Helsinki, 24 May 2022

Addressees
Registrant(s) of 5-amino-o-cresol listed in the last Appendix of this decision

Registered substance subject to this decision (the 'Substance')
Substance name: 5-amino-o-cresol
EC number: 220-618-6
CAS number: 2835-95-2

Decision number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)

DECISION ON SUBSTANCE EVALUATION

Under Article 46 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below:

A. Information required to clarify the potential risk related to Mutagenicity

1. An *in vivo* mammalian alkaline comet assay test (OECD TG 489) in liver, gastro-intestinal tract (glandular stomach and duodenum) and urinary bladder performed in rats via oral route using the Substance, as further specified in Appendix A (section 2.1.b).

Deadlines

The information must be submitted by **29 August 2023**.

Conditions to comply with the information requested

To comply with this decision, you must submit the information in an updated registration dossier, by the deadlines indicated above. The information must comply with the IUCLID robust study summary format. You must also attach the full study report for the corresponding study/ies in the corresponding endpoint of IUCLID.

You must update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You will find the justifications for the requests in this decision in the Appendix entitled ''Reasons to request information to clarify the potential risk'.
You will find the procedural steps followed to reach the adopted decision and some technical guidance detailed in further Appendices.

Appeal

This decision may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Approved[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Basis for substance evaluation

The objective of substance evaluation under REACH is to allow for the generation of further information on substances suspected of posing a risk to human health or the environment ('potential risk').

ECHA has concluded that further information on the Substance is necessary to enable the evaluating Member State Competent Authority (eMSCA) to clarify a potential risk and whether regulatory risk management is required to ensure the safe use of the Substance.

The ECHA decision requesting further information is based on the following:

(1) There is a potential risk to human health or the environment, based on a combination of hazard and exposure information;
(2) Information is necessary to clarify the potential risk identified; and
(3) There is a realistic possibility that the information requested would allow improved risk management measures to be taken.

The Appendix entitled 'Reasons to request information' describes why the requested information is necessary and appropriate.

ECHA
EUROPEAN CHEMICALS AGENCY

Appendix A – Reasons to request information to clarify the potential risk related to Mutagenicity

1. Potential risk

1.1 Potential hazard of the Substance

Following its assessment of the available relevant information on 5-amino-o-cresol, the evaluating MSCA and ECHA have identified the following potential hazard which must be clarified.

Potential mutagenicity

The available information suggests that the Substance may have a mutagenic effect. However, the available information you reported is not sufficient to clarify the identified concern.

In particular, the available *in vitro* data showed the ability for the Substance to induce prevalently clastogenicity and the potential to induce gene mutation and aneugenicity cannot be excluded. The available *in vivo* data are considered inconclusive.

Therefore a concern on potential mutagenicity of the Substance cannot be excluded. In your comments to the draft decision, you provided an expert's statement arguing that "*From the significant toxicological database available on 5-amino-o-cresol, […] the two studies requested by ECHA would essentially be repeats of already available animal studies*." The eMSCA took your comments into account and the decision was partially modified (see below).

Genotoxiciy *in vitro*

The *in vitro* available studies assessing the potential genotoxicity of the Substance are the following:

- A bacterial reverse mutation test (OECD TG 471) was performed to investigate the potential of the Substance to induce gene mutations using the *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 and concentrations up to 5000 µg/plate. No biologically relevant increase in revertant colony numbers was observed in any tested strain following treatment with and without metabolic activation ([redacted], 2005). Mixed results are reported in literature in TA98 and TA1538 strains, these data are also reported in the chemical safety report.
- An *in vitro* gene mutation test on mammalian cells (L5871Y mouse lymphoma cells) was carried out according to OECD TG 476 ([redacted], 2002a) at the following (12 + 12) concentrations: from 0 to 1500 µg/mL, with metabolic activation and from 0 to 500µg/mL without metabolic activation. The treatment period was about 4 hours. A biologically relevant increase of mutant frequencies was observed both with (between a concentration range of 250 µg/mL and 1000 µg/mL) and without (at the highest concentrations) metabolic activation. In addition, in the assay also the size/optical density of the colony was determined. A shift toward small colonies was observed in the absence of S9 mix, indicating a clastogenic potential of the Substance. In the presence of S9 mix, this shift was not observed.

  Therefore, the Substance was considered mutagenic in the Thymidine Kinase +/- locus in L5178Y mouse lymphoma cells, with and without metabolic activation. Considering the absence of a shift toward small colonies in the presence of S9 mix, the potential of the Substance to induce gene mutation is also shown.
- Chromosome aberration (CA) test according to OECD TG 473 was performed in order to assess the potential clastogenicity of the Substance ([redacted], 1988). The test

was performed with human peripheral blood lymphocytes treated with 8 concentrations (from 62.14 to 3000 µg/mL). Based on the mitotic index, the test concentrations selected for CA analysis were 1267.5, 1950 and 3000 µg/mL. Without metabolic activation, treatment of cells with the Substance resulted in large increases in aberration frequencies which were statistically significant at the the top two doses (1950 and 3000 µg/mL) in the absence of S9. With metabolic activation, increases were statistically significant following treatment in the presence of S9 at all test concentrations. Under the test conditions, the Substance induced chromosomal aberrations in human peripheral blood lymphocytes (*in vitro*), with and without metabolic activation. The Substance is considered clastogenic in human peripheral blood lymphocytes.

- An *in vitro* micronucleus (MN) assay according to OECD TG 487 was performed on human lymphocytes ([redacted], 2005). The cells were exposed for 3 or 20 hours period, and followed by 28 or 45 hours of recovery with or without S9 mix. A significantly elevated frequency of binucleated cells with micronuclei was observed at 24 and 48 hours for all concentrations both with and without S9. The Substance induced MN in human lymphocytes with and without metabolic activation. A clearer response was seen without metabolic activation especially when treatment was started 48hrs rather than 24hrs after mitogen stimulation.

For the key studies reported above, you also provided the original reports to the eMSCA.

Moreover, the following non-guideline compliant *in vitro* tests are reported in the IUCLID as robust study summaries for the Substance:

- An *in vitro* comet assay was performed in Chinese hamster V79 cells with and without metabolic activation at 0, 308, 616 and 1232 µg/mL during 3 hours of treatment ([redacted], 2005). DNA damage was measured as % tail DNA appearing as a comet with head and tail. The Substance induced a clear dose-dependent increase in DNA damage of the cells in the presence and absence of metabolic activation.
- The Substance was evaluated for cell transformation assay on Syrian Hamster Cells (SHE; [redacted], 2005). After a preliminary study (dose range finding study), SHE cells were exposed during 24 hours and subsequent 7 days incubation to 8 doses (from 0 to 120 µg/mL). The average number of colonies per dish was calculated. Each colony was evaluated and recorded as either normal or morphologically transformed (MT). There was a dose-related trend ($p<0.05$) in the number of transformed colonies. The results were considered as positive. Two of the concentrations (80 and 120 µg/mL) gave a statistically significant increase in the transformation frequency ($p<0.05$) when compared to the solvent control.

The evaluating MSCA considers that the Substance is able to induce gene mutation on mammalian cells (L5871Y mouse lymphoma cells) and both CA and MN (clastogenicity/ aneugenicity) in human lymphocytes. The available information suggests that the Substance may have a mutagenic effect.

Genotoxiciy *in vivo*

- A micronucleus test was performed according to OECD TG 474 using male and female NMRI mice which were treated by gavage with 0, 125, 250 and 500 mg/kg bw for 24 h preparation interval and 500 mg/kg bw for 48 hours preparation interval ([redacted], 2005). In comparison to the corresponding vehicle controls, there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any harvest time (24 and 48 h) and dose level after administration of the Substance. Some signs of systemic toxicity, such as reduction of spontaneous activity, ruffled fur and orange colored urine, was observed in the

animals treated at 250 and 500 mg/kg bw. The ratio of PCE/NCE in BM was not decreased after treatment with the Substance as compared to the control. A negative result in a MN test in the absence of clear toxicity to target organ (toxicity to Bone marrow cell, PCE/NCE ratio) should be considered inconclusive. Moreover, no conclusion can be drawn on the effects at first site of contact (i.e. GI tract after oral administration) where the Substance concentration could be higher than at the distal site. Then, the eMSCA considers that it cannot to conclude whether the Substance administered at a higher dose could be genotoxic at the site of first contact. On the other hand, the assay ([redacted], 2005) was performed at MTD dosage and dosing up to 500 mg/kg resulted in signs of systemic toxicity. Thus, it is unlikely that a new *in vivo* MN study would produce different results.

- A micronucleus test was performed according to OECD TG 474 using male and female NMRI mice which were treated by intraperitoneal injection with 20, 100 and 200 mg/ kg bw for 24 hours and with 200 mg/kg bw for 48 hours ([redacted], 2002b). In comparison to the corresponding negative controls there was no statistically significant enhancement ($p < 0.05$) in the percentage of cells with micronuclei at any harvest time or dose level of the test substance. In comparison to the historical controls, the relative PCE was decreased in some treatment groups, especially in male mice, but also in the negative controls of female mice. As these effects were not dose related, they were not used as an unequivocal indicator for the test substance having reached bone marrow. Based on above, the Substance was non-mutagenic in the micronucleus test with bone marrow cells of the mouse when administered intraperitoneally. Although the systemic availability of the Substance can be assumed because of the application route used, no conclusion can be reached, due the very low dosage used, corresponding to 1/10 of the maximum dose to be tested and lower than the dosage used in MN in gavage study ([redacted], 2005).
- An in vivo UDS study was performed on male Sprague Dawley rats (according to OECD TG 486) which were treated by oral gavage with 0, 500, 1000, 2000 mg/kg bw ([redacted], 2005). The Substance was administered to 5 male rats/ dose level/ exposure time (i.e. either 2 to 4 h or 12 to 16 h) giving a total of 10 animals at each dose. The only exception was in 2000 mg/kg bw group where 7 rats/ exposure time were used giving a total of 14 animals. No exposure period induced unscheduled DNA synthesis in primary rat hepatocytes in an autoradiographic *in vivo* unscheduled DNA synthesis assay.

  In your comment to the draft decision, you considered, as provided in the expert's statement, that the negative results in the assay proves that the Substance is not able to induce mutagenicity in the liver. The eMSCA does not support this conclusion because the UDS test is known to have limited sensitivity in particular with some types of DNA lesions. As reported in the ECHA guidance (p. 572, 2017): "*A negative result in a UDS assay alone is not a proof that a substance does not induce gene mutation*". Although the liver could appear as the target organ of the Substance, the limits of the UDS to reveal only some DNA repair mechanisms makes the negative UDS study inconclusive regarding the ability of the substance to induce genotoxicity.
- An *in vivo* comet assay was performed on Wistar male rat before the OECD TG 489 publication ([redacted], 2005) with the analysis of liver, stomach, and urinary bladder. The authors declared they followed the robust method, scientifically agreed, reported in Hartmann *et al.*, 2003. Five male Wistar rats per group were treated twice via gavage with 0, 500, 1000 and 2000 mg/kg bw, respectively, with a time difference of 20 hours between administrations. Animals were sacrificed 3 hours after the second administration. Histopathology of the liver, stomach, and urinary bladder was assessed in a parallel experiment with three male rats for each concentration. Inconclusive results were reported at all doses for the first comet assay, and groups of 10 male Wistar rats were treated in an additional assay analogously with 0 and

2000 mg/kg bw. Tail length, tail moment and tail intensity (% tail DNA) were used as assessment parameters for genotoxicity.

In the first comet the results showed: in the liver, a statistically significant increase in mean tail length, mean tail moment, and mean tail intensity at low-dose, as well as only a statistically increase of the mean of tail moment at mid-dose. The mean tail moment showed similar results also in stomach and urinary bladder. No dose-response was observed in all treated organs in all parameters analysed. The author assigned this finding to a loss of cells due to hepatotoxicity. A hepatotoxic response was observed only at the high dose (2000 mg/kg) while no cytotoxicity was reported at any other doses.

A second experiment with only the high dose tested is also reported. The authors concluded that the Substance is not genotoxic in the stomach and urinary bladder while a primary genotoxic effect cannot be excluded in liver cells. However, considering that a positive effect was observed also in stomach, although only at the lowest dose, the eMSCA considers the test as inconclusive also in this organ.

In your comments to the draft decision, you argued that the data of this study are difficult to interpret, as provided in the expert's statement. The eMSCA considers the assay inconclusive: in brief, the comet run 1 should be considered as equivocal (you deem these data were inconclusive); in the comet run 2, the results should be interpreted with caution: it is not clear why the second comet was done only at the high dose (2000 mg/kg) where effects were observed only in the stomach (mean of tail intensity, not in liver and urinary bladder) in the comet run 1. As also reported in the expert statement, the data of the low dose group were very heterogenous and a repetition (as for the high dose) could help to interpret correctly the results.

Finally, the negative results reported in the UDS assay were not considered sufficient to rule out the concern for genotoxicity for the reasons reported above. Therefore, to reach a final conclusion, the study must be repeated under appropriate experimental conditions.

The Substance was originally selected by ECHA to be jointly evaluated with other aminophenols during the substance evaluation process considering that the Substance is structurally very similar to the aminophenols (i.e. ortho-, meta- and para-aminophenol isomers), differing from the meta isomer (3-aminophenol, EC number 209-711-2) only for the presence of a methyl substituent. In this regard, preliminary structure-activity considerations point to the possibility for the Substance to share a genotoxic mechanism of action with aminophenols, highlighted in particular by the isomers 4-aminophenol (EC number 204-616-2) and 2-aminophenol (EC number 202-431-1), through the formation of DNA reactive electrophilic intermediates. These considerations take into account that the methyl substituent could stabilize the electrophilic intermediate and increase the genotoxic potential of the Substance compared to 3-aminophenol. However, the eMSCA has decided to evaluate the substances separately, due to metabolic differences for the structural analogues.

In your comments to the draft decision, you argued (as provided in the expert's statement) that "*[…] with regards to chemical and biological structure-activity, 5-amino-o-cresol lacks an inherent propensity to form intermediary reactive quinones compared to 4-aminophenol (CAS RN 123-30-8) and 2-aminophenol (CAS RN 95-55-6), which therefore excludes a potential for a relevant shared mode of action as postulated* in the draft decision".
Although we agree that the mechanism of genotoxicity of 4-aminophenol (EC number 204-616-2) and 2-aminophenol (EC number 202-431-1), via quinoneimine formation, is not relevant for the Substance, this is not sufficient to exclude possible alternative pathways. For instance, primary aromatic amines, such as 5-amino-o-cresol, can be metabolically

activated to reactive electrophiles involving the activation of N-hydroxylamine metabolites with enzymatic reaction and eventual formation of highly reactive nitrenium ions which can covalently bind to DNA.

Nevertheless, having analyzed the overall evidence the eMSCA conclusions are based primarily on the experimental findings you reported for the Substance. The data showed a relevant gene mutation and clastogenic and/or aneugenic potential for the Substance *in vitro* while no firm conclusion can be drawn *in vivo*.

In your comments to the draft decision, you claimed that the systemic availability of the Substance after oral application based on toxicokinetic and NTP studies is demonstrated. The eMSCA considers that while some systemic exposure is demonstrated, no conclusion can be reached regarding a possible effect at the first site of contact where the exposure is assumed to be higher.

In your comments to the draft decision, you also referred to European Commission's SCCP conclusion stating that the Substance has no relevant mutagenic potential *in vivo*. However, the eMSCA notes that also the SCCP in its conclusions states that: "*primary genotoxicity of 4-amino-2-hydroxytoluene [the Substance] could not be excluded in rat liver, where the comet assay indicated an increase in DNA strand breakage*." The final SCCP conclusion "*On the basis of the available data, the substance has no relevant mutagenic potential in vivo*" is not explained and appears to the eMSCA not justified.

In your comments to the draft decision, you also referred to [redacted] studies yielding negative results for genotoxicity. Regarding the negative *in vivo* MN test, the eMSCA considers that in this test. no toxicity in the bone marrow was reported and therefore the target exposure is not demonstrated. Therefore, the assay should be considered inconclusive for the reasons reported above. Also, the eMSCA notes that the [redacted] reported also an Ames study in which three out of five bacterial strains gave positive results for mutagenicity.

You concluded in your comments that "*based on a weight of evidence analysis 5-amino-o-cresol is not considered to be an in vivo genotoxin*." However, the eMSCA considers that no firm conclusion can be drawn for mutagenicity as reported above in the description of each study.

Considering that the available data does not allow a firm conclusion the eMSCA deems requesting further data is the most adequate approach to clarify the mutagenicity concern.

In conclusion, the available information showed a relevant gene mutation and clastogenic potential for the Substance *in vitro* while no firm conclusion can be drawn *in vivo*. Therefore, an *in vivo* mammalian alkaline comet assay test (OECD 489) in liver, gastro-intestinal tract (glandular stomach and duodenum), and urinary bladder performed in rats via oral route using the Substance is requested.

1.2 Potential exposure

According to the information you submitted in chemical safety reports (CSR) the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 100–1000 tonnes per year.

Furthermore, you reported that among other uses, the Substance is used:

- by consumers in cosmetics and personal care products, and in indoor use as processing aid;
- by professional workers in cosmetics and personal care products and [redacted] products, and in indoor use (e.g. [redacted]

[redacted]).

Therefore exposure to workers and consumers cannot be excluded.

1.3 Identification of the potential risk to be clarified

Based on all information available in the registration dossier and information from the published literature, there is sufficient evidence to justify that the Substance may have genotoxic/mutagenic effects on somatic and/or germ cells.

The information you provided on manufacture and uses demonstrates a potential for exposure of workers and consumers.

Based on the hazard and exposure information the Substance poses a potential risk to human health.

As explained in Section 1.1 above, the available information is not sufficient to conclude on the potential hazard. Consequently, further data is needed to clarify the potential risk related to the mutagenicity of the Substance.

1.4 Further risk management measures

If the mutagenicity of the Substance is confirmed, the evaluating MSCA will analyse the options to manage the risk(s). New regulatory risk management measures could be harmonisation of the classification for the mutagenicity concern and, as a consequence, improved measures at manufacturing sites, better waste management and revised instructions on safe use, if appropriate.

The results from the request will, amongst other relevant and available information, be used by the evaluating MSCA to assess whether the Substance should be classified as germ cell mutagen as defined in the CLP Regulation.

The potential classification of the Substance as germ cell mutagen would have consequences for the classification of mixtures containing the Substance due to cut-off/ concentration limits triggering classification and acceptability of consumer products.
If classified as germ cell mutagen revised instructions on safe use could be applied, if appropriate.

2. How to clarify the potential risk

2.1 *In vivo* mammalian alkaline comet assay (OECD TG 489) performed in rats via oral route on specific tissues

a) Aim of the study

A comet assay will clarify the *in vivo* genotoxicity/mutagenicity of the Substance as further specified below.

To address the missing information identified above, the OECD TG 489 required will allow to obtain information on genotoxicity/mutagenicity in somatic cells and possibly in germ cells.

b) Specification of the requested study

Test material: the Substance

Route of exposure

The oral route (by gavage) is the most appropriate to investigate local gastro-intestinal tract related effects and systemic genotoxicity potential for the Substance.

Tissues to be investigated

- Liver, gastro-intestinal tract (glandular stomach and duodenum) and urinary bladder.

In line with the OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular glandular stomach and duodenum as first sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.
Moreover, to compare the data with the available comet *in vivo* where the urinary bladder was investigated, you are requested also to collect and analyse this organ because it represents a distal site where the Substance or its metabolites can accumulate before their elimination.

- Gonadal cells

You may consider to collect the male gonadal cells collected from the seminiferous tubules at the same time as the other tissues, to optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light.

Following the generation and analysis of data on somatic cells, you should consider analysing the slides prepared with gonadal cells, using the comet assay. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation. In case of positive results in any of the somatic tissues, you must analyse the collected gonadal cells.

As reported in the OECD TG 489, "*positive results in whole gonad are not necessarily reflective of germ cell damage, nevertheless, they indicate that tested chemical(s) and/or its metabolites have reached the gonad*".

You are reminded that a subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required if 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

Request for the full study report

You must submit the full study report which includes:

- a complete rationale of test design and
- interpretation of the results
- access to all information available in the full study report, such as implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.

This will enable the evaluating MSCA to fully and independently assess all the information provided, including the statistical analysis, and to efficiently clarify the potential hazard for the Mutagenicity for the Substance.

c) Alternative approaches and how the request is appropriate to meet its objective

The request for an *in vivo* mammalian alkaline comet assay test (OECD TG 489) is:

- appropriate, because it will provide information which will clarify the genotoxicity/mutagenicity *in vivo* also at the site of contact. The *in vivo* mammalian alkaline comet assay (OECD TG 489) is suitable to follow up the positive *in vitro* result for gene mutation and chromosomal aberrations and can be applied in many tissues including “site of contact” tissues and gonadal cells. This will enable the evaluating MSCA to conclude on potential classification for mutagenicity.
- the least onerous measure because there is no equally suitable alternative method available to obtain the information that would clarify the potential mutagenicity hazard, amongst the *in vivo* tests.
- Two possible alternative *in vivo* are available, the TGR assay (OECD TG 488) and the spermatogonial assay (OECD TG 483). The TGR is not the most adequate because it is only able to detect gene mutation *in vivo* and is also a more expensive test. The spermatogonial assay is able to detect clastogenic effects but only on germ cells.

2.2 References relevant to the request (which are not included in the registration dossier)

ECHA, 2017: Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance.

[redacted], 2002a: *In vitro* Mammalian Cell Gene Mutation Assay (Thymidine Kinase Locus/TK +/-) in Mouse Lymphoma L5178Y Cells (study report).

[redacted], 2002b: Mammalian Micronucleus Test of Murine Bone Marrow Cells with 23032 (study report).

Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V, Tice RR; 4th International Comet Assay Workshop. Recommendations for conducting the in vivo alkaline Comet assay. 4th International Comet Assay Workshop. Mutagenesis. 2003 Jan;18(1):45-51. doi:10.1093/mutage/18.1.45. PMID: 12473734.

[redacted], 2005: Micronucleus Assay in Bone Marrow Cells of the Mouse with 4-Amino-2-Hydroxytoluene (WR 23032) (study report).

[redacted], 1988: Study to evaluate the chromosome damaging potential of B177 by its effects on cultured human lymphocytes using an in vitro cytogenetics assay (study report).

[redacted] Technical Report on the Toxicity Studies of 5-amino-o-cresol (cas. N.2835-95-2) administered dermally To f344/NTac Rats and B6C3F1/N mice. 2015.

[redacted], 2005: SHE Cell Transformation Assay (study report).

[redacted], 2005: *In Vivo* Unscheduled DNA Synthesis (UDS) Test in Rats (study report).

Scientific Committee on Consumer Products (SCCP), 2006: Opinion on 4-Amino-2-hydroxytoluene COLIPA N° A27 (publication), European Commission, Health & Consumer Protection Directorate-General. Report no: SCCP/1001/106. Report date: Oct 10, 2006.

[redacted], 2005: Salmonella Typhimurium Reverse Mutation Assay with 4-Amino-2-Hydroxytoluene (WR 23032) (study report).

[redacted], 2005: 4-Amino-2-hydroxytoluene: Induction of micronuclei in cultured human peripheral blood lymphocytes (study report).

[redacted], 2005: Comet Assay in vivo In Liver, Stomach and Urinary Bladder Epithelium of Male Rats (study report).

[redacted], 2005: Single cell gel electrophoresis analysis (comet assay) to detect DNA damage induced by 4-amino-2-hydroxytoluene ([redacted]) and its acetylated derivative 4-acetylamino-2-hydroxytoluene ([redacted]) in Chinese hamster V79 lung cells (study report).

Appendix B - Procedure

This decision does not imply that the information you submitted in your registration dossier(s) are in compliance with the REACH requirements. ECHA may still initiate a compliance check on your dossiers.

12-month evaluation

Due to initial grounds of concern for Mutagenicity, Sensitisation (skin) and other hazard-based concern, the Member State Committee agreed to include the Substance (EC No 220-618-6, CAS RN 2835-95-2) in the Community rolling action plan (CoRAP) to be evaluated in 2020. The National Institute of Health (ISS), Italy is the competent authority ('the evaluating MSCA') appointed to carry out the evaluation.

In accordance with Article 45(4) of REACH, the evaluating MSCA carried out its evaluation based on the information in the registration dossier(s) you submitted on the Substance and on other relevant and available information. The evaluating MSCA completed its evaluation considering that further information is required to clarify the following concerns: Mutagenicity

Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA on 18 March 2021.

Decision-making

ECHA notified you of the draft decision and invited you to provide comments.

For the purpose of this decision-making, dossier updates made after the date the draft of this decision was notified to you (Article 50(1) of REACH) will not be taken into account.

Registrant(s)' commenting phase

ECHA received your comments and forwarded them to the evaluating MSCA. The evaluating MSCA took your comments into account (see Appendix A).

The request(s) and the deadline to provide information were amended: the initially requested *in vivo* micronucleus test (OECD TG 474) was removed from the current decision

Proposals for amendment by other MSCAs and ECHA

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

As no amendments were proposed, ECHA took the decision according to Articles 52(2) and 51(3) of REACH.

Appendix C - Technical Guidance to follow when conducting new tests for REACH purposes

Test methods, GLP requirements and reporting

Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[2].

Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. *Selection of the Test material(s)*

   The Test Material used to generate the new data must be selected taking into account the following:

   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

2. *Information on the Test Material needed in the updated dossier*

   a) You must report the composition of the Test Material selected for each study, under the 'Test material information' section, for each respective endpoint study record in IUCLID.
   b) The reported composition must include all constituents of each Test Material and their concentration values.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual "How to prepare registration and PPORD dossiers"[3].

[2] https://echa.europa.eu/practical-guides
[3] https://echa.europa.eu/manuals