (ATP12). Pyrocatechol is currently classified as Acute Tox. 4 (*) (H312), Acute Tox. 4 (*) (H302), Eye Irrit. 2 (H319) and Skin Irrit. 2 (H315) according to Annex VI of CLP regulation.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal is based on the information as available in the registration dossiers of pyrocatechol and on literature search. Pyrocatechol has shown genotoxicity and carcinogenicity in available animal experiments.

Most of *in vitro* studies available indicated a genotoxic effect of pyrocatechol on different animals and human somatic cells lines studied. However, *in vivo* experiment showed contradictory results about the potential genotoxicity of pyrocatechol. Clear evidences of genotoxicity on rat (Study report n°18255 2008; Mirvish et al. 1985) and clastogenicity on mice (Marrazzini et al. 1994) have been shown. Two others supportive experiments confirmed the ability of pyrocatechol to induce micronuclei formation in bone marrow cells, although some contradictory results were observed without clear explanation.

Carcinogenicity, co-carcinogenicity or tumour promotion studies with pyrocatechol demonstrated the carcinogenic effect on catechol on glandular stomach on rats with formation on adenomas or adenocarcinomas. It is important to notice that effects appeared at high dose of 0.4% and mainly 0.8%. The lowest doses tested presented submucosal hyperplasia indicating that repeated administration of important dose at the site of application (stomach) lead to toxic effect for which the severe form at high dose were carcinoma and adenocarcinoma. Among three species studied (rat, mouse and hamster), rat was clearly the most sensitive. According to data available, catechol did not exert carcinogen effect on other organs than the site of application after oral administration: esophagus and stomach (glandular and forestomach) of rat.

IARC (1999) classify pyrocatechol as possibly carcinogenic to humans (Group 2B).

2.2.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Acute Tox. 4 (*) H312

Acute Tox. 4 (*) H302

Eye Irrit. 2 H319

Skin Irrit. 2 H315

GHS07 Wng

2.2.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification: Xn; R21/R22

Xi; R36/38

Labelling: Xn

R: 21/22-36/38

S: (2-)22-26-37

2.3 Current self-classification and labelling

2.3.1 Current self-classification and labelling based on the CLP Regulation criteria

Self-classification for mutagenicity as Mut. 2 and carcinogenicity as Carc. 2. were done by 124 and 47 notifiers, respectively. A summary is provided in the table below.

Table 4: Hazard Class, Category Codes(s), Hazard statement Code(s) according to notifiers

Hazard Class and Category Code(s)	Hazard Statement Code(s)	Number of Notifiers
Acute Tox. 4	H302	1569
Acute Tox. 4	H312	67
Acute Tox. 4	H332	9
Skin Irrit. 2	H315	1711
Eye Irrit. 2	H319	1641
Eye Dam. 1	H318	60
Skin Sens. 1	H317	64
Muta 2	H341	124
No classification Mutagen		1588
Carc. 2	H351	47
No classification carcinogen		1665

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A substance with the classification of Mut. 2 (H341) and Carc. 2 (H351) is normally subject to harmonised classification (CLP article 36.1). Based on the available data from the registration dossier and scientific literature, classification for the endpoint mutagenicity (Mut.2; H341) and carcinogenicity (Carc. 2; H351) are warranted.

Pyrocatechol is currently classified as Acute Tox. 4 (*) (H312), Acute Tox. 4 (*) (H302), Eye Irrit. 2 (H319) and Skin Irrit. 2 (H315) according to Annex VI of CLP.

Acute toxicity data from oral and dermal route of pyrocatechol are also presented in this report in order to update the minimum acute toxicity classification of pyrocatechol with the new criteria of CLP regulation.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

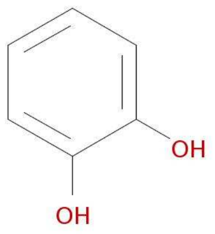
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	204-427-5
EC name:	***1,2-dihydroxybenzene; pyrocatechol***
CAS number:	120-80-9
CAS name:	1,2-dihydroxybenzene; 1,2-Dihydroxybenzene; 1,2-Benzenediol
IUPAC name:	Pyrocatechol
CLP Annex VI Index number:	604-016-00-4
Molecular formula:	$C_6H_6O_2$
Molecular weight range:	110 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Pyrocatechol	≥ 98.5 %	985 – 1000 g/kg	/

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No data			

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
/				

1.2.1 Composition of test material

The minimum purity is specified to be minimum 98.5%.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Beige brown solid	Ferron, 2010	
Melting/freezing point	105°C	Several references	Review of different handbooks
Boiling point	245.5°C	Several references	Review of different handbooks
Relative density	1.3 to 1.4 at 15°C	Several references	Review of different handbooks
Vapour pressure	133 hPa at 176°C; 266 hPa at 197.7°C; 533 hPa at 221.5°C	Several references	Review of different handbooks
Surface tension	Data waiving		Based on the structure
Water solubility	235 to 584 g/L at 20 - 25°C.	Several references	Review of different handbooks
Partition coefficient n-octanol/water	0.84 to 1.03.	Several references	Review of different handbooks
Flash point	127°C	Several references	Review of different handbooks
Flammability	Not flammable	Ferron, 2010	Method EU A 10 was used
Explosive properties	Data waiving		Based on the structure

Self-ignition temperature	510°C	Kirk-Othmer, 1981	handbook
Oxidising properties	Data waiving		Based on the structure
Granulometry	D10: 60.5 µm D50: 113.1 µm D90: 197 µm	Masson, 2010	Laser diffraction
Stability in organic solvents and identity of relevant degradation products	Solubility at 20°C: 660 g/L in acetone; 582 g/L in ethanol; 19 g/L in chloroform; 1 g/L in carbon tetrachloride; 8 g/L in benzene. Solubility at 25°C: 740 g/L in ethyl alcohol; 653 g/L in ethyl ether; 752 g/L in acetone; 73 g/L in benzene; 7 g/L in chloroform.	Several references	Review of different handbooks
Dissociation constant	pKa1 = 9.23 and pKa2 = 13.05	Sergeant E.P., Dempsey B, 1979	Not indicated
Viscosity			

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Pyrocatechol is a major intermediate for synthesis of molecules for agrochemicals use. It is an intermediate for perfumes, cosmetics, aromas. It is also used in various areas such as: anticorrosion agent; antioxidant for rubber, olefins and polyofins, polyurethanes; therapeutic agent; bonding agents; tanning agent, synthetic tannins or photography; catalysts.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not relevant for this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

In the CLH report, catechol is used synonymously with pyrocatechol.

Studies selected for evaluating acute toxicity, mutagenicity and carcinogenicity of pyrocatechol are collected from registration dossier, and also from literature.

3.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

From the dissemination website, some data are available that are presented here as supportive data for other endpoints. The reliability of the data has not been challenged.

<u>After injection</u>

There are 2 publications of Greenlee (1981a, 1981b) recorded on the dissemination website evaluating the distribution of pyrocatechol. They are all performed *in vivo* and quoted of reliability 2 according to Klimisch scales by the registrant.

- [^{14}C] Catechol in saline solution were administered into the lateral vein of male rats at concentration of 1.2 mg/kg alone or with simultaneous administration with non-labelled catechol at dose of 12 mg/kg bw. It was shown that, the radioactivity was concentrated in the bone marrow and lymphoid organs (Greenlee, 1981b).

- [^{14}C] Catechol was administered for 2 hours into the lateral vein of male rats at concentration of 14 mg/kg in rats pre-treated or not five days prior with 500 mg/kg ip injection of Arochlor 1254 solution in corn oil. Rats were sacrified 2 or 24 hours after dosing. Liver, thymus and bone marrow were analysed for total radioactivity. After 2 h, the amount of soluble radioactivity measured in liver, thymus, and bone marrow ranged from 149 to 370 dpm/mg protein. After 24 h, the amount of soluble radioactivity measured in liver and thymus was markedly lower than at 2 h., whereas in bone marrow no statistically significant difference was found.

According to these two studies, after IV administration, bone-marrow seems to be exposed. No data after oral or inhalation exposure are available on distribution of catechol in bone marrow or reproductive organs.

The following summary is included in the monograph of IARC on pyrocatechol vol. 71: “Proposed metabolic pathways of catechol are summarized in Figure 1. The major metabolic pathways in experimental animals are sulfation and glucuronidation. Catechol may be oxidized by peroxidases to the reactive intermediate benzo-1,2- quinone, which readily binds to proteins (Bhat et al., 1988); this process, catalysed by rat or human bone-marrow cells in the presence of H_2O_2 (0.1 mM), is stimulated by phenol (0.1–10 mM), and decreased by hydroquinone and by glutathione, which conjugates with benzo-1,2-quinone. These phenols (phenol, catechol and hydroquinone) may play a role in benzene toxicity to bone marrow: all three are formed as benzene metabolites (Smith et al., 1989) and they interact in several ways as far as their bioactivation by (myelo)peroxidases is concerned (Smith et al., 1989; Subrahmanyam et al., 1990).

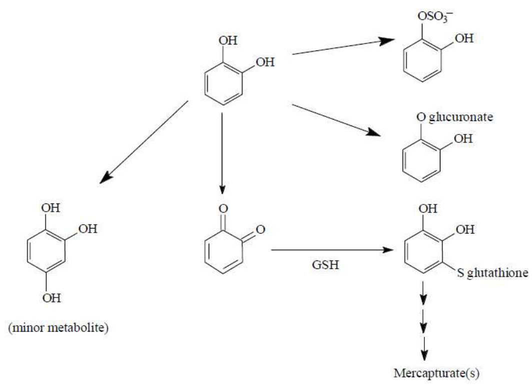


Figure 1: metabolism of catechol (IARC monographs vol. 71)

<u>After inhalation</u>:

- [3H] catechol was used to follow the kinetics and metabolic fate of inhaled catechol in cigarette smoke in Mice. 2 to 2.3 µCi [3H] catechol was present in reconstituted cigarette. Mice were exposed to 10% (v/v) 2R1 cigarette smoke on nose only and received 35mL puff volume, 2 sec/puff, 10 puffs/cigarette for 0, 5, 10, 30, 60 and 120 minutes. The deposition and distribution of inhaled catechol were determined in all internal tissues, urine and faeces. Data showed that clearance was occurring during the 10 minutes smoke exposure period. Immediately after exposure, over 50% of the radioactivity was found in the blood, with 10% found in the lung, and approximately 12% in the respiratory tract. Over 91% of the inhaled radioactivity was found in the urine 120 minutes after exposure. Less than 0.5% of the total dose was found in the lung at this time. Catechol is rapidly absorbed, redistributed, and excreted from mice exposed to whole cigarette smoke (Hwang, 1982).

13 workers were exposed to vapours of catechol and phenol (Hirosawa, 1976). The dose for catechol was 1.8 ppb and occasionally 70 ppb. Main complaints of the workers were related to upper respiratory tract, such was confirmed by CMI health questionnaire and clinical examination. The biological half-life of catechol measured was 3 -7h, similar than for phenol. Although catechol is well-known inhibitor of catecholamine o-methyl transferase, the exposure at the level studied did not modify catecholamine metabolism; the daily excretion of catecholamines and their metabolites in urine remained within the normal range except for a slight decrease in noradrenaline excretion. Blood pressure and body temperature during and right after exposure also remained normal. There were no signs of hepatic and renal dysfunction. Regarding result obtained in this study, catechol was rapidly eliminated *via* urine after inhalation exposure (half-life 3 -7h) and seemed not to bioaccumulate.

After inhalation exposure, catechol was rapidly and largely absorbed and found in the blood, lung, and eliminated via urine. Catechol was rapidly eliminated via urine after inhalation exposure (half-life 3 -7h).

3.2 Acute toxicity

This part is a copy/paste from the dissemination website. eMSCA did not access the original studies/ publication.

3.2.1 Non-human information

3.2.1.1 Acute toxicity: oral

The results of studies on acute toxicity after oral administration are summarised in the following table. Only the two references that were published are included below. The two other studies were not included as they were secondary literature and no details were provided.

Table 10: Studies on acute toxicity after oral administration

Method	Results	Remarks	Reference
rat (albino) male oral: gavage equivalent or similar to Federal register 5 males/dose	LD_{50}: 300 mg/kg bw (male) (95% C.L. 200- 500 mg/kg bw) Mortality: 0/5 at 158 mg/kg 2/5 at 316 mg/kg bw 5/5 at 630 mg/kg bw 5/5 at 1260 mg/kg	2 (reliable with restrictions: The purity of the test substance and the strain of rat used are not known. The administration volume and the use of vehicle are not specified.) key study experimental result **Test material (EC name): pyrocatechol**	Flickinger C.W. (1976)
Rat	LD_{50}: 358 mg/kg bw	2 (reliable with	Lewis RJ (1996a)

Method: other		restrictions) weight of evidence experimental result **Test material (EC name): pyrocatechol**	Anonymous (1972)

3.2.1.2 Acute toxicity: inhalation

Not relevant for this dossier.

3.2.1.3 Acute toxicity: dermal

The results of studies on acute toxicity after dermal administration are summarised in the following table:

Table 11: Studies on acute toxicity after dermal administration

Method	Results	Remarks	Reference
rat (Crj: CD(SD)) male/female Coverage: openequivalent or similar to OECD Guideline 402 (Acute Dermal Toxicity)	LD_{50}: 600 mg/kg bw (male/female)	2 (reliable with restrictions) key study experimental result **Test material (EC name): pyrocatechol**	Study reportn° 16948 (1973)
rabbit (albino) male equivalent or similar to Federal register	LD_{50}: 800 mg/kg bw (male)	2 (reliable with restrictions) key study experimental result **Test material (EC name): pyrocatechol**	Flickinger C.W. (1976)

3.2.1.4 Acute toxicity: other routes

3.2.2 Human information

No data available.

3.2.3 Summary and discussion of acute toxicity

- Oral route:

Four studies were available but two of them were retained: one as key study and another as supportive evidence because they were reliability 2.

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

No death occurred at 158 mg/kg bw, 2/5 died at 316 mg/kg bw and 5/5 at 630 mg/kg bw within 1 hour.

No clinical signs were reported and the autopsy revealed for rats died during the study: hyperaemia of the stomach and intestines. None of the rats sacrificed at the end of the experiment revealed gross pathology after pathological examination. No summary was provided by Lewis, 1996, the data originated from an Estonian study from 1972.

- Dermal route:

Two studies were available, from one study report and from one publication. They were selected as key studies and had reliability 2. The summary of the study report is the following:

In an acute dermal toxicity study (Study n° 16948, 1973), CD male and female rats (5/sex/group) were exposed to Catechol by dermal route for a maximum of 24 hours, at doses of 125, 875 and 1125 mg/kg bw. Animals then were observed for 15 days.

0/10 died at 125 mg/kg and no clinical signs, and 10/10 died at 875 and 1125 mg/kg, the rats presented tremors 5 minutes after dermal application, and died within 30 minutes after clonic convulsions.

In the second study (Flickinger, 1976), 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 catechol for a maximum period of 24 h. The number of death at each dose levels and time of death was as follows: no death occured at the dose of 250 mg/kg bw, 1/4 rabbit died at the dose of 500 mg/kg bw on day 2, 2/4 rabbits died at the dose of 1000 mg/kg bw by day 2, 4/4 rabbits died at the dose of 2000 mg/kg bw on day 1.

The effects observed after dermal administration were local effect (All the rabbits that died during the observation period revealed subdermal hyperaemia and oedema) and death with LD_{50}= 800 mg/kg bw (95% C.I.: 500-1400) in male rabbits.

3.2.4 Comparison with criteria

Oral LD_{50} Males = 300 mg/kg bw (95% C. L. 200-500 mg/kg). Catechol is considered as toxic based on the LD_{50} obtained, and classified as category 3 (H301: Toxic if swallowed) according to classification criteria of EC regulation 1272/2008 and current EC regulation in Annex VI 3.1.

Dermal $LD_{50\ Combined}$ = 600 mg/kg bw. Catechol is considered as toxic in contact with the skin and should be classified as category 3 (H311: Toxic in contact with skin) according to classification criteria of EC regulation 1272/2008. Currently the harmonised classification is category 4 H312: Harmful in contact with skin in the Annex VI 3.1.

Criteria for CLP classification of substances as acutely toxic:

Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal route according to the numeric criteria shown in Table 12. Acute toxicity values are expressed as (approximate) LD 50 (oral, dermal) values or as acute toxicity estimates (ATE). Explanatory notes are shown following Table 12.

Table 12: Acute toxicity hazard categories (oral and dermal) and acute toxicity estimates (ATE) defining the respective categories

Exposure route	Category 1	Category 2	Category 3	Category 4
Oral (mg/kg bodyweight) See: Note (a)	ATE ≤ 5	5 < ATE ≤ 50	50 < ATE ≤ 300	300 < ATE ≤ 2 000
Dermal (mg/kg bodyweight) See: Note (a)	ATE ≤ 50	50 < ATE ≤ 200	200 < ATE ≤ 1 000	1 000 < ATE ≤ 2 000

(a) The acute toxicity estimate (ATE) for the classification of a substance is derived using the LD_{50} /LC_{50} where available.

3.2.5 Conclusions on classification and labelling

The following information is taken into account for any hazard assessment:

LD_{50} oral (rat): 300 mg/kg Acute toxicity - oral: Acute Tox. 3 - H301

LD_{50} dermal (rat): 600 mg/kg Acute toxicity – dermal: Acute Tox. 3 - H311

3.3 Specific target organ toxicity – single exposure (STOT SE)

Not relevant for this dossier.

3.4 Irritation

Not relevant for this dossier.

3.5 Corrosivity

Not relevant for this dossier.

3.6 Sensitisation

Not relevant for this dossier.

3.7 Repeated dose toxicity

Not relevant for this dossier.

3.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not relevant for this dossier.

3.9 Germ cell mutagenicity (Mutagenicity)

4.9.1. Non-human information

4.9.1.1. *In vitro* data

Studies selected for evaluating mutagenicity of pyrocatechol *in vitro* are mainly collected from CSR report and also from literature. As a considerable number of papers (around 60) are proposed in the registration dossier of pyrocatechol, data with not enough details on test conditions and results are not reported. Most of the reported studies are reliability 2 (n=21) according to CSR and only few studies are reliability 3 (n=6) because of the lack of details about controls.

In vitro gene mutation assays on bacterial cells

Mutagenicity of pyrocatechol was studied on bacteria strains using bacterial reverse mutation assay. This assay is similar to OECD 471 but none of the studies was performed according to the current OECD guideline 471. Five studies were considered of reliability 2 (with restrictions). Results from 3 studies indicated no mutagenic activity with and without metabolic activation in all strains of *Salmonella Typhimurium* tested (TA 98, TA 100, TA 1535, TA 1537, TA 1538) (Study report n° FSR-IPL 060904-01 2007; Study report n°7960 05 1983; Study report n°7961 03 1983; Study report n° BOA/PA T/73 988, 1983; Haworth et al. 1983). Only two studies showed mutagenic activity. In a screening micromethod 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 of strain TA 102 is probably due to a substitution of AT to GC by 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, negative response was observed with WP2 uvrA/pKM101 strain IC188 (with and without S9-mix).

In vitro gene mutation assays on mammalian cells

Mutagenicity of pyrocatechol was also studied on mammalian cells. Results were positive for the 3 studies selected (reliability 2) performed according to a protocol similar to OECD guideline 476. In the study of Tsutsui et al. (1997), mutation induction was observed on SHE cells starting from 3 µM (0.33 µg/ml) and 10 µM (1.1 µg/ml) of catechol for HPRT and 10 µM and 30 µM for Na^+/K^+ ATPase locus, respectively. However, mutation inductions of Na^+/K^+ ATPase locus were observed at cytotoxic concentrations (decreased cell survival to 28.8% and 1.4% of untreated cells at the concentration of 10 and 30 µM, respectively). The study of Mc Gregor et al. (1988) revealed similar results with mutation induction at cytotoxic concentration on mouse lymphoma cells. They observed that the lowest observed effective dose based on mutagenic potencies or producing cytotoxic effect (without metabolic activation) was 2.5 µg/ml (25 µM). The mutagenic potential of catechol was completely negated by coincubation with Superoxide Dismutase (SOD). It was noticed that SOD had little effect upon cytotoxicity. Another study on mouse lymphoma cells showed that catechol was found to increase the mutation frequency in a non-dose dependent manner, without metabolic activation (Wangenheim and Bolcsfoldi 1988).

In vitro mammalian chromosome Aberration test

All studies selected for clastogenic endpoint were considered of reliability 2 (with reliable restrictions) and showed positive genotoxic results.

In the study of Tsutsui (1997), Syrian Hamster Embryo (SHE) cells were treated overnight without metabolic activation system with catechol at doses of 0.11 - 0.33 - 1.1 - 3.3 - 11 µg/ml (1 - 3 - 10 - 30 - 100 µM) in presence of BrdU. Similar experiments were performed 2 or 3 times, and the results obtained were reproducible. Negative results were obtained at 1 and 3 µM. Sister Chromatid Exchange (SCE) in SHE cells occurred with catechol at cytotoxic concentrations of 10 µM (11.06 SCEs/cell) and 30 µM (15.40 SCEs/cell). At 100 µM, catechol was overly toxic to obtain SCEs data. This study assessed also chromosome aberration or aneuploidy on SHE cells after 6, 24 or 48 h of exposure to catechol (3, 10, and 30 µM) without metabolic activation system. Even though no significant effect was observed at 3 and 10 µM, a slight aneuploidy in the near diploid range of SHE cells was significantly induced by catechol at 30 µM after 48 h.

Another study on chromosomal aberration was performed on lung fibroblasts (V79 cells) exposed to different concentration of catechol (0 - 20 - 40 - 60 - 80 µM) at different pH values (6.0, 7.4 and 8.0, with and without metabolic activation system from Wistar rat liver induced with Aroclor 1254 at pH 7.4) (Do Ceu et al. 2003). Results showed that clastogenic effect of catechol was dependent on the pH: non-significant increase in chromosomal aberrations at pH 6.0, at any dose-level. Catechol induced significant chromosomal aberrations at the 3 three highest concentrations tested (40-60-80 µM) and at the 2 highest concentrations tested (40-60 µM) at pH values of 7.4 and 8.0, respectively. It showed also a significant induction of multi-aberrant cells (with more than 10 chromosomal aberrations), which represent in some cases more than 50% of the aberrant cells.

Clastogenic effect of catechol was confirmed on micronucleus test on L5178Y mouse lymphoma cells with micronucleus test (micromethod) (Study report n° FSR-IPL 060505-01, 2007). Results showed that catechol (from 5 to 156 µg/ml) induced a significant genotoxic effect on L5178Y mouse lymphoma cells, both without and with metabolic activation (by means of liver or kidney S9-mix). Even though the positive controls induced the appropriate responses in the corresponding assays, nevertheless this experiment was performed without repetition.

The capacity of catechol to induce chromatid breaks and exchanges in Chinese Hamster Ovary (CHO) cells was evaluated by Stich et al. (1981). Catechol (50 µg/mL or 454 µM) exhibited a chromosome-damaging potential (chromatid break or exchange) and the addition of an S9 mixture reduced its clastogenic activity. These authors showed that treatment with S9 mix, catalase + SOD, and catalase or SOD alone did not lead to a significant reduction on the level of chromosomal aberration induced by the catechol. However, the addition of S9 mix with catalase + SOD leads to a reduction in the number of multi-aberrant cells. It was also observed that OH* radicals were produced, so a participation of a radical-type mechanism cannot be excluded in the genotoxicity of catechol.
In the study of Sze et al., 1996, negative results were shown on CHO cells exposed to catechol at concentrations as high as 250 µM without metabolic activation and no one DNA strand breaks were produced.

Regarding clastogenicity of catechol on human cells, Human lymphocytes were treated to a range of concentration of catechol (from 0.5 to 250 µM) without metabolic activation system (Yager et al., 1990). Statistical significant increase of micronucleated cells was observed starting from 0.5 µM and decrease of cell viability was measured starting from 100 µM. A significant dose related increases in kinetochore-positive micronucleated cells was noticed suggesting that catechol was likely aneuploidy-inducing agents in Human lymphocytes.

Other studies on DNA damage

Genotoxicity of catechol is also assessed by several methods such as: measuring single/double strand break DNA, alkali-labile sites, unscheduled DNA synthesis, inhibition of DNA synthesis or inhibition of repair system of DNA.

In the study of Cahill (2004), DNA damage and repair was checked in microplates method with Saccharomyces cerevisiae. The relative total growth of Saccharomyces cerevisiae was assessed by comparing the extent of proliferation of treated and untreated cells. The measurement of total growth was performed by fluorescence collection. Two strains were tested (GENC01 and GEN T01) at concentration of catechol from 177 µg/mL to 880 µg/mL without metabolic activation system. A clear positive response was measured in growth inhibition rate with strain GENC01 at 177 µg/mL, and clear genotoxicity with GFP induction with strain GEN T01 at 599 µg/mL.

In the study of Solveig Walles (1992), performed on rat hepatocytes, the results showed an increase in the rate of elution of DNA corresponding to the formation of single strand breaks (SSB) in DNA. The dose-response curve showed a threshold value of 1 mM after which the DNA damage increased. The viability was about 75% and unchanged after treatment. DNA damage increased slightly with the period of exposure (0, 20, 40 and 60 min) at 3000 µM catechol. When the hepatocytes were pre-treated for 30 min with the Ca^{2+}-chelator Quin-2 AM, there was a decrease of the DNA damage, indicating probable oxidative damage. The mechanism for repairing the DNA damage induced was challenged by post-treatment of the hepatocytes with an inhibitor of poly(ADP-ribose) polymerase (3-aminobenzamide - 3AB). Upon such treatment, the level of DNA damage by catechol was increased.

In the study of Pellack-Walker (1985) on mouse L5178YS cells, DNA synthesis inhibition was 65% after a treatment of 30 min at 1000 µM of catechol. Beyond 60 min, a small recovery was observed. However, an irreversible inhibition of DNA synthesis was observed at >1.0 mM. A specific dose-dependent inhibition of DNA synthesis was shown following 30 min of exposure to catechol and 60 min washout which was correlated to the oxidative potential of catechol. Cell viability results showed that concentrations as high as 1.0 mM had no effect on protein synthesis and no effect on membrane integrity (trypan blue dye exclusion).

In the study of Pellack-Walker (1986), concentrations of catechol as high as 1.0 mM did not increase the percentage of single-stranded DNA observed on mouse lymphoma cells line L5178YS.

However, catechol was able to inhibit 52% of the nuclear synthetic activity at 24 µM (IC_{50} = 23 µM) on mouse bone marrow cell (Lee et al. 1989), In a cell-free DNA synthetic system, catechol did not inhibit the incorporation of 3H-thymidine triphosphate up to 24 µM. The viability of the cells was not modified at all test concentrations. For all these *in vitro* studies, cell viability is not affected when genotoxicity is observed.

Three studies on Human cells were available and they were considered of reliability of 2 or 3 according to Klimisch scale. In the study of Fabiani (2001), Human Peripheral Blood Mononuclear Cells (PBMC) was exposed to catechol at following concentrations: 0 - 1.1 - 5.5 - 11 - 22 - 66 µg/mL (0 - 10 - 50 - 100 - 200 - 600 µM) for 4 hours without metabolic activation system. After lysis of cells and elution in specific comet assay conditions, DNA damage was evaluated in single cell gel electrophoresis by fluorescence. The different concentration tested did not reduce cell viability to less than 95% (Trypan blue assay). Catechol did not induce DNA damage at concentrations of 10, 50 and 100 µM. Catechol was only genotoxic at 200 and 600 µM when cells were incubated in PBS (in conditions not pertinent for hazard evaluation for humans because no proteins were present). Very little genotoxic effects of catechol were observed when cells were incubated in RPMI + 5% FCS. In the presence of 5% of serum, cells were completely protected from catechol effects in both media. The positive response was observed when cells were incubated with the 2 highest concentrations of catechol in phosphate serum buffer only. No such results were obtained in RPMI medium, and under more physiological conditions, i. e. following the addition of foetal calf serum. Therefore, the positive result obtained in very simplified medium, without any proteins, appears not relevant.

Another study exposed Human DNA fragments to catechol at doses of 0 - 0.011 - 0.022 - 0.055 - 0.110 - 0.220 - 0.550 - 1.101 - 2.202 µg/mL (0 - 0.1 - 0.2 - 0.5 - 1.0 - 2.0 - 5 - 10 - 20 µM) without metabolic activation system (Hirakawa et al. 2002). DNA damage and measurement of O2- generation were evaluated. Catechol was tested with or without the addition of Cu^{2+} and/or NADH. Catechol alone at the concentration of 20 µM (without the addition of Cu^{2+} or NADH) did not produce any DNA damage. Catechol can induce DNA damage only in specific conditions: in presence of NADH and Cu^{2+}. The DNA damage induced by catechol was inhibited by catalase and bathocuprine, a specific chelator of Cu^{2+}. Neither OH* scavenger nor SOD could inhibit this DNA damage, suggesting the induction of DNA damage mediated cooperatively by H_2O_2 and Cu^{2+}. DNA clivage was observed at Guanine and Thymine sites, and it was due to reactive oxygen species. DNA damage induced by catechol was dependent on specific conditions (presence of NADH and Cu^{2+}) suggesting that the DNA damage was mediated by H_2O_2 and Cu^{2+}.

Oikawa et al. (2001) assessed the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and its hydrogen peroxide (H_2O_2)-resistant clone HP-100 by using an electrochemical detector coupled to HPLC. Human Leukaemia cell line HL-60 and HP 100 were exposed to different concentrations of catechol without metabolic activation system: 0 - 1.1 - 2.2 - 5.5 µg/mL (0 - 10 - 20 - 50 µM). HP100 cells, known to have a high level of catalase activity, were used to assess if H_2O_2 participates in catechol-induced DNA lesion. Catechol treatment resulted in increased 8-oxodG content in a dose dependent manner in HL-60 cells, but not in HP-100 cells. This increase of 8-oxodG content is indicative of oxidative base damage. DNA ladder (associated with apoptosis) were observed at 50 µM (20 µM just visible) in HL-60 cells, but not in HP-100 cells. Catechol caused DNA damage depending of its concentration in presence of Cu^{2+}. DNA damage was enhanced in presence of 100 µM NADH. Catechol frequently induced piperidine labile sited at thymine residues and it increased 8-oxodG content in calf thymus DNA in presence of Cu^{2+}, but not in absence of Cu^{2+} or in the presence of other metal ions (Fe^{3+}, Co^{2+}, Ni^{2+}, Mn^{2+} or Mg^{2+}).

Table 13: Summary table of relevant *in vitro* mutagenicity studies for pyrocatechol

Test	Results	Remarks	Reference
***In vitro* gene mutation in bacterial**			
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) - *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538 - Concentrations tested : 0 - 3.9 - 15.6 - 62.5 - 250 - 1000 µg/plate - Metabolic activation: with and without Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay); non-GLP Reliability 2: reliable with restrictions	**Negative** with and without metabolic activation	**Negative** for *S. typhimurium*, other: TA 98, TA 100, TA 1535, TA 1537, TA 1538; Met. Act.: with and without Cytotoxicity: yes (at 1000 µg/plate) Vehicle controls valid: yes; Negative controls valid: not examined; Positive controls valid: yes	Study report n°7961 03 (1983) Study report n°7960 05 (1983)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) - *S. typhimurium*: TA 1535, TA 1537, TA 1538, TA 98, TA 100 - Concentrations tested: 0 - 62.5 - 125 - 250 - 500 - 1000 µg/plate equivalent - Metabolic activation: with and without Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay);	**Negative** with and without metabolic activation	**Negative** for *S. typhimurium*, other: TA 1535, TA 1537, TA 1538, TA 98, TA 100; Met. Act.: with and without Cytotoxicity: yes (> 1000 µg/plate) Vehicle controls valid: not examined; negative controls valid: yes; positive controls valid: yes	Study report n° BOA/PA T/73 988 (1983)

non-GLP Reliability 2: reliable with restrictions			
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) - *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 - Concentrations tested: Lab. 1: 0 - 11 - 35 - 104 - 333 - 1000 µg/plate Lab. 2: 0 - 33.3 - 100 - 333.3 - 1000 - 3333.3 µg/plate - Metabolic activation: with and without Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay): only 4 strains tested instead of 5, positive control used for TA 98 strain without S9 is not the one recommended by the OECD guidelines, 2-AA should not be used as the sole indicator of the efficacy of the S9 Mix; non-GLP Reliability 2: reliable with restrictions	**Negative** with and without metabolic activation	**Negative** for *S. typhimurium*: TA 1535, TA 1537, TA 98 and TA 100; Met. Act.: with and without Cytotoxicity: yes, at 3333.3 µg/plate (without metabolic activation) Vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	Haworth S. et al. (1983)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) - *S. typhimurium* TA1537, TA98, TA100 and TA102 - 10 concentrations tested from 0 to 5000 µg/plate - Metabolic activation: with S9 mix and without Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay): 4 strains tested instead of 5, Ames test according to micromethod, no repetition performed; non-GLP Reliability 2: reliable with restrictions	**Positive** for TA 102, with and without metabolic activation	**Positive:** Clear mutagenic activity in strain TA102 without and with metabolic activation (no repetition performed) **Negative** for *S. typhimurium*: TA1537, TA98 or TA100 in absence or in presence of metabolic activation system Cytotoxicity: yes, at 2500 and 5000 µg/plate Vehicle controls valid: yes ; negative controls valid: not examined; positive controls valid: yes	Study report - n° FSR-IPL 060904-01 (2007)
WP2 Mutoxitest - Bacterial reverse mutation assay (gene mutation) - E. Coli : WP2uvrA/pKM101; IC203 - Concentrations tested: 5 doses including 1000 - 2000 - 3000 µg/plate - Metabolic activation : with and without S9 WP2 bacterial reverse mutation assay analogous to the Ames test, compare the sensitivity of strain IC203 with that of IC188 for the detection of mutagenesis by oxidants No details about controls; Non-GLP Reliability 2: reliable with restrictions	**Positive** without metabolic activation	**Positive** for IC203 strain **Negative** for IC188 strain Cytotoxicity: at the dose of 2000 µg/disc, the inhibition (millimeters) was 6 mm for IC188, 12 mm for IC203 and 7 mm for IC 203+S9. Mutagenesis and cytotoxicity are inhibited by S9 for IC203 **Catechol considered as an oxidative mutagen** Vehicle controls valid: not examined; negative controls valid: no data; positive controls valid: yes	Martinez et al. (2000)
***In vitro* gene mutation in mammalian cells**			
In vitro mammalian cell gene mutation assay (HPRT and Na^+/K^+ ATPase loci) - Syrian Hamster Embryo (SHE) cells - Concentrations tested: 1-3-10-30 µM	**Positive** without metabolic activation	Catechol induced gene mutation at the two loci in SHE cells: both ouabain-resistant (Na^+/K^+-ATPase) and 6-thioguanine resistant (hprt) mutant frequencies	Tsutsui et al. (1997)

- Metabolic activation: without Equivalent or similar to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test) No details about controls; Non-GLP Reliability 2: reliable with restrictions		were increased in dose dependent manner for TG. HPRT locus : mutations induced starting from 0.33 µg/ml (3µM) Na^+/K^+ ATPase locus: mutations induced starting from 10 µM (1.1 µg/ml) Strong cytotoxic effects: 85.2% (1µM), 70.2% (3 µM); 28.8% (10 µM) and 1.4% (30 µM) of survival (Number of colonies scored is between 4160 et 68. Ratio small/ large colonies is not reported.) Vehicle controls valid: not examined; negative control valid: not examined; positive controls valid: not examined	
In vitro Mouse Lymphoma L5178Y Assay - L5178Y mouse lymphoma cell - Catechol concentrations tested: 2.5-4-5.5-7-8.5 µg/ml - Metabolic activation: without - Superoxide dismutase (SOD) coincubation treatment : 100 units/ml during 4 hours Equivalent or similar to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test) No metabolic activation and no positive control used; Non-GLP Reliability 2: reliable with restrictions	**Positive** without metabolic activation	Lowest dose effect: 2.5 µg/ml Mutagenic effects not dose-dependent SOD treatment: Inhibition of mutagenic effects starting from the lowest concentration (2.5 µg/ml) Cytotoxic effect observed with or without SOD treatment (starting from 2.5 µg/ml) Negative control valid: yes	Mc Gregor et al. (1988)
In vitro Mouse Lymphoma L5178Y Assay - L5178Y mouse lymphoma cell - Concentrations : 0 - 1.145 - 2.874 - 5.516 - 11.450 - 28.736 µg/mL - Exposure time : 4 hours - Metabolic activation: without Equivalent or similar to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test) No metabolic activation and no positive control used; Non-GLP Reliability 2: reliable with restrictions	**Positive** without metabolic activation	**Positive** results : Increase of the mutation frequency in a non-dose dependent manner at all concentrations tested, without metabolic activation Cytotoxicity: not examined Vehicle controls valid: yes; negative control valid: not examined; positive controls valid: not examined	Wangenhein and Bolcsfoldi (1988)
***In vitro* clastogenic effects in mammalian cells**			
In vitro Sister chromatid exchange assay - Syrian Hamster Embryo cells - Concentrations tested:1-3-10-30-100 µM - Metabolic activation: without Equivalent or similar to OECD Guideline 479 (Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells) No metabolic activation, no positive control mentioned and positive responses obtained only at cytotoxic concentrations;	**Positive** without metabolic activation	Significant increase of sister chromatid exchange at 10 µM (1.1 µg/ml) and 30 µM (3.3 µg/ml) Cytotoxicity: inhibition of growth at doses greater than 10 µM (1.1 µg/ml) Vehicle controls valid: not examined; negative control valid: yes; positive controls valid: not examined	Tsutsui et al. (1997)

Non-GLP Reliability 2: reliable with restrictions			
In vitro sister chromatid exchange assay - Human T-Lymphocytes (collected from 1 single donor) - Material purity: 99+% - Concentrations: 5-50-70-100-300 µM - Metabolic Activation: Without Equivalent or Similar to OECD 479 (Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells) Results obtained with the lymphocyte of a single donor ;Test performed without metabolic activation and no details about positive controls ; the number of replicated tested is not mentioned; Non-GLP Reliability 2: reliable with restrictions	**Positive** without metabolic system	Significant increase of SCE frequency (concentration-dependent), Decreases in mitotic indices, and Inhibition of cell cycle kinetics. Lowest effective dose: 5 µM Cytotoxic effects: 300 µM Negative control: not examined; vehicle control: yes (valid); positive control: not examined	Erexson et al. (1985)
In vitro sister chromatid exchange assay (SCE) - Human lymphocytes - Concentrations:0- 1.6 – 8 -40 -200 - 1000 µM - Exposure time: 72h - Metabolic Activation: Without Equivalent or similar to OECD 479 (Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells) Test only performed with metabolic activation; induction of SCE observed at cytotoxic concentration; 50 cells scored for SCE's and no data about replicates and positive controls; Non-GLP Reliability 3	**Positive** without metabolic activation	Induction of sister chromatid exchanges and delays cell division at 40 and 200 µM Estimated concentration to induce SCE doubling: 30 µM (3.3 µg/ml) Cytotoxicity: yes at 40 µM Negative control: yes (valid); vehicle control: not examined; positive control: not examined	Morimoto and Wolff (1980)
In vitro sister chromatid exchange assay - Human lymphocytes - Concentration: 0.3 mM - Time: 2h - Metabolic activation: without Similar or Equivalent to OECD 479 (Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells) Only without metabolic activation; Cytotoxicity not checked ; only one concentration tested ; Non-GLP Reliability 3	**Positive** without metabolic activation	<u>GSH treatment (3 mM):</u> No induction of sister chromatid <u>No GSH treatment:</u> Significant induction of sister chromatid exchanges by catechol at 0.3 mM without metabolic activation Cytotoxicity: not evaluated Vehicle controls valid: yes; negative control: not examined; positive controls valid: not examined	Morimoto (1983)
In vitro chromosomal aberrations assay - Syrian Hamster Embryo cells - Concentrations tested: 1-3-10-30 µM - Exposure time: 6-24-48h - Metabolic activation: without Equivalent or similar to OECD Guideline 473 (*In Vitro* Mammalian Chromosomal	**Positive** without metabolic activation	Significant increase of aberrant metaphases starting from 3 µM (0.33 µg/ml) Slight but significant induction of aneuploidy in the near-diploid range at 30 µM (3.3 µg/ml) Cytotoxicity: inhibition of growth at doses greater than 10 µM (1.1	Tsutsui et al. (1997)

Aberration Test) No metabolic activation, no positive control mentioned and only 100 metaphases scored per experimental group (instead of 200 with replicate); Non-GLP Reliability 2: reliable with restrictions		µg/ml) Vehicle controls valid: not examined; negative control valid: yes; positive controls valid: not examined	
In vitro chromosomal aberrations assay - Chinese Hamster Ovary (CHO) cells - Concentrations tested: 50 µg/mL (454 µM) - Exposure time: 3h - Metabolic activation: with and without - 200 metaphases scored for each sample Equivalent or similar to OECD Guideline 473 (*In Vitro* Mammalian Chromosomal Aberration Test) No data on cytotoxicity; Non-GLP Reliability 2: reliable with restrictions	**Positive** without metabolic activation	**Positive**: Induction of chromatid breaks and exchanges at 50 µg/mL without metabolic activation **Negative results** with metabolic activation Vehicle controls valid: not examined; negative control valid: yes; positive controls valid: yes	Stich et al. 1981
In vitro chromosomal aberrations assay - Chinese hamster lung fibroblasts (V79 cells) - Concentrations tested: 0-20-40-60-80 µM, pH values : 6.0, 7.4 and 8.0 - Exposure time: 3h - Metabolic activation: with and without - 100 metaphases scored for each dose-level group (2 independent experiments) Equivalent or similar to OECD Guideline 473 (*In Vitro* Mammalian Chromosomal Aberration Test) Reliability 2: reliable with restrictions	**Positive** without metabolic activation	Clastogenic effect of catechol pH-dependent: **Positive:** Significant chromosomal aberrations at 40-60-80 µM, pH 7.4 **Positive**: Significant chromosomal aberrations at 40-60 µM, pH8.0 **Negative**: non-significant increase at any dose tested, pH 6.0 Addition of S9 mix with SOD+ catalase lead to a reduction in the number of multi-aberrant cells Cytotoxicity was only observed at 80 µM at pH8.0 Vehicle controls valid: not examined; negative control valid: yes; positive controls valid: yes	Do Ceu et al. 2003
In vitro mammalian cell micronucleus test (chromosome aberration) - Mouse lymphoma L5178Y cells - Concentrations tested: <u>Without S9, 0h:</u> 39.06, 19.53 and 9.77 µg/ml <u>Without S9, 20h:</u> 19.53, 9.77 and 4.88 µg/ml <u>With S9, 24h (liver S9-mix):</u> 156.25, 78.12, 39.06 and 19.53 µg/ml <u>With S9, 24h (kidney S9-mix):</u> 156.25, 78.12, 39.06 and 19.53 µg/ml - Metabolic Activation: with and without Equivalent or similar to OECD Guideline 473 (*In vitro* Mammalian Chromosome Aberration Test) Micronucleus *in vitro* using micromethod assay; Non-GLP	**Positive** with or without Metabolic Activation	<u>Without S9, 0h:</u> **positive** effect at the 3 concentrations tested with a clear dose-effect relationship <u>Without S9, 20h:</u> **positive** effect at the highest and lowest concentrations tested <u>With S9, 24h (liver S9-mix):</u> **positive e**ffect at the 4 concentrations tested <u>With S9, 24h (kidney S9-mix):</u> **positive** effect at the 4 concentrations tested Cytotoxicity: yes (up to 19.53 µg/ml, depending of the assay) ; Vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	Study report n° FSR-IPL 060505-01 (2007)

Reliability 2: reliable with restrictions			
In vitro Micronucleus test, - Human lymphocytes - Material purity: 99+% - Concentrations: 0- 0.5- 5.0-50-100-200-250 µM - Activation metabolic: Without Only tested without metabolic activation system; only one donor of lymphocytes; Non-GLP Reliability 2: with restrictions	**Positive** without metabolic activation	Genotoxicity: Significant dose-related increases of micronuclei formation (starting from 0.5 µM or 0.05 µg/ml) Cytotoxicity: Decrease of cell viability starting from 100 µM (11 µg/ml) Positive controls: yes; Negative controls: yes; vehicle controls: yes - Results valid	Yager et al. (1990)
***In vitro* DNA damage in mammalian cells**			
DNA strand breaks/cross-links, mouse lymphoma cells *in vitro* - Mouse lymphoma L5178Y/TK +/- cells - Concentrations tested: 0- 0.5- 1.5- 5- 15 mM - Metabolic activation: with and without Method: Alkaline unwinding elution and chromatography on hydroxyapatite Not performed according to a standard guideline; Non-GLP Reliability 2 : reliable with restrictions	**Positive** with and without metabolic activation	Without Metabolic Activation: **Positive** results only at 1.5 mM (160 µg/ml) Cytotoxic effect at 5 and 15 mM without S9 With Metabolic Activation: **Positive** at doses higher than 0.5 mM (55 µg/mL) with a clear dose-response relationship Cytotoxic effect at 15 mM with S9 Vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: not examined	Gardberg et al. (1988)
In vitro DNA damage Comet assay - Human Peripheral Blood Mononuclear Cells (PBMC) - Concentrations: 0 - 1.1 - 5.5 - 11 - 22 - 66 µg/mL (0 - 10 - 50 - 100 - 200 - 600 µM) - Exposure time: 2 hours - Metabolic Activation: Without Test not performed according to recognized guidelines; No details about positive controls; Non-GLP Reliability 2: with restriction	**Positive** in non relevant conditions (PBS)	Genotoxic effects observed at 200 and 600 µM in PBS Very low genotoxicity of catechol in media composed of RPMI+5% FCS No DNA damage observed with catechol in medium with 5% of serum Cell viability: No cytotoxic effects (viability>95% with blue dye exclusion method) Vehicle controls valid: not examined; negative control: yes; positive controls valid: not examined	Fabiani et al. (2001)
DNA content determined after alkaline treatment of the cells and neutralisation - Rat hepatocytes - Concentrations tested: 0 - 110 - 220 - 330 µg/mL (0 - 1000 - 2000 - 3000 µM) - Exposure time: 1 hour - Metabolic activation: without Not performed according to a standard guideline; only without activation metabolic; Non-GLP Reliability 2: reliable with restrictions	**Positive** without metabolic activity	**Positive** Increase of single strand breaks (SSB) with a dose response: threshold value=1000 µM Slight increase of SSB with the period of exposure (0, 20, 40 and 60 min) at 3000 µM Cotreatment with Ca^{2+}-chelator Quin-2 AM decrease DNA damage Cytotoxicity: viability about 75% and no change after treatment at any dose tested Vehicle controls valid: not	Solveig Walles (1992)

		examined; negative controls valid: yes; positive controls valid: yes	
DNA strand breaks/ Fluorimetric analysis of DNA unwinding - CHO-K1 cells - Concentrations tested: 0-50-100-150-200-250 µM - Exposure time: 45 min - Metabolic activation: without Not performed according to a standard guideline; only without activation metabolic and no details about controls; Non-GLP Reliability 3	**Negative** without metabolic activation	Catechol did not produce DNA strand breaks. Cytotoxicity: 50% of cell death is 100 µM Vehicle controls valid: not examined; negative control valid: not examined; positive controls valid: not examined	Sze et al. (1996)
DNA damage and repair/ Inhibition of synthesis - Mouse L5178YS cells - Concentrations tested: 0.11 to 110 µg/mL (0.001 mM to 1 mM including 10 concentrations) - Exposure time: 30 min - Metabolic activation: without Not performed according to a standard guideline; only without activation metabolic and no details about controls; Non-GLP Reliability 2: reliable with restrictions	**Positive** without metabolic activity	Strong DNA synthesis inhibition at 0.1 mM (65%) When doses increase, irreversible inhibition of DNA synthesis at >1 mM Cytotoxicity: no effect on membrane integrity and protein synthesis as high as 1 mM (90% of cell viability) Vehicle controls valid: not examined; negative control valid: not examined; positive controls valid: yes	Pellack-Walker et al. (1985)
DNA damage and repair/ Inhibition of synthesis - Mouse L5178YS cells - Concentrations tested: 0.11 to 110 µg/mL (0.001 mM to 1 mM) - Exposure time: 30 min - Metabolic activation: without Not performed according to a standard guideline; only without activation metabolic and no details about controls; Non-GLP Reliability 3	**Negative**	No increase of Single Strand Breaks as high as 1 mM <u>Cytotoxicity:</u> 0.01mM is the highest nontoxic dose according to blue trypan dye exclusion 0.001mM is the highest nontoxic dose according to protein synthesis Vehicle controls valid: not examined; negative control valid: not examined; positive controls valid: not examined	Pellack-Walker et al. (1986)
Inhibition of DNA synthesis - Mouse bone marrow cells - Concentrations tested: 0 - 0.66 - 1.321 - 1.982 - 2.642 µg/mL (0 - 6 - 12 - 18 - 24 µM) - Exposure time: 60 min - Metabolic activation: without Not performed according to a standard guideline; only without activation metabolic and no details about controls; Non-GLP Reliability 3	**Positive** without metabolic activation	**Positive:** Inhibition of 52% of the nuclear synthetic activity at 24 µM; IC_{50}=23µM <u>Cytotoxicity:</u> over 94% of cells viable at all concentration tested Vehicle controls valid: not examined; negative control valid: not examined; positive controls valid: not examined	Lee et al. (1989)
DNA damage and repair: GreenScreen assay (GSA) - *Saccharomyces cerevisiae:* GENC01 and GENT01 strains	**Positive** without metabolic activation	**Positive response** in growth inhibition rate with strain GENC01 at 177 µg/mL	Cahill et al. (2004)

- Concentrations tested: from 177 to 800 µg/mL - Metabolic activation: without Induction of the RAD54 promoter due to DNA damage results in production of the extremely stable green fluorescent protein (GFP), which is fluorescent in the green spectrum when illuminated by blue light. Relative total growth assessed by comparing the extent of proliferation of treated cells with that of untreated cells. Not performed according to a standard guideline; only without activation metabolic; Non-GLP Reliability 2: reliable with restrictions		Clear genotoxicity with GFP induction with strain GEN T01 at 599 µg/mL Vehicle controls valid: yes; negative control valid: yes; positive controls valid: yes	
In vitro DNA damage ^{32}P-labeled DNA fragment - Human DNA fragments - Concentrations: 0-0.1-0.2-0.5-1-2-5-10-20 µM - With or without the addition of Cu^{2+} ions (20 µM) and/or NADH (100 µM) - Metabolic Activation: without The test was not carried out according to the international recognized guidelines; no details about positive control; Non-GLP Reliability 2: with restrictions	**Positive** without metabolic activation	<u>Catechol alone:</u> no genotoxic effects <u>Catechol + Cu^{2+} ions:</u> small DNA damage at 10 and 20 µM <u>Catechol + Cu^{2+} ions + NADH:</u> High level of DNA damage at 5 µM of catechol ⇨ DNA damage resulted from base modification at guanine and thymine residues in addition to strand breakage induced by Cu^{2+} and H_2O_2 Cytotoxicity: not determined Negative control: yes (valid); vehicle control: not examined; positive control: not examined	Hirakawa et al. (2002)
In vitro, 8-oxodG production - Human Leukaemia cell line HL-60 and HP 100 - Concentration : 0 - 1.1 - 2.2 - 5.5 µg/mL (0 - 10 - 20 - 50 µM) - Exposure time: 120 min - Metabolic activation : without Test was not carried out according to recognized international guidelines; No metabolic activation ; Cell viability not checked; No details about controls; Non-GLP Reliability 3	**Positive** without metabolic activation	<u>HL-60 cells:</u> Increase of 8-oxodG content in a dose dependent manner (indicative of oxidative base damage); significant increase at 20 µM; and DNA ladder (associated with apoptosis) at 50 µM (4 hours treatment) <u>HP-100 cells:</u> No increase of 8-oxodG content and no DNA ladder Cytotoxicity: not examined Vehicle controls valid: not examined; negative control: yes; positive controls valid: not examined	Oikawa et al. (2001)

4.9.1.2. *In vivo* data

Studies selected for evaluating mutagenicity of pyrocatechol *in vivo* are mainly collected from CSR report and also from literature.

<u>*Micronucleus assays*</u>

Five *in vivo* studies were performed with a protocol similar to OECD Guideline 474 ("*In vivo* Mammalian Erythrocyte Micronucleus Test"), only two of them were considered of reliability of 2 according to Klimisch scale (Marrazzini et al., 1994; Ged-El-Karim et al., 1985).

- Oral route:

In the study of Gad-El-Karim et al. (1985), group of 3 to 5 males mice received by oral route 0 or 150 mg/kg bw of catechol. After 30 hours of dosing, animals were sacrificed and bone marrow from femur was used for the micronucleus test. The statistical analysis did not revealed difference between treated and negative controls animals in micronucleated PCE/NCE (PCE: Polychromatic Erythrocytes). This study revealed that pyrocatechol at 150 mg/kg bw was not considered to induce micronucleus.

Ciranni et al. (1988a) exposed mice (4 animals /group) to catechol at 40 mg/kg bw in a single time by oral route. The proportion of PCE in bone marrows smears was calculated by counting both NCE and PCE. Until 3000 PCE had been scored for the presence of micronuclei after 18, 24, 42 and 48h. During oral route experiment, catechol produced a significant increase of micronuclei at 24h with evident bone marrow depression.

- Intraperitoneal route

In the study of Marrazzini et al. (1994), 3 males mice per group received single administration of catechol by intraperitoneal route at concentration of 0, 10, 20, 30 mg/kg bw. Catechol statistically induced micronuclei in a dose-dependent manner in bone marrow of femur. No variation of the PCEs/NCEs (NCE: Normochromatic Erythrocytes) ratio after 18h of exposure was observed.

Ciranni et al. (1988b) exposed mice (4 animals /group) to catechol at 40 mg/kg bw in a single time by intraperitoneal route. The proportion of PCE in bone marrows smears was calculated by counting both NCE and PCE. Until 3000 PCE had been scored for the presence of micronuclei after 18, 24, 42 and 48h. Significant genotoxic effects were observed at 24h after intraperitoneal injection and they were more pronounced than by oral route.

- Subcutaneous injection

In the study of Tunek et al. (1982) catechol was tested at 9 doses ranging between 5 and 42 mg/kg bw/d, injected once daily for 6 consecutive days. Cellularity did not significantly differ from control values at any dose and no increased frequency of micronuclei was observed (data not presented in the publication). As no toxicity was observed, they are no proof bone marrow exposure.

<u>*In vivo comet assays*</u>

An*in vivo* comet assay study is available and showed negative results in stomach and positive results in duodenum cells. Even if catechol is hence devoid of genotoxic activity on the stomach cells from rat, it induced statistically significant increase in DNA strand breaks at non-lethal doses on rat duodenum cells after oral administration. The highest increase of median olive tail moment (OTM) was observed at the lowest dose tested of 100 mg/kg/day (x2) (Study report n°18255, 2008). Furthermore, the very low cell density observed at the two highest doses tested (200 and 400 mg/kg/day x2) indicated a probable cell lysis due to cytotoxicity and /or highly damaged cells with loss of information. Under these conditions, catechol was considered as a DNA strand breaks and/or alkali-labile sites inducer on duodenum cells.

<u>*Unscheduled DNA synthesis*</u>

Another *in vivo* study showed the genotoxicity of catechol on esophageal epithelial cells of rats exposed through drinking water and diet (Mirvish et al. 1985).

Catechol at concentration of 1-8 g/l significantly enhanced the uptake of tritium-labelled thymidine relative to untreated rats in a dose dependent manner, so catechol was able to stimulate the DNA synthesis. Cells from gut of rodents (duodenum and esophageal epithelial cells) appear sensitive to gentoxicity of catechol while no DNA damages were observed in stomach cells of rats (Furihata et al. 1989). In this study on stomach cells, results indicated an absence of induction of unscheduled DNA synthesis in the pyloric mucosa of the stomach of rats treated with catechol. In the same study, the administration of catechol at doses from 37.5 to 90 mg/kg bw did not induce DNA single strand scission in the pyloric mucosa of the stomach as determined by the alkaline elution method after 2 and12 h. The fraction of DNA remaining on filter 2 and 12h after administration of catechol at the dose of 75 mg/kg remains in the same range than distilled water (0.8 to 1.0). The elution rate constant did not increase after administration of catechol suggesting that catechol did not induce single break scission of DNA in the pyloric mucosa.

Mouse spot test

In the study of Fahrig et al. (1984) a Mouse Spot Test equivalent or similar to OECD Guideline 484 was performed. Female mouse received i.p. injections of catechol at concentration of 22 mg/kg bw on days 9, 10 and 11 postconception with or without co-treatment with ENU. Catechol alone did not modified the apparition of color spots 2/216 (1%) compared to negative controls, so there was the same mutation rate in both conditions. When catechol was co-administered with ENU (ethylnitrosourea), the effects of ENU was slightly but not statistically significant enhanced. So catechol was considered as non-mutagen during *in vivo* exposure.

Other in vivo assays

Pyrocatechol was also considered not to have genotoxic effect on DNA repair system against E. coli K-12 uvr B/recA DNA repair in the Mice organs (Hellmer and Bolcsfoldi 1992).

Table 14: Summary of relevant *in vivo* mutagenicity studies for pyrocatechol

Test	Results	Remarks	Reference
***In vivo*, Mouse spot test**			
Gene mutation test, mouse spot test - Mouse embryos (122 females treated) - Intraperitoneal injection - Dose: 22 mg/kg on days 9, 10, 11 post-conception with or without treatment of ethylnitrosurea (ENU), a carcinogen, at 30 mg/kg (IP) Equivalent or similar to OECD Guideline 484 (Genetic Toxicology: Mouse Spot test) Only one dose tested, Non-GLP Reliability 2: with restriction	**Negative**	Without NRU: mutation rate same as control mutation rate (2%) With NRU: Catechol enhances slightly the mutagenic effect of ENU Toxicity: no effects Vehicle controls valid: yes; negative control: yes; positive controls valid: yes	Fahrig (1984)

In vivo, **DNA damage assay**			
DNA damage and/or repair; Alkaline Comet assay - Rat (Sprague-Dawley) male (5-10 weeks old); 5 animals/dose - Oral gavage - Doses tested: 100 mg/kg/day - 200 mg/kg/day - 400 mg/kg/day (nominal in water) - 2 treatments at 24 hours interval, one sampling time 3 to 6 hours after last treatment Equivalent or similar to OECD guideline 489 *(in vivo* Comet assay on mammalian cells), GLP Reliability 2: reliable with restriction	**Positive** in duodenum cells	Genotoxicity: Stomach cell: no significant increase of DNA strand breaks Duodenum cells: Significant increase in DNA strand breaks at 100 and 200 mg/kg/day (x2); highest increase at the lowest dose (100 mg/kg/day x2) Cytotoxicity: yes, highly damaged cells at 200 and 400 mg/kg/day x2 Toxicity: yes Maximum Tolerated Dose= 400 mg/kg/day Vehicle controls valid: yes; positive controls valid: yes	Study report n° 18255 (2008)
DNA damage/repair, Unscheduled DNA synthesis ; Alkaline elution of DNA - Rat male (344/DuCrj), 6-8 weeks old; 4-5 rats/group (pyloric mucosa of stomach) - Oral gavage - Single dose: 0-10-20-37.5-75-90 mg/kg bw in the presence of tritiated thymidine for UDS experiment - Exposure time: 2, 12, 24h Equivalent or similar to OECD Guideline 486 (Unscheduled DNA Synthesis or UDS Test with Mammalian Liver Cells *in Vivo*) Cells examined are pyloric mucosa of stomach instead of liver cells (UDS); only male animals treated; no data about positive controls (UDS); Non-GLP Reliability 2: reliable with restriction	**Negative**	Unscheduled DNA synthesis (UDS) Negative results: no induction of UDS Dose-dependent stimulation of replicative DNA synthesis Vehicle controls valid: yes; negative control: not examined; positive controls valid: not examined Alkaline elution assay No increase of elution rate at 2h and 12h suggesting no increase of single break scission of DNA Vehicle controls valid: yes; negative control: yes; positive controls valid: yes	Furihata et al. (1989)
DNA damage/repair, Unscheduled DNA synthesis - Rat Wistar male, 4-5 weeks, 4 animals/group, esophageal epithelial cells - Doses tested daily: 1, 2, 4, 8 g/L/day (drinking water) - Or 4 g/kg/day (diet semipurified)Exposure time: 7 days - Injection of tritium-labelled thymidine 1 hour before sacrifice into the esophageal epithelial Not performed according to a standard guideline; Only male animals treated; no details about controls; Non-GLP; no data on food or water consumption Reliability 2: reliable with restriction	**Positive**	Drinking water: catechol significantly enhanced the uptake of tritium-labelled thymidine incorporation into esophageal epithelial DNA relative to that in untreated rats in a dose dependent-manner. Diet: catechol significantly enhanced the uptake of tritium-labelled thymidine relative to that in untreated rats. This effect is more consistent than in drinking water. Vehicle controls valid: not examined; negative control: yes; positive controls valid: not examined	Mirvish et al. (1985)

DNA damage/repair; E. Coli K-12 DNA repair host-mediated assay - Mouse NMRI (male), 7 animals/group Bacteria strain: E. Coli K-12 343/636 and 343/591 - Oral route - Single dose: 200mg/kg - Exposure time: 2h Not performed according to a standard guideline; Only one dose tested; only male mice treated; route of administration not specified (oral route?); no positive control; Non-GLP Reliability 3	**Negative**	No difference between treated and control for the ratio: number of colonies of DNA repair deficient strain/ number of colonies of DNA repair proficient strain in any organs of the mice (blood, liver, lungs, kidneys, testes) Vehicle controls valid: yes; negative control: not examined; positive controls valid: not examined	Hellmer and Bolcsfoldi (1992)
***In vivo*, micronucleus assay**			
In vivo Micronucleus assay - Mouse CD-1 male, 6-8 weeks old (3 animal/group) - Intraperitoneal - Single dose: 10-20-30 mg/kg bw (3 animals/dose) - Exposure time: 18h - At least 3000 PCEs (Polychromatic erythrocytes) Equivalent or similar to OECD guideline 474 (Mammalian Erythrocyte Micronucleus Test) Low relevance of administration route; only male mouse treated; Non-GLP Reliability 2: with reliable restrictions	**Positive**	Genotoxicity: **positive** with significant induction of micronuclei in bone marrow cells, with a dose dependent No variations of PCEs/NCE Vehicle controls valid: not examined; negative control: yes; positive controls valid: not examined	Marrazzini et al. (1994)
In vivo Micronucleus test - Mouse CD-1 male, bone marrow cells from femur (3-5 animal/group) - Oral gavage - 1 single dose tested: 150 mg/kg - Exposure time: 30 hours Equivalent or similar to OECD guideline 474 (Mammalian Erythrocyte Micronucleus Test) Only male mice treated ; only one dose tested; Non-GLP Reliability 2: with restrictions	**Negative**	Genotoxicity: **negative** (male) According to the authors, the dose of pyrocatechol was a very toxic single dose and convulsive seizures occur after administration. Vehicle controls valid: yes; negative control: not examined; positive controls valid: yes	Gad-El-Karim et al. 1985

In vivo Micronucleus test - Male NMRI mice (polychromatic erythrocytes) *in vivo* (4 mice per group) - Concentration tested: 9 doses between 5 and 42 mg/kg bw - Subcutaneous injection: one per day for 6 consecutive days Equivalent or similar to OECD guideline 474 (Mammalian Erythrocyte Micronucleus Test) Only male mice treated; Administration route not relevant; No details about controls; Non-GLP; very short reporting Reliability 3	**Negative**	No increase of frequency of micronucleus in polychromatic erythrocytes Cellularity did not differ from control values at any dose Vehicle controls valid: yes; negative control: not examined; positive controls valid: not examined	Tunek et al. (1982)
In vivo Micronucleus test - Pregnant female CD-1 mouse bone marrow and fetal liver - One single dose tested: 40 mg/kg - Oral route: gastric intubation - Time: 0-15-18-24-30-36-40h Equivalent or similar to OECD guideline 474 (Mammalian Erythrocyte Micronucleus Test) Only pregnant females; only one dose tested; Only micronuclei in fetal liver cells presented; Non-GLP Reliability 3	**Positive**	Pregnant female: Significant increase of micronuclei in the polychromatic erythrocytes from bone marrow after 24h, 36h and 48h. Fetal liver: Induction of micronuclei (1-3 fold over the control values), only significant at 18h Toxicity observed in fetal liver cells: Reduction of the PCE/PNE ratio (reduction between 30 and 60% compared to control) after 9 and 12h. Vehicle controls valid: not examined; negative control valid: yes; positive controls valid: not examined	Ciranni et al. (1988a)
In vivo Micronucleus test - Male CD-1 mouse (6-8 weeks; 4 animals per group), bone marrow smears - Single dose tested: 40 mg/kg - Oral route or intraperitoneal - Exposure time: 18-24-42-48h Equivalent or similar to OECD guideline 474 (Mammalian Erythrocyte Micronucleus Test) Only one dose tested; no details about controls; Non-GLP Reliability 3	**Positive**	Oral route: significant increase of micronuclei in the polychromatic erythrocytes only at 24h with evident bone marrow depression. Intraperitoneal: significant increase of micronuclei in the polychromatic erythrocytes from bone marrow only at 24h and evident bone marrow depression starting from 18h after treatment. Vehicle controls valid: not examined; negative control: not examined; positive controls valid: not examined	Ciranni et al. (1988b)

4.9.2. Human information

No data available

4.9.3. Other relevant information

Three other *in vitro* studies were performed to evaluate the genotoxicity of catechol using topoisomerase inhibition. These 3 studies are considered of reliability of 2 according to Klimisch scale. Results from topoisomerase assay I indicated no inhibition effect of catechol observed at the only high dose tested 1000 µM. However, a significant inhibition effect was only observed at 1000 µM in topoisomerase assay II Chen and Eastmond (1995). Another study showed that catechol required bioactivation by peroxidase (presence of hydrogen peroxide) to inhibit topoisomerase II at 10 µM and 100 µM (Frantz et al. 1996). However, catechol had no effect on inhibition of topoisomerase II at concentrations up to 300 µM even though inhibition of peroxidase activation was observed with catechol at 30 µM (Baker et al. 2001).

In conclusion, the inhibition effect of catechol was only observed on Topoisomerase II and in specific *in vitro* conditions.

4.9.4. Summary and discussion of mutagenicity

Mutagenicity effects of pyrocatechol in the different *in vitro* models indicated positive effects mainly on mammalian cells without metabolic activation system. High mutagenic frequency was observed at concentrations of pyrocatechol below 10 µg/ml. Mutagenic and cytotoxic effects may be induced by independent chemical species with probably superoxide anion-mediated for mutagenicity. Only one study on bacteria (TA102) showed mutagenicity of pyrocatechol suggesting oxidative properties (Study report n° FSR-IPL 060904-01 2007). During *in vitro* experiment, pyrocatechol is also able to induce genotoxic effects on mammal's and human cells with or without metabolic activation: chromatid breaks, chromatid exchange and micronucleus production. Genotoxic effect of pyrocatechol seems to be dose-dependent and link to specific mechanism of oxidative property. It had not been clearly demonstrated whether or not this genotoxic effect had a threshold.

Results collected from *in vivo* experiments revealed that pyrocatechol is able to induce the production of single strand breaks on duodenum cells and esophageal epithelial cells of rodents after oral treatment (Study report n° 18255 2008; Mirvish et al. 1985). However, no genotoxic effects have been observed on stomach cells of rodent (Study report n° 18255 2008, Furihata et al. 1989).

Pyrocatechol induced micronuclei formation on erythrocytes after oral and intraperitoneal administration in a dose-dependent manner (Marrazzini et al. 1994; Ciranni et al. 1988a, 1988b). However, contradictory results have been noticed by assessing the micronuclei formation on mouse exposed by oral route to pyrocatechol. On one hand, one author didn't observe any genotoxic effects of pyrocatechol on mouse exposed to 150 mg/kg after 30 hours (Gad-El-Karim et al. 1985); the dose was stated to be very toxic in the publication. On the other hand, a significant increase of micronuclei in the PCE was measured on male and female mice exposed to 40 mg/kg of pyrocatechol after 24 hours by oral route (Ciranni et al. 1988a, 1988b). This study was reliability 3 because it was performed without any positive control, while the first one (Gad-El-Karim et al. 1985) was reliability 2 and validated by a vehicle and a positive control. Nevertheless, a significant induction of micronuclei was measured on mice exposed to 10-30 mg/kg bw of pyrocatechol per i.p. during 18h (Marrazini et al. 1994). By summarizing the overall studies performed during *in vivo* experiments, 3/5 positive micronucleus studies and a positive screening comet assay in duodenum cells suggest a potential genotoxic effect of pyrocatechol.

4.9.5. Comparison with criteria

According to CLP classification of a substance as mutagen **Category 1B** is based on the following criteria.

- Positive result (s) from *in vivo* heritable germ cell mutagenicity test in a mammals; or
- Positive result (s) for *in vivo* somatic cell mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- Positive result from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cell of exposed people.

Classification into **Category 2** according to CLP is required for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans based on:
- Positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:
    - Somatic cell mutagenicity tests *in vivo*, in mammals.
    - Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

4.9.6. Conclusions on classification and labelling

No genotoxic effects of pyrocatechol have been observed on germinal cells, so pyrocatechol can't be classified in Category 1B mutagen.

There is no ADME data in the registration dossier showing availability of Pyrocatechol in reproductive tissues.
The few available data on the registration dossier and a rapid search do not provide evidence of effects of pyrocatechol on reproductive organs.

All in *vitro* studies available indicated a genotoxic effect of pyrocatechol on different animals and human somatic cells lines studied. However, *in vivo* experiment showed contradictory results about the potential genotoxicity of pyrocatechol. Clear evidences of genotoxicity in rat by oral route on duodenum (Comet) and esophageal tissue (UDS) have been shown (Study report n° 18255 et al. 2008; Mirvish et al. 1985). A study has shown the ability of pyrocatechol to induce clastogenic effect after an intraperitoneal injection on mice: a significant induction of micronuclei in bone marrow cells with a dose dependant was observed (Marrazzini et al. 1994). Two others supportive experiments confirmed the ability of pyrocatechol to induce micronuclei formation in bone marrow cells by I.P. and oral routes, although some contradictory results were observed without clear explanation .

On this basis, according to classification criteria of EC regulation 1272/2008 the pyrocatechol should be classified in **Category 2 mutagen** (H341: Suspected of causing genetic defects).

4.10. Carcinogenicity

4.10.1. Non-human information

4.10.1.1. Carcinogenicity: oral

The carcinogenic and co-carcinogenic effect of pyrocatechol in animals was evaluated by 38 studies of reliability 2 (according to Klimisch scale) including 8 carcinogenicity studies. Only validity 2 studies were selected and reported in table 18 of the CLH report. They were performed on rodents by oral feeding of pyrocatechol. The objectives of these studies were mainly to assess effects of pyrocatechol on specific organs such as stomach. According to data available, there is no study investigating effects of pyrocatechol on all organs from the whole body of rodents.

Carcinogenicity studies

- In rats

The potential reversibility of glandular stomach lesions induced by catechol was studied by Hirose et al. (1992). Male F344 rats were treated continuously with 0.8% catechol in the diet for 12, 24, 48, 72, or 96 weeks followed by a return to basal diet for 84, 72, 48, 24, and 0 weeks, respectively. 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 time dependently. 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 adenoma and non-tumorous areas decreased significantly after cessation of catechol treatment. Results indicate that neoplastic lesions (adenoma and hyperplasia) from short time exposure to catechol (12 to 24 weeks) have the potential to regress after a long recovery period (basal diet) of 72 or 84 weeks (see table below).

Table 15: Histopathological findings in the glandular stomach from the carcinogenity study with recovery period (Hirose et al. 1992)

Table 1 ***Histopathological findings in the glandular stomach: incidence data***

Group	Treatment (wk) Catechol	Basal diet	No. of rats	No. of rats with (%) Hyperplasia	Adenoma	Adenocarcinoma
1	12	0	10	9 (90)	2 (20)	0
2	12	84	17	6 (35.3)[a]	2 (11.8)	0
3	24	0	10	10 (100)	10 (100)	0
4	24	72	16	10 (62.5)	12 (75)	1 (6.3)
5	48	0	10	10 (100)	10 (100)	1 (10)
6	48	48	14	14 (100)	14 (100)	3 (21.4)
7	72	0	10	10 (100)	10 (100)	4 (40)
8	72	24	18	18 (100)	18 (100)	9 (50)
9	96	0	15	15 (100)	15 (100)	11 (73.3)
14	0	96	12	0	0	0

[a] Significantly different at $P < 0.02$ *versus* group 1.

Biological changes of rat's glandular stomach after catechol treatment were also studied at short (7 days) and mid-long term (24 weeks) time of exposure (Hirose et al. 1999). Males' rats (5 per group) were treated at different concentrations of catechol: 0.01, 0.1, 0.5 or 1% in diet for 7 days and sacrified after 12h, 1, 2, 3 and 7 days; and 0.8% for 24 weeks. Short time exposure (7 days) showed that catechol induced first apoptosis, inflammation and erosion or ulceration in pyloric region. Catechol induced toxicity and continuous strong cell-proliferation responsible of glandular stomach carcinogenesis. This effect had a threshold of 0.01%. Experiment at 24 weeks confirmed these observations with apparition of polyploid hyperplasia and adenoma at the end of the treatment.

Carcinogenicity of catechol on stomach was also investigated on male and female F344 rats (Hirose et al. 1993a). Rats (30/group) were treated with 0.8% (around 480 mg/kg bw/d) of catechol in powdered diet continuously for 104 weeks. At necropsy, neoplastic lesions were mainly observed in glandular stomach of animals (both sexes). Adenomas and submucosal hyperplasia were found in all rats. Moreover, 15/28 (54%) of male rats and 12/28 (43%) female rats had well differentiated adenocarcinomas. Although there was no significant increase of papilloma in the forestomach epithelium, incidences of squamous cell hyperplasia were significantly increased in forestomach of rats from both sexes. This study showed that catechol exerts clearly a carcinogenic activity in rat glandular stomach epithelium.

Carcinogenicity of catechol was studied by using different strains of rats (Tanaka et al. 1995): Wistar, Lewis, Sprague Dawley or WKY. They were treated with 0.8% of catechol in diet (equivalent to around 320 mg/kg bw/d using default standard food intake value for rat of 40 g/kg bw/d), for 104 weeks. Neoplastic lesions were observed in forestomach with hyperplasia (Wistar, WKY and SD) and papilloma only in SD strain. Glandular stomach appears more sensitive to catechol effect with a clear strain differences in the induction of adenocarcinomas was noticed. Even though submucosal hyperplasia, adenoma and ulceration were observed in glandular stomach of all rats' strains, only 3 strains (Wistar, Lewis, Sprague Dawley) showed adenocarcinomas in their glandular stomach.

Another carcinogenicity study on male rats (104-weeks of exposure to 0.16%) indicated a significant decrease of body weight (-13%) and a slight increase (3%) of forestomach papillomas in catechol treated group as compared to the basal diet group (not statistically different) (Hirose et al. 1997). However, this study confirmed the sensitivity of glandular stomach to catechol as a significant increase of incidence of submucosal hyperplasia and adenoma was measured.

In the study of Hagiwara (2001), the potential carcinogenesis of catechol was investigated on glandular stomach of male F344 rats during 104 weeks. Strong retardation of body weight (-17%) was observed in the 0.8% group, but no adverse effects were found in terms of survival. Results demonstrated that dietary levels of 0.4% and 0.8% of catechol (141 and 318 mg/kg bw/d average intake, respectively) long-term exposure (104 weeks) induced not only an increase of submucosal hyperplasias and adenomas but also a low number of adenocarcinomas and sarcomas were observed (non-significant) in the pyloric glands, while 0.1 and 0.2% groups showed only benign proliferative lesions (submucosal hyperplasia and adenoma), all accompanied by a significant increase of serum gastrin levels.

- In mice

Carcinogenicity of catechol was also studied on mice in this study (Hirose et al. 1993a). Mice (30 per group) were fed with diet containing 0.8% (ca. 960 mg/kg bw/d) of catechol during 96 weeks. The authors observed a slight reduction of survival rate, a significant diminution of body weight (males: -22%; females: -41%) and a significant increase in relative liver body weight. Main lesions were observed in stomach with significant increase of squamous cell hyperplasia in forestomach and glandular stomach. Incidence of adenomas was significantly increased in glandular stomach but no adenocarcinoma was observed in glandular stomach or forestomach. Mice appear less sensitive than rats to carcinogenicity of catechol (Hirose et al. 1993a).

<u>Initiation-Promotion models</u>

Tumour promotion was studied in rats pre-exposed to a single intragastrin dose of 150 mg/kg bw/d of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), then rats received or not 1.5% of catechol diet for 4 weeks and 0.8% for 47 weeks (Hirose et al. 1987, rapid communication). 10-20 male F344 rats/group were treated with catechol alone or basal diet. A significant increase of incidence of squamous cell carcinoma was measured in the forestomach of rats exposed to MNNG and catechol. A slight but non-significant hyperplasia and papillomas were also observed in catechol and MNNG + catechol groups. Glandular stomach appears very sensitive to catechol with a significant increase of incidence of adenomatous hyperplasia and adenocarcinoma in MNNG + catechol group. Rats treated with catechol showed only an increase of incidence of adenomatous hyperplasia. These results underline the fact that catechol is a potential carcinogen for glandular stomach.

A similar study on tumour promotion study on rats was performed by Wada (1988). Incidence of hyperplasia, papillomas and squamous cell carcinomas were strongly increased in the forestomach of rats treated with MNNG and catechol (0.8%) after 52 weeks of treatment. Catechol alone significantly increased only incidence of hyperplasia (mild to severe) in the forestomach of rats compared to basal diet. Lesions of the glandular stomach were predominantly developed in the pyloric region: all rats treated with MNNG and catechol showed submucosal hyperplasia and adenoma. Adenocarcinomas were observed in glandular stomach of 73% of rats treated with MNNG and catechol. Catechol alone significantly increased incidence of adenoma and submucosal hyperplasia in the glandular stomach of rats. Adenocarcinomas were observed in only one rat (7%). This study confirmed the potential carcinogenesis of catechol on stomach, especially glandular stomach.

Yamagushi et al. (1989) showed a significant increase of incidence of carcinoma in esophagus, a significant increase of incidence of preneoplastic hyperplasia and papilloma in tongue of rats exposed to MNAN (methyl-N-amylnitrosamine) and catechol (0.8%). A significant decrease of alveolar hyperplasia was observed in lung and no effect on nasal cavity was noticed.

Tumour promotion study of catechol was also studied on urinary bladder (Kurata et al. 1990). Incidence of papillomas and carcinoma in bladder were slightly increased when rat received BBN + catechol during 32 weeks compared to rat that received only BBN (4 weeks pre-treatment) or only catechol (0.8% of catechol).

One tumour promotion study (Hasegawa et al.1990) showed that treatment with catechol (0.8% for 30 weeks) alone had no effect (carcinoma or adenoma) on kidneys, bladder and thyroid of rats. Catechol induced submucosal and adenomatous hyperplasia in pyloric region of glandular stomach. Anti-carcinogenetic effects of catechol with DHPN were observed in lung and thyroid: a significant decrease of number and areas of lung neoplastic lesions and a low decrease of carcinoma in thyroid (non-significant). This study revealed that treatment with DHPN and catechol seemed to decrease slightly the incidence of carcinogenic effect (thyroid and lung) observed with DHPN alone.

In the tumour promotion study of Fukushima (1991), rats were feed for 16 weeks with or without 0.8% of catechol after a pre-treatment of 4 weeks to DEN, MNU and DHPN. Catechol decreased significantly the number of GST-P positive foci. DMD+Catechol treatment induced significantly hyperplasia and papilloma on forestomach and submucosal hyperplasia on glandular stomach. Catechol had no effect on urinary bladder, thyroid, and esophagus.

Maruyama et al. (1991), studied female Syrian golden hamsters. They observed a significant decrease of body weight and liver weight of female hamster treated to catechol (0.75 to 1.5% by oral feed) for 20 weeks with pre-treatment to BOP. No neoplastic lesions were evident in female hamsters administered catechol after saline or BOP pre-treatment. The numbers of atypical pancreatic hyperplasias and adenocarcinomas in hamsters treated with 0.75% catechol after BOP were significantly decreased when compared to control group (BOP). Liver lesions were not found in hamsters administered with catechol after saline. A slight decrease of hepatocellular carcinomas was noticed in the group treated with 1.5% catechol after BOP compared to BOP control group. According to the authors, results indicate that catechol exerted a weak inhibitory effect on pancreatic carcinogenesis after initiation of female hamsters with BOP at low dose of 0.75%.

Another tumour promotion study exposed rats to a lower dose of catechol (0.2%) than previous studies during 36 weeks (after MNNG pre-treatment) (Hirose et al. 1991). These authors noticed no significant effect on forestomach with only a significant increase of incidence of hyperplasia and adenomas in glandular stomach. No adenocarcinomas were observed in forestomach or glandular stomach of rats treated with catechol (0.8%).

Hasegawa et al. (1992) exposed F344 male rats to 0.2 and 0.8% in diet with catechol for 6 weeks only. Rats were pre-treated by i.p. with 200 mg/kg bw/d of diethylnitrosamine (DEN) during 2 weeks. The body weight was decreased (-20%) in group treated at 0.8% catechol. Rats pre-treated with DEN and treated with catechol at 0.8% showed a significant tumour inhibition (medium-term bioassay method using preneoplastic glutathione S-transferase-positive liver cell foci as the endpoint marker lesion).

A similar study was performed by Kajimura (1992) where rats (15 per group) were exposed to catechol at 0.8% for 16 weeks after a DMD pre-treatment. Strong hyperplasia and papillomas in forestomach and submucosal hyperplasia in glandular stomach were observed after DMD pre-treatment with catechol. Slight cell hyperplasia and papilloma were observed in urinary bladder, and low incidence of alveolar/bronchiolar adenoma in lung of rats pre-treated with DMD and catechol.

In the study of tumour promotion of Hirose et al. (1993b), rats were treated with catechol in diet at 0.8% (ca. 480 mg/kg bw/day) for 28 weeks (including 4 weeks of multiple initiation periods with DEN, DHPN and NaNO2). A significant decrease of final body weights of animals treated with catechol was noticed (with or without pre-treatment). All rats treated with catechol alone had submucosal hyperplasia, most of them had adenomas in the glandular stomach. A significant increase of adenocarcinomas was also observed in rats pre-treated with carcinogen and catechol. Forestomach results showed a mild to severe hyperplasia and a significant increase of number of carcinoma *in situ* and squamous cell carcinoma in rats pre-treated with mutagens + catechol with or without $NaNO_2$ treatment. Numbers of GSTP-P-positive foci in liver were significantly reduced in rats pre-treated with mutagens and catechol ($p<0.05$). In the other organs examined, catechol was shown to significantly reduce the incidence of thyroid follicular cell hyperplasia from 64% (basal diet) to 7% (catechol and catechol+NaNO2; $p < 0.01$), the incidence of follicular cell adenoma from 29% (basal diet) to 0% (catechol +NaNO2; $p < 0.05$) and the incidence of kidney nephroblastoma from 36% (basal diet) to 0% (catechol; $p < 0.05$). At the opposite, papillomas of oesophagus were increased to 50% after treatment with catechol +NaNO2 ($p < 0.01$).

Another tumour promotion study performed on rats exposed to 0.8% of catechol during a similar time, 36 weeks (after pre-treatment to 0.1% of EHEN administered in drinking water for 3 weeks) (Okasaki et al. 1993). Nevertheless, this study revealed no carcinogen effects but only toxic effects on liver, kidney and body weight.

These results are confirmed by Kawabe et al. (1994). 20 F344 male rats were given 150 mg/kg bw of MNNG by gavage. One week later, rats were exposed for 51 weeks to 0.8% of catechol in diet with and without $NaNO_2$ in the drinking water. Further 15 rats/group received catechol with and without $NaNO_2$ without MNNG pre-treatement. The stomach, esophagus, liver, kidneys and macroscopic lesions were weighted and examined histopathologicaly. Treatment with MNNG alone result in only small nodules in the forestomach. In rats treated with catechol without MNNG, significant development of hyperplasia in the forestomach. In the glandular stomach, submucosal hyperplasia (27%), adenomas (100%) and adenocarcinomas (33%) were observed. Additional exposure to $NaNO_2$ further increased the degree of hyperplasia and papilloma development in the catechol group in forestomach but no influence of $NaNO_2$ was found in the lesion in the glandular stomach. After MNNG initiation, catechol enhanced the incidence of carcinomas but additional treatment with $NaNO_2$ did not further enhance lesion development. In the other organs, no histopathological findings suggestive of the influence of catechol and $NaNO_2$ were evident.

Table 16: Lesion in the forestomach in rats with and without MNNG treatment (Kawabe et al. 1994)

Treatment			Effective no of rats	Incidence of rats		
MNNG	$NaNO_2$	chemical		Hyperplasia	Papilloma	SCC
+	+	Basal diet	20	20	15	5
+	+	Catechol	20	14	15	19
+	-	Basal diet	18	18	9	6
+	-	Catechol	20	19	18	17
-	-	Basal diet	15	0	0	0
-	-	Catechol	15	6	0	0
-	+	Basal diet	15	0	0	0
-	+	Catechol	15	15	4	1

SCC: Scamous cell carcinoma

Table 17: Lesion in the glandular stomach in rats with and without MNNG treatment (Kawabe et al. 1994)

Treatment			Effective no of rats	Incidence of rats			
MNNG	$NaNO_2$	chemical		Hyperplasia	adenoma	adenocarcinoma	sarcoma
+	+	Basal diet	20	0	3	0	0
+	+	Catechol	20	10	15	15	0
+	-	Basal diet	20	0	3	0	0
+	-	Catechol	18	8	15	15	1

In the study of Maruyama et al. (1994), male hamster were fed (diet) for 30 weeks with 1.5% of catechol. Animals received a pre-treatment by subcutaneous injection once a week for 5 weeks at 500 mg/kg of BHP (an inducer of pancreatic tumours). Body weight decreased significantly and liver weight increased. The combined multiplicity of pancreatic atypical hyperplasias and adenocarcinomas in hamsters from group treated with 1.5% catechol after BHP were significantly ($p < 0.02$) decreased when compared to control group. The multiplicity of cancers was lower in catechol-treated group than in BHP alone-treated group. Liver lesions were not found in hamsters administered catechol. The incidences of gall bladder papillomas and carcinomas were not significantly different between group treated with 1.5% catechol after BHP and BHP alone-treated group.

Hagiwara et al. (1996) exposed rats to catechol (0.8%) during 104 weeks in diet. This experiment showed neither hepatocellular adenoma nor carcinoma; catechol is considered to have a low hepatocyte tumour incidence.
Short-term multi organ carcinogenesis study in rat (28-weeks exposure to 0.16 and 0.032% of catechol) revealed that low dose group (0.032%) had no one significant variation of the number of adenoma/carcinoma/papilloma in any organs tested (thyroid, lung, tongue, forestomach, small and large intestine, liver, kidney, urinary bladder) when compared to basal diet group values (Hirose et al. 1997). This study showed a significant increase of incidences of forestomach papillomas and hyperplasia at 0.032 and 0.8% of catechol co-exposed with 4 other mutagens.

In the tumour promotion study of Kobayashi (1999), mice received catechol in diet for 50 weeks at concentrations of: 0.48, 2.4, 12 or 16 mg/kg bw/d. Animals were pre-treated with MNU in drinking water for three weeks period at 120 ppm. In the MNU-catechol treated groups receiving catechol at 12 or 16 mg/kg bw/d, a significant and appreciable enhancement of pepsinogen 1 altered pyloric gland was noted. The administration of catechol in the diet enhanced only pre-neoplastic with adenomatous hyperplasia, lesion development in glandular stomach but no neoplastic lesion development. Another experiment with mice treated with MNU and catechol during 35 weeks showed a significant increase of incidence of adenomas in all catechol+MNU-treated groups and a significant increase of PAPG (pepsinogen isoenzyme 1 –altered pyloric gland) and incidence of carcinomas in glandular stomach only at 0.8% (Kobayashi et al. 1997).

Other mechanistic studies

DNA labelling methods was also used to assess the potential carcinogenity of catechol on rats (Shibata et al. 1990a). They were exposed to catechol (0.8%) for 8 weeks. The authors observed an elevation of DNA synthesis and increase of labelling index in forestomach and pyloric gland epithelium and only hyperplasia in forestomach. These authors observed also an increase of hyperplasia and an increase of DNA synthesis in the forestomach epithelium of rats after only 4 weeks of exposure to 0.8% of catechol (Shibata et al. 1990b). Glandular stomach results showed a slight induction of submucosal growth and an elevation of DNA synthesis in 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.

Methylation may play an important role in the early stage of stomach carcinogenesis. Tatematsu et al. (1993) have exposed male rats to catechol (0.8%) for 60 weeks. The aim of the study was to assess the methylation patterns of the rat pepsinogene1 (Pg1). Catechol induced adenomatous hyperplasia but no adenocarcinomas in glandular stomach. An increase of specific methylation of CCGG sites of Pg1 gene was noticed in pyloric mucosa. The alteration of methylation of the Pg1 gene is considered as an early carcinogenic process and progressive methylation changes occur with tumour development.

Table 18: Summary of relevant carcinogenicity studies by oral exposure (feed)

Method	Main Results	Remarks	Reference
Carcinogenicity studies			
Rat (Fischer 344/DuCrj) male, 5 weeks old Organs: Blood, stomach, liver, lymph nodes, pancreas Doses tested :0, 0.1, 0.2, 0.4 and 0.8% (0, 33, 65, 141 and 318 mg/kg/day) (nominal in diet), 30 rats/group (5 rats/cage) Exposure: 34 weeks (continuously): 5 rats 104 weeks (continuously): 25 rats Equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Neoplastic effects: positive - benign tumours** - No adverse effect on survival; Significant moderate retardation of body weight in the 0.8% group (-17%) - Significant increase of serum gastrin (blood) in the 0.2, 0.4 and 0.8% group (week 34) and all doses tested at 104 weeks - Forestomach: Significant increase of squamous **cell hyperplasia** et 0.4% (5/25) and 0.8% (10/25) (104 weeks) - Glandular stomach: 34 weeks: Significant increase of submucosal **hyperplasia** at 0.2, 0.4, 0.8% (5/5) and significant increase of adenoma at 0.4 and 0.8% (5/5) 104 weeks: Significant increase of submucosal **hyperplasia** at all doses tested (14/25 at 0.1%; 14/25 at 0.2% and 25/25 at 0.4 and 0.8%, significant increase of **adenoma** at 0.2% (23/25), 0.4% (25/25) and 0.8% (25/25) and significant **ulceration** at 0.4% (9/25) and 0.8% (15/25), low increase of adenocarcinoma (non-significant: 1/25 at 0.4 % and 2/25 at 0.8% vs 0 at 0%) - Lymph nodes (104 weeks):Cystic enlargement; significant increase in size of regional lymph nodes of the stomach in 0.4 and 0.8% group - Pancreas (104 weeks): Significant increase of acinar cell **adenoma** at 0.8% (6/25 vs 0/25 at 0%)	Reliability 2: with restrictions, Key study Experimental result **Test material (EC name): pyrocatechol**	Hagiwara et al. (2001)

Rat (Fischer 344) male/female, 5 weeks old Organs: Forestomach, Glandular stomach, Liver Dose tested: 0.8% (about 480 mg/kg bw/day) (nominal in diet), 30 rats/group Exposure: 104 weeks (continuously) Equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Neoplastic effects: positive – benign and malign tumours** - Slight reduction in survival rate (both sexes), - Significant decrease of final body weight (males: -17%; females: -25%); significant increase of relative liver weight in rats treated with catechol - Forestomach: Significant increase of **hyperplasia** in rats of both sexes (♀82% vs 17% in basal diet; ♂: 86% vs 3% in basal diet) - Glandular stomach: significant increase of **submucosal hyperplasia**, **adenomas** (♀100% vs 0% in basal diet; ♂: 100% vs 0% in basal diet) and **adenocarcinomas** in rats (♀43% vs 0% in basal diet; ♂: 54% vs 0% in basal diet) of both sexes - Liver: Non-significant increase of hyperplastic foci and significant decrease of number of foci in male rat (0.34 vs 1.18 in basal diet) and in female rats (1.82 vs 4.38 in basal diet)	Reliability 2: with restrictions Key study Experimental result **Test material (EC name): pyrocatechol**	Hirose et al. (1993a) Hirose et al. (1990)
Mouse (B6C3F1) male/female; 5 weeks old Organs studied : liver, forestomach and glandular stomach Dose tested: 0.8% in the diet (ca. 960 mg/kg bw/d) (nominal in diet); 30 mice/group/sex Exposure: 96 weeks (continuously) Equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Neoplastic effects: positive - benign tumours** - Slight reduction in survival rate in female, - Significant decrease of final body weight (males: -22%; females: -41%); significant increase of relative liver weight (both sexes) - Forestomach: Significant increase of incidence of **hyperplasia** for both sexes: ♀86% vs 10% in basal diet; ♂: 53% vs 4% in basal diet - Glandular stomach: Significant increase of **submucosal hyperplasia** (♀90% vs 0% in basal diet; ♂: 100% vs 0% in basal diet) and **adenomas** (♀72% vs 0% in basal diet; ♂: 97% vs 0% in basal diet) but no adenocarcinomas (both sexes). - Liver: no effect on incidence of hyperplastic nodules/foci and hepatocellular carcinoma	Reliability 2: with restrictions Supporting study: Only one dose tested, low number of organism, only 96 weeks of exposure instead of 104 weeks Experimental result **Test material (EC name): pyrocatechol (purity >99%)**	Hirose et al. (1993a) Hirose et al. (1990)
Rat (Wistar or Lewis or Sprague Dawley or WKY) male, 6 weeks old, 20-30 animals/group Organs: Stomach, liver, kidneys Dose tested: 0.8% (nominal in diet), Exposure: 104 weeks (continuously) Equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Neoplastic effects: positive – benign and malign tumours** - Significant decrease of body weight in all strains (from -15% to -40%) - Increase of final liver (Lewis, WKY) and kidney weight (Lewis) - Forestomach: Significant increase of **hyperplasia** (Wistar, WKY, Sprague Dawley; 73-70% vs 5-7% in basal diet), and **papilloma** (Sprague Dawley; 20% vs 0% in basal diet) - Glandular stomach: significant increase of **submucosal hyperplasia** (100% vs 0% in basal diet), adenoma (100-97%) and **erosion/ulcer** in all strains (43-80% vs 0%); significant increase of **adenocarcinoma** (Wistar, Lewis, Sprague Dawley; 67-77% vs 0% in basal diet)	Reliability 2: with restrictions Supporting study: strong decrease of body weight (up to -40%); only male and one dose tested, no data on food consumption Experimental result **Test material (EC name): pyrocatechol (purity >99%)**	Tanaka H. et al. (1995)

Carcinogenicity study: Rat (Fischer 344) male, 6 weeks old Organs: Forestomach, glandular stomach Dose tested: 0.16% (ca. 19 mg/kg bw/d) Exposure: 104 weeks Medium-term multi organ carcinogenesis study: Rat (Fischer 344) male, 6 weeks old Organs: Liver, kidney, lung, esophagus, stomach, urinary bladder, intestines Doses tested: 0.16 and 0.032% (ca. 19 and 3.8 mg/kg bw/d) (nominal in diet) (continuously); 10-15 animals/group Exposure: 28 weeks DMBDD pre-treatment (5 mutagens): DEN, MNU,DMH,BBN, DHPN (4weeks)	Carcinogenicity study: **Neoplastic effects: positive – benign tumours** • Significant decrease of body weight (-13%) and significant decrease of relative kidney weight (-6%) • Forestomach: Slight increase of incidence of Papillary or Nodular (PN) hyperplasia and papillomas (Non-significant), no carcinoma observed • Glandular stomach: Significant increase of incidence of **submucosal hyperplasia** and **adenoma** Medium-term multi organ carcinogenesis study: **Neoplastic effects: positive – benign tumours** **-** No effect on body weight Without DMBDD pre-treatment: no effects on adenoma, carcinoma and papilloma in any organs studied at 0.032% and 0.16% With DMBDD pre-treatment: forestomach: significant increase of incidence of **papillomas** (61% vs 4%) and hyperplasia (43% vs 0%) at 0.032% and significant increase of incidence of **papillomas** (67% vs 0%) and **hyperplasia** (93% vs 13%) at 0.16%	Reliability 2: with restrictions Supporting study: only male tested; only one dose tested for carcinogenicity study; only 28 weeks for Medium-term multi organ carcinogenesis study Experimental result **Test material (EC name): pyrocatechol**	Hirose M et al. (1997) Ito N. et al. (1998)
Rat (Fischer 344) male, 5 weeks old Organs: Glandular stomach Dose tested: 0.8% (about 480 mg/kg bw/day) (nominal in diet), 9 groups of 10-18 rats Exposure: 12 - 24 - 48 - 72 or 96 weeks (continuously) + Recovery period of 0,12, 24, 48,72 and 84 weeks Carcinogenesis study with recovery period (reversibility) – Non-GLP	**Neoplastic effects: positive - malign tumours** - Significant reduction in body weight, slight increase of relative liver and kidney weights (ns) - Multiple polyploid lesions: hyperplasia, adenoma and adenocarcinoma (ns) (Pyloric region) - Time effect: increase of incidence of hyperplasia, adenomas and adenocarcinoma from 12 to 96 weeks. After 96 weeks, all rats had hyperplasia and **adenomas** and high level of **adenocarcinomas** (up to 73.3% vs 0% in basal diet) - High pyloric gland thickness during catechol treatment (significant) - Number of tumour per rat (up to 11.4 tumours/rat) and size of tumours increase (up to 93.3% of rats with tumour>2mm) with time.	Reliability 2: with restrictions Key study: Only 10-18 rats per group were used; only males treated at only one dose; This study was not carried out according to recognized international guidelines. Experimental result **Test material (EC name): pyrocatechol**	Hirose et al. (1992)
Rat (Fischer 344) male, 6 weeks old Organs: Glandular stomach (pyloric region) Dose tested: 7 days: 0, 0.01, 0.1, 0.5, 1% (12hrs to 7 days) (ca. 0 - 6 - 60 - 300 - 600 mg/kg bw/d) 24 weeks: 0.8% in diet (ca. and 480 mg/kg bw/d) (nominal in diet); 5-6 rats/group Oral administration Exposure: 7 days and 24 weeks (continuously) Sequential morphologic changes studied. Non-GLP	**Neoplastic effects: positive – benign tumours** - 7 days study: significant increase of labelling index at all doses tested starting 12hrs; significant increase of thickness at 0.5% and 1% (1 day to 7 days);Significant increase of **apoptotic index** at all doses tested until 3 days - 24 weeks study: significant increase of the thickness of mucosa, of the labelling index and apoptotic index from 4 to 24 weeks; increase of **ulceration/erosion** (67%), **submucosal hyperplasia** (100%); **adenomas** (83%), **polyploid hyperplasia** (50%)	Reliability 2: with restrictions Supporting study: Only 5 rats/group; The variation of bw was not mentioned; The exposure period was short (7 days and 24 weeks instead of 104 weeks). Non guideline. Experimental result Test material (EC name): pyrocatechol	Hirose M. et al. (1999)

Rat (Fischer 344/DuCrj) male, 6 weeks old Organs: Liver Dose tested: 0.8 % (about 480 mg/kg bw/day) (nominal in diet), 30 animals/dose Exposure: 104 weeks (continuously) Long term feeding (carcinogenicity) study and medium term liver bioassays	**Neoplastic effects: negative** - Significant decrease of body weight (-17%); significant increase of liver absolute and relative weight in treated group, - No neoplastic lesions: no hepatocellular adenoma (0) or carcinoma (0)	Reliability 2: with restrictions Supporting study: Only male and one dose tested; strong decrease of body weight Experimental result **Test material (EC name): pyrocatechol (purity >99%)**	Hagiwara et al. (1996)
Tumour promotion studies			
Rat (Fischer 344) male, 5 weeks old Organs: Stomach, liver, Doses tested: 1.5% for 4 weeks, then 0.8% for 47 weeks (ca. 480 mg/kg bw/day) (nominal in diet), 65 male rats divided in 4 groups MNNG Pre-treatment: single intragastric administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) at 150 mg/kg (1 group at day 0 and another group 1 week later) Exposure: 52 weeks (continuously)	**Neoplastic effects: positive – malign tumour** - Decrease of final body weight in rats exposed to MNNG and catechol (-22% compared to MNNG only) - Forestomach: Catechol alone induces slight increase of hyperplasia and papilloma (non-significant) MNNG + Catechol treatment increases significantly the incidence of **squamous cell carcinoma** (100% vs 0% in basal diet group). - Glandular stomach: Catechol: Significant increase of adenomatous hyperplasia (100% of rats) and slight increase (non-significant) of **adenocarcinoma** in 20% of the rats. MNNG + Catechol: Significant increase of incidence of adenomatous hyperplasia (100% of rats) and **adenocarcinoma** in 94.7% of the rats.	Reliability 2: with restrictions Supporting study: Only male; test period is only 52 weeks; strong decrease of body weight Experimental result **Test material (EC name): pyrocatechol (purity >99.8%)**	Hirose M. et al. (1987)

Rat (Fischer 344) male, 5 weeks old Organs: Stomach Dose tested: 0.8% (ca. 480 mg/kg bw/day) (nominal in diet), 15 rats/group (10 groups) Pre-treatment: single intragastric administration of MNNG at 150 mg/kg at day 0 (1 week later: administration of catechol at 0.8% or basal diet for 51 weeks) Other groups: 0.8% catechol exposure or basal diet without MNNG pre-treatment Exposure: 52 weeks (continuously)	**Neoplastic effects: positive – benign and malign tumours** - Significant decrease of body weight (catechol group: -20%; catechol + MNNG:-25%) and slight decrease of food intake of rats exposed to catechol with or without MNNG pre-treatment compared to basal diet - Significant increase of liver weight of rats treated with catechol with or without MNNG; significant decrease of kidney weight of rats treated with catechol with or without MNNG pre-treatment - Forestomach: Catechol: Significant increase of incidence of hyperplasia compared to basal diet (mild to moderate hyperplasia) (100% vs 7% in basal diet) Catechol + MNNG pre-treatment: Significant increase of incidence of papilloma (100% vs 0%) and **squamous cell carcinoma** compared to basal diet (100% vs 0% in basal diet) - Glandular stomach: Lesions in the pyloric region Catechol: Significant increase of incidence of submucosal hyperplasia (100% of rats) and adenoma (100% of rats); **adenocarcinoma** observed in only one rat (7% of rats). Catechol + MNNG pre-treatment Significant increase of submucosal hyperplasia (100% vs 0%), **adenoma** (100% vs 0%) and **adenocarcinoma** (73% of rats vs 0% in basal diet)	Reliability 2: with restrictions Supporting study: Only male; test period is only 52 weeks; strong decrease of body weight experimental result **Test material (EC name): pyrocatechol (purity>98%)**	Wada S. et al. (1998)
Rat (Fischer 344) male, 5 weeks old Organ: Stomach Dose tested: 0.8% (ca. 480 mg/kg bw/day) (nominal in diet), 15-20 rats/group MNNG pre-treatment: 150 mg/kg bw (1 week later: administration of catechol at 0.8% or basal diet with or without 0.2% $NaNO_2$ for 51 weeks) Other groups: 0.8% catechol exposure or basal diet without MNNG pre-treatment Exposure: 52 weeks (continuously)	**Neoplastic effects: positive - malign tumours** - Significant decrease of body weight (-14% in catechol + $NaNO_2$ group), slight increase of relative liver and kidney weights - Forestomach: Catechol + $NaNO_2$: **Hyperplasia** moderate to severe (15% vs 0% in basal diet) Catechol + MNNG: Significant increase of incidence of **papillomas** (90% vs 50% in basal diet) and **squamous cell carcinoma** (85% vs 33% in basal diet) Catechol + $NaNO_2$+ MNNG: Significant increase of incidence squamous cell **carcinoma** (95% vs 25% in basal diet) - Glandular stomach: Catechol: Significant increase of **adenoma** (100%) and **adenocarcinoma** (33% vs 0% in basal diet) Catechol +/- $NaNO_2$: Significant increase of **adenoma** (85-75% vs 0% in basal diet) Catechol + MNNG +/- $NaNO_2$: Significant increase of **submucosal hyperplasia** (50%), **adenoma** and **adenocarcinoma** (75%vs 0% in basal diet)	Reliability 2: with restrictions – non GLP Supporting study: Only male tested ; test period is only 52 weeks; Experimental result **Test material (EC name): pyrocatechol (purity>99%)**	Kawabe M. et al. (1994)

Rat (Fischer 344) male, 5 weeks old Organs: Nasal cavity, lung, tongue, esophagus Dose tested: 0.8% (ca. 480 mg/kg bw/day) (nominal in diet), 10-15 rats/group 1 group pre-treated by I.P. with 25 mg/kg bw of Methyl-N-amylnitrosamine (MNAN) at week 0,1 and 2 followed by 1 week later 0.8% catechol treatment or basal diet for 49 weeks Exposure: 52 weeks (continuously)	**Neoplastic effects: positive - benign and malign tumours** - Significant decrease of final body weight by 21 to 24%, significant increase of relative liver and kidney weights (+/- MNAN pre-treatment) - Tongue: MNAN + catechol: Significant increase of incidence of **PN hyperplasia** (28.6% vs 18.2% in basal diet) and **papilloma** (57.1% vs 9.1% in basal diet) - Esophagus: MNAN+ catechol: Significant increase of incidence of **carcinoma** (64.3% vs 0% in basal diet) - Lung: MNAN + catechol: Significant decrease of incidence of **alveolar hyperplasia** (0% vs 54.5% in basal diet) - Nasal cavity: no effect on incidence of PN hyperplasia or papilloma	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, only one dose tested, short time of exposure; strong decrease of body weight Experimental result **Test material (EC name): pyrocatechol (purity>99%)**	Yamagushi S.et al. (1989)
Rat (Wistar) male, 5 weeks old Organs: Liver, kidney Dose tested: 0.8% in powdered basal diet (ca. 480 mg/kg bw/day), 15-20 rats/group Pre-treatment: N-ethyl-N-hydroxyethyl nitrosamine (EHEN) at 0.1% for 3 weeks; 1 week later, rats treated with 0.8% catechol or basal diet for 36 weeks Exposure: 40 weeks (continuously)	**Neoplastic effects: negative** - Significant decrease of final body weight (-15 or 28%) - Low increase of relative liver weight and kidneys weight - Liver: No significant effect on incidence of hepatocellular adenoma and hepatocellular carcinoma - Kidneys: Slight significant decrease of number of atypical renal tubules in rats pre-treated with EHEN (82.3% vs 100%). No effect on microadenoma or renal cell tumour	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, only one dose tested, short time of exposure; strong decrease of body weight Experimental result **Test material (EC name): pyrocatechol**	Okazaki S. et al. (1993)
Rat (Fischer 344) male, 5 weeks old Organs: Forestomach, glandular stomach Dose tested:0.2% (ca. 120 mg/kg bw/day) (nominal in diet); 15 rats/group Pre-treatment: MNNG at 150 mg/kg bw Exposure: 36 weeks (continuously)	**Neoplastic effects: positive – benign tumours** - Significant decrease of final body weight (-6 to -7%); significant increase of liver and kidney weight - Forestomach: no significant effect of catechol on papilloma, carcinoma *in situ* or squamous cell carcinoma, only mild and moderate hyperplasia (+/- MNNG) - Glandular stomach: Catechol: Significant increase of submucosal hyperplasia (50% vs 0% in basal diet) and adenomas incidences (60% vs 0% in basal diet); No adenocarcinoma observed Catechol + MNNG: Significant increase of submucosal hyperplasia (26% vs 0% in basal diet) and adenomas incidences (26% vs 0% in basal diet). No adenocarcinoma observed.	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, only one dose tested, short time of exposure; low number of rats per group; The study was not carried out according to recognized international guidelines. Experimental result **Test material (EC name): pyrocatechol (purity >98%)**	Hirose M. et al. (1991)

Rat (Fischer 344) male, 6 weeks old Organs: Esophagus, forestomach, glandular stomach, liver, thyroid, kidney, lung, intestine, urinary bladder Dose tested: 0.8% (ca. 480 mg/kg bw/day) (nominal in diet), 10-15 rats/group 4 weeks of pre-treatment to carcinogens: MNU, UDMH, DEN, BBN, DHPN followed by 3 days later 0.8% catechol treatment or basal diet for 24 weeks With or without $NaNO_2$ treatment Exposure: 28 weeks (continuously)	**Neoplastic effects: positive - benign and malign tumours** - Significant decrease of final body weight (-9 to -20%: with or without pre-treatment); significant increase of relative liver weight - Forestomach: Catechol: Mild **hyperplasia** (50%) Catechol + $NaNO_2$: Mild to severe **hyperplasia** (100%) Catechol + pre-treatment: Significant increase of **papillomas** (73% vs 0% in basal diet**)**, **squamous cell carcinoma** (33% vs 0% in basal diet), and **carcinoma *in situ*** (9% vs 0% in basal diet).Catechol + pre-treatment + $NaNO_2$: Significant increase of **carcinoma *in situ*** (50%) and **squamous cell carcinoma** (100%) - Glandular stomach: Significant increase of submucosal **hyperplasia** in all group tested; significant increase of **adenoma** (catechol + pre-treatment, catechol+ $NaNO_2$, catechol); significant increase of **adenocarcinoma** in catechol + pre-treatment group (27% vs 0% in basal diet). - Liver: Significant **decrease of numbers of GST-P positive foci** in catechol+ pre-treatment group. No effect on hyperplastic nodule or hepatocellular carcinoma. - Thyroid: Catechol: Significant decrease of incidence of follicular cell hyperplasia (1 vs 9 in basal diet) Catechol + $NaNO_2$: Significant decrease of incidence of **follicular cell adenoma** (0 vs 3 in basal diet); Significant decrease of incidence of **follicular cell hyperplasia** (1 vs 9 in basal diet) - Esophagus: Significant increase of incidence of **papilloma** in catechol+$NaNO_2$ group (7 vs 0 in basal diet) - Kidney: Significant decrease of incidence of nephroblastoma in catechol group (0 vs 5 in basal diet) - Lung, Tongue, intestine, urinary bladder: no effects	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, only one dose tested; short time of exposure; low number of rats per group; strong decrease of body weight; The study was not carried out according to recognized international guidelines. Experimental result **Test material (EC name): pyrocatechol (purity >98%)**	Hirose M. et al. (1990a) Hirose M. et al. (1993b)
Rat (Fischer 344) male, 6 weeks old Organs: Urinary bladder Dose tested: 0.8% (ca. 480 mg/kg bw/day) (nominal in diet); 20 rats/group Exposure: 32 weeks of treatment (continuously) after 4 weeks of pre-treatment with BBN or tap water	**Neoplastic effects: negative** - Significant decrease of final body weight (-13%) in rats pre-treated with BBN - Urinary bladder: Slight (non-significant) increase of incidence of papilloma (4 vs 2) and carcinoma (2 vs 1) in catechol+BBN group	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, only one dose tested; short time of exposure; strong decrease of body weight; The study was not carried out according to recognized international guidelines Experimental result **Test material**	Kurata Y. et al. (1990)

		(EC name): pyrocatechol (purity >98%)	
Rat (Fischer 344) male, 5 weeks old Organs: liver Doses tested: 0.2; 0.8% in powdered basal diet (ca. 120 and 480 mg/kg bw/d) (nominal in diet); 15 rats/group Pre-treatment to DEN (200 mg/kg bw) => 2 weeks after DEN injection, 6 weeks of exposure to catechol (continuously) Exposure: 8 weeks	**Neoplastic effects: positive** - Significant decrease of final body weight (20%) and significant increase of relative liver weight only in group of 0.8% catechol + DEN - Liver: Significant increase of BUdr labelling index at both dose; significant decrease of number and area of GST-P positive liver foci only in group 0.8% catechol +DEN	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, only one dose tested; very short time of exposure; strong decrease of body weight; The study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol (purity >99%)**	Hasegawa R. et al. (1992)
Rat (Fischer 344/DuCrj) male Organs: Lung, thyroid, urinary bladder, kidneys, glandular stomach Doses tested: 0.8 % (ca. 480 mg/kg bw/day) (nominal in diet); 20 rats/group Exposure: 2 weeks pre-treatment DHPN at 0.1% followed by 30 weeks treatment with antioxidant compound (continuously) Tumour inhibition	**Neoplastic effects: negative** - Significant decrease of body weight (-11%) and low increase of liver weight (non-significant) only in rat pre-treated with DHPN + catechol - Glandular stomach: non-significant submucosal and adenomatous hyperplasia in the pyloric region observed - Lung: significant **decrease of number** and areas of **lung neoplastic lesions** per unit section in group pre-treated with DHPN - Thyroid gland: Low decrease of carcinoma (non-significant) - Urinary bladder, kidneys: no effect on adenoma, papilloma or carcinoma	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, only one dose tested; very short time of exposure; strong decrease of body weight; The study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol**	Hasegawa R. et al. (1990)

Rat (Fischer 344/DuCrj) male; 5 weeks old Organs: Liver, forestomach, glandular stomach, bladder, thyroid, oesophagus Dose tested: 0.8 % (ca. 480 mg/kg bw/day) (nominal in diet); 15 animals/group DMD pre-treatment: DEN + MNU+DHPN Exposure: 16 weeks (continuously)	**Neoplastic effects: positive – benign tumours** - Increase of liver weight - Liver: Significant decrease of the number of GST-P+ liver foci; - Forestomach: Catechol: Low (non-significant) increase of cell hyperplasia DMD + Catechol: Significant increase of incidence of **squamous cell hyperplasia** and **papillomas** - Glandular stomach: Catechol: low (non-significant) increase of **submucosal hyperplasia** (90%) and **adenoma** (40% vs 0%) DMD+ catechol: Significant induction of incidence of **submucosal hyperplasia** - Urinary bladder, thyroid and esophagus: no effect	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, low number of rats per group; only one dose tested; very short time of exposure; The study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol**	Fukushima S. et al. (1991)
Rat (Fischer 344/DuCrj) male; 6 weeks old Organs: Lung, urinary bladder, forestomach, glandular stomach, seminal vesicle Dose tested: 0.8% (601.6 mg/kg bw/d, calculated by the authors) (nominal in diet); 15-16 rats/group Exposure: 16 weeks (continuously) DMD pre-treatment (4weeks): DEN + MNU+DHPN	**Neoplastic effects: positive - benign tumours** - Significant decrease of body weight (-20%) in DMD + catechol group; significant decrease of food consumption - Forestomac: Catechol: slight increase of squamous cell carcinoma (non-significant)DMD + catechol: Significant increase of **squamous cell hyperplasias** and **papillomas** - Glandular stomach: Development significant of **submucosal growth pyloric glands** (DMD + catechol group) and atypical glandular epithelium observed (non-significant) - Lung: low incidence (non-significant) of alveolar/bronchiolar adenoma in DMD + catechol group - Urinary bladder: low incidence (non-significant) of cell hyperplasia and cell papilloma in DMD + catechol group - Seminal vesicle: hyperplasia observed (non-significant)	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, low number of rats per group; only one dose tested; very short time of exposure; strong decrease of body weight; the study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol**	Kajimura T. et al. (1992)
Mouse (Balb/c) male; 6 weeks old Organs: Glandular stomach **(**pyloric gland) Dose tested: 4, 20, 100, 500 ppm in basal diet (ca. 0.48 - 2.4 - 12 - 60 mg/kg bw/d) (nominal in diet); 10-30 animals/group Pre-treatment: MNU at 120 ppm during 3 weeks. One week after the last MNU treatment, exposure to catechol or basal diet for 50 weeks Exposure: 50 weeks (continuously)	**Neoplastic effects: negative** - No significant decrease of body weight - Slight increase but not significant of incidence of adenomatous hyperplasia and carcinomas in any catechol-treated group - Significant increase of the number of PAPG in group MNU + catechol (100 or 500 ppm) => Only induction of **pre-neoplastic lesions**	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, low number of mouse per group; short time of exposure; the study was not carried out according to recognized international guidelines Experimental result **Test material: pyrocatechol**	Kobayashi K. et al. (1999)

Mouse (Balb/c) male; 6 weeks old Organs: Glandular stomach Dose tested:0.05, 0.2, 0.8%; 15-45 animal/group Pre-treatment: MNU at 120 ppm during 3 weeks. One week after the last MNU treatment, exposure to catechol or basal diet for 29 weeks Exposure:20- 35 weeks (continuously)	**Neoplastic effects: positive - benign and malign tumours** - Significant decrease of body weight (-10 to -20%) at 0.8% of catechol +/- MNU pre-treatment Catechol: - No effect on incidence of adenoma, adenocarcinoma or PAPG number MNU + catechol: - Significant increase of **PAPG** at 0.8% (week 20) - Significant increase of incidence of **adenomas** at all dose tested at week 35 (20 to 32% vs 0% in basal diet) - Significant increase of incidence of **adenocarcinomas** 0.8% at week 35 (70% vs 0% in basal diet) ⇨ Catechol strongly enhanced the pre-neoplastic and neoplastic lesions	Reliability 2: with restrictions – non GLP Supporting study: Only male tested, low number of mouse per group; short time of exposure; strong decrease of body weight; the study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol**	Kobayashi K. et al. (1997)
Hamster, Syrian (Syrian golden) male; 6 weeks old Organs: Liver, gall bladder, pancreas Dose tested: 1.5% in diet (ca. 1800 mg/kg bw/d) (nominal in diet); 20 animals/ group Pre-treatment with BHP for 5 weeks (5 injections of 500 mg/kg) or saline solution Exposure: 30 weeks (continuously)	**Neoplastic effects: negative** - Significant decrease of body weight in all catechol-treated group (-10.5% and -13%); significant increase of liver weight in BHP/catechol group - Pancreas: Significant decrease of the combined multiplicity of pancreatic atypical hyperplasias and adenocarcinomas in BHP + catechol group - Liver: No lesions - Gall bladder: No significant modifications of incidences of gall bladder papillomas and carcinomas ⇨ Weak inhibitory effect of catechol on pancreatic carcinogenesis	Reliability 2: with restrictions – non GLP Supporting study: Only male tested; only one dose tested; low number of hamster per group; short time of exposure; strong decrease of body weight; the study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol (purity>98%)**	Maruyama H. et al. (1994)
Hamster, Syrian (Syrian golden) male; 6 weeks old Organs: Liver, pancreas Dose tested: 0.75%, 1.5 % in diet (ca. 900, 1800 mg/kg bw/d) (nominal in diet); 5 animals/ group Pre-treatment with BOP (70 mg/kg) Exposure: 20 weeks (continuously)	**Neoplastic effects: negative** - Significant decrease of final body weight (-12 to -18%); significant decrease of liver weight in all catechol-treated group - Liver: No liver lesions, low incidence of hepatocellular carcinoma (non-significant) in BOP+ 1.5% catechol group - Pancreas: Significant decrease of the number of atypical pancreatic hyperplasia and adenocarcinomas in BOP+0.75% catechol group	Reliability 2: with restrictions – non GLP Supporting study: Only male tested; low number of hamster per group; short time of exposure; strong decrease of body weight; the study was not carried out according to recognized international guidelines	Maruyama H. et al. (1991)

		Experimental result **Test material (EC name): pyrocatechol (purity>98%)**	
Rat (Fischer 344) male; 6 weeks old - Drinking water experiment 0.05% (about 50 mg/kg bw/day) Exposure: 78 weeks (continuously) - Direct instillation into urinary bladder 1-2% by direct instillation (nominal in water) in the urinary bladder Exposure: 15 weeks (twice a week) 5 groups of 30 rats Tumour promotion study	**Neoplastic effects: negative** - Drinking water experiment: no macroscopic or microscopic lesions - Direct instillation: only one animal (5% incidence) was found to have developed carcinoma of the urinary bladder	2 (reliable with restrictions) supporting study: Because the test performed by direct instillation of catechol in the urinary bladder is not relevant ;only 30 animals/ group ;only males; only 1 dose tested. The exposure period was short Experimental result **Test material (EC name): pyrocatechol (purity >99%)**	La Voie E.J. et al. (1985)
Other studies			
Rat (Wistar Kyoto, WKY) male Organs: Pyloric glandular stomach Dose tested: 0.8% (about 480 mg/kg bw/day) (nominal in diet), 10-11 rats/group Positive control: MNNG, known as carcinogen Exposure: 30, 60 weeks (continuously) Test the hypothesis that the altered methylation may play an important role in the early stage of the stomach carcinogenesis.	- Increase of adenomatous hyperplasia, no adenocarcinoma found - Increase of the number of PAPG of pyloric mucosa per cm increase with time - Increase of specific methylation in CCGG sites of the Pg1 gene in adenomatous hyperplasia (same phenotype than MNNG); no effect on CGCG sites	Reliability 2: with restrictions – non GLP Supporting study: Only male tested; low number of rat per group; only one dose tested; short time of exposure; the study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol**	Tatematsu M. et al. (1993)
Rat (Fischer 344) male; 6 weeks old Organs: Forestomach, glandular stomach Dose tested: 0.8% in powdered basal diet (ca. 398 mg/kg/d, based on final body weight) (nominal in diet); 5 rats/group Exposure: 8 weeks (continuously) DNA labelling method	- Significant decrease of final body weight (20% lower than control); reduction of food and water consumption - Forestomach epithelium: Significant hyperplasia (from slight to moderate);Significant elevation of DNA synthesis; slight increase of labelling index - Pyloric glandular epithelium: significant increase of the number of Pg1-1decreased; significant elevation of DNA synthesis associated with significant increased crypt height	Reliability 2: with restrictions – non GLP Supporting study: Only male tested; low number of rat per group; only one dose tested; very short time of exposure; the study was not carried out according to recognized international	Shibata MA. et al. (1990a)

		guidelines Experimental result **Test material: pyrocatechol**	
Rat (Fischer 344) male/female; 6 weeks old Organs: Forestomach, glandular stomach Dose tested: 0.8% in powdered basal diet (ca. 480 mg/kg bw/d) (nominal in diet); 5 rats/group/sex Exposure: 4 weeks (continuously) DNA labelling method	- Significant decrease of final body weight (males: -20%; females:-10%); reduction of food and water consumption - Forestomach: Significant increase of the occurrence of mild hyperplasia (both sex), significant elevation of DNA synthesis and significant increase of labelling index - Glandular stomach: Slight increase of submucosal growth (ns); Elevated DNA synthesis observed in association with increased crypt height of pyloric gland (ns); significant increase of BrdU-incorporating cells/crypt and significant increase of crypt height (both sexes)	Reliability 2: with restrictions – non GLP Supporting study: Low number of rats per group; very short time of exposure; only one dose tested; strong decrease of body weight; the study was not carried out according to recognized international guidelines Experimental result **Test material (EC name): pyrocatechol**	Shibata M.-A et al. (1990b)
Study type: The effect of catechol on the growth of 3 prostate cancer cell lines:: LNCaP, PC3 and DU145 cells Endpoint addressed: carcinogenicity	Cell proliferation: Catechol was found to significantly inhibit the proliferation of PC3 cells. LNCaP cells were less sensitive to this effect. In DU145 cells, catechol produced only partial inhibition. Catechol acts preferentially on the two cell lines possessing non-functional (PC3) or functional (LNCaP) androgen receptor. Results of competition experiments indicate that catechol is a very weak competitor of androgen binding. Preincubation with catechol resulted in an increased resistance of DU145 and PC3 cells to H_2O_2 toxicity. In LNCaP cells, this effect was observed only at H_2O_2 concentrations < 1 mM. Production of ROS: In PC3 cells, a significant decrease of ROS production was observed. At long incubation time (> 40 min), this effect was also observed in the DU145 cell line. LNCaP cells did not show any modification of ROS production after catechol preincubation. • Production of NO: Catechol decreases the production/secretion of NO.	Reliability 2: with restrictions – non GLP Supporting study: *in vitro* experiment; the study was not carried out according to recognized international guidelines Experiment results **Test material (EC name): pyrocatechol**	Kampa et al. (2000)

4.10.1.2. Carcinogenicity: inhalation

No data available.

4.10.1.3. Carcinogenicity: dermal

No data available.

4.10.2. Human information

No data available

4.10.3. Other relevant information

No data available

4.10.4. Summary and discussion of carcinogenicity

Results collected from carcinogenicity studies revealed that pyrocatechol induced benign (adenoma hyperplasia) and malign tumours (adenocarcinoma) on stomach of rats after 104 weeks (Hagiwara et al. 2001; Hirose et al. 1999, 1997; Tanak et al. 1995; Hirose et al. 1992; Hirose et al. 1993a, 1990). These benign/malign tumours were only found in glandular stomach while pre-neoplastic lesions (e.g. hyperplasia and/or papilloma) were mainly found in forestomach.. Recent literature has shown that forestomach tumours should be evaluated in details before discarding continuous induction of cell proliferation, hyperplasia, and ultimately carcinomas. An analysis scheme is proposed by Proctor et al (2007), in which the route of exposure, the dose levels, the genotoxicity, the tissues in which effects of the substance are observed should be considered all together so as to evaluate the relevance for humans of forestomach tumours observed in rodents.

Tumours in forestomach were only induced after co-treatment to carcinogens (Wada et al. 1998; Kawabe et al. 1994; Hirose et al. 1993, 1990a; Hirose et al. 1987). Benign tumours were also found in pancreas (Hagiwara et al. 2001) and malign tumours were present in the esophagus after pre-treatment to carcinogen (Yamagushi et al. 1989). Neoplastic lesions (papillomas, hyperplasia) were found in the tongue, esophagus and lungs in tumour promotion studies (Hirose et al. 1993b, 1990a; Yamagushi et al. 1989). No carcinogenic effect of pyrocatechol on liver was reported on rodents (Hagiwara et al. 2001, 1996; Okazaki et al. 1993; Hirose et al. 1993a, 1990).

Rat was not the only species studied: mouse and hamster were also used to assess the potential carcinogenicity of pyrocatechol. Results showed malign tumours (adenocarcinoma) in glandular stomach of mice after 104 weeks of exposure to 0.8% of pyrocatechol without pre-treatment (Hirose et al. 1990; 1993a) and after only 35 weeks exposure to 0.05%-0.8% of pyrocatechol with a pre-treatment to carcinogens (Kobayashi et al. 1997). However, tumour promotion studies on hamster were negative and decrease tumours or neoplastic lesions were observed in liver, pancreas or gall bladder after pyrocatechol exposure by oral feeding (Maruyama et al. 1991, 1994).

Overall, tumours were mainly induced on stomach of rodents at a dose of 0.8% (ca 480 mg/kg/day) of pyrocatechol. Survival of rodents was not affected by pyrocatechol exposure. However, a strong and significant decrease of body weight (from -10% to -41% at the end of most of the experiment) has been noticed at this dose. Loss of body weight was observed in male and female rats or mice and also in male hamsters (no study on female hamster). These results suggest that tumours may have been induced at a dose higher than the Maximum Tolerated Dose (MTD). As hyperplasia is a neoplastic lesion observed in most cases, cell proliferation appears as a determinant factor in the induction of cancer in rodent by pyrocatechol. Irritating and genotoxic properties of pyrocatechol could also contribute to its ability to generate tumours in rodents.

Data collected from all these studies on carcinogenic and co-carcinogenic effect of pyrocatechol on rodents were consistent. They revealed that stomach is the main target organs with benign and malign tumours observed at concentrations of 0.2% and 0.8% respectively. It is known that oral gavage with its physical nature of repeated administration of excessive dose volumes through the use of an oral gavage needle can result in irritation to the forestomach mucosa and/or abnormal compound absorption. In the case of pyrocatechol, all the data presented are in diet. No experimental study was carried out by gavage. A meta-analysis of forestomach carcinogens has shown that a majority (84% of the 120 evaluated), also induced tumours at other sites, while only 19 chemicals (16%) induced tumours exclusively in the forestomach. This analysis helps to appreciate if the lesions found in forestomach can be found elsewhere: in this case, it increases the possibility of a non rodent specific effect. In the case of Pyrocatechol alone, it induces only tumours in glandular stomach. However, after initiation, tumours were found also in forestomach. Finally, Understanding the mode-of-action causing forestomach tumours as either a genotoxic (or mutagenic) or nongenotoxic (not a promutagenic) mechanism is an important consideration for assessing the relevance of forestomach tumours to human cancer chemically induced.

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 body 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). In the case of pyrocatechol, irritant properties can be suspected as well as a genotoxic mode of action. There is no sufficient data available to conclude on the precise mechanism leading to the observed carcinogenic effects.

Finally, there is sufficient evidence to classify pyrocatechol for its carcinogenicity.

4.10.5. Comparison with criteria

Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

A substance is classified in **category 1** for carcinogenicity on the basis of epidemiological and/or animal data. The substance is known or presumed human carcinogens.

- A substance is classified in **category 1A** if it is known to have carcinogenic potential for humans. The classification in this category is largely based on human evidence, human studies that establish a causal relationship between human exposure to a substance and the development of cancer.

- A substance is classified in **category 1B** if it is presumed to have carcinogenic potential for humans. The classification in this category is largely based on animal evidence, animal experiments for which there are sufficient evidence to demonstrate animal carcinogenicity.

A substance is classified in **category 2** if it is suspected human carcinogens. The placing of a substance in category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in category 1A or 1B.

4.10.6. Conclusions on classification and labelling

29 studies of reliability 2 are oral carcinogenicity studies or tumour promotion study with pyrocatechol. They all demonstrated the carcinogenic effect on pyrocatechol on glandular stomach on rats with formation on adenomas or adenocarcinomas. It is important to notice that effects

appeared at high dose of 0.4% and mainly 0.8%. The lowest doses tested presented submucosal hyperplasia indicating that repeated administration of important dose at the site of application (stomach) lead to toxic effect for which the severe form at high dose were carcinoma and adenocarcinoma. Three species were studied: rat, mouse and hamster. It was clearly demonstrated that rat was the most sensitive.

Co-carcinogenicity studies permitted to better understand the mechanisms of co-carcinogenesis and tumour promotion. They all confirmed the carcinogenic effect of pyrocatechol on glandular stomach in rats, and indicated that pyrocatechol could inhibited carcinogen effect of some substances on specific organs like the effect of BOP on pancreas of hamster (Maruyama et al. 1991). According to data available, pyrocatechol did not exert carcinogen effect on other organs than the site of application after oral administration: esophagus and stomach (glandular and forestomach) of rat.

The specific carcinogenic effect of pyrocatechol on rat glandular stomach after oral administration, at high dose was the result of 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. The co-effect of the genotoxic properties of pyrocatechol cannot be excluded.

Pyrocatechol may play a role in human gastric cancer development, so it makes this compound an obvious target for carcinogenesis classification. IARC (1999) classify pyrocatechol as possibly carcinogenic to humans (Group 2B).

According to results from all carcinogen studies showing induction of tumours in one organ in one species and IARC classification, we propose to classify **pyrocatechol as carcinogen of category 2 or suspected human carcinogen.**

4.11. Toxicity for reproduction

Not relevant for this dossier

4.12. Other effects

Not relevant for this dossier

5. ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier

6. OTHER INFORMATION

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