Helsinki, 24 May 2022

**Addressees**
Registrant(s) of 3-aminophenol listed in the last Appendix of this decision

**Registered substance subject to this decision (the 'Substance')**
Substance name: 3-aminophenol
EC number: 209-711-2
CAS number: 591-27-5

**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 (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.

Authorised[1] by 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 3-aminophenol, 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 reported by the Registants is not sufficient to clarify the identified concern.

In particular, the available *in vitro* and *in vivo* data were analysed using a weight of evidence approach. The *in vitro* results showed a clear ability for the Substance to induce clastogenicity and/or aneugenicity in mammalian cell lines. Moreover, the *in vitro* data showed a potential of the Substance to induce gene mutation only in bacteria. The available *in vivo* data reported in the chemical safety report (CSR) are not sufficient to drawn a firm conclusion on this endpoint. Therefore a concern for potential mutagenicity of the Substance cannot be excluded.

The original reports of the studies cited in the CSR were provided by the Registrant(s). Also the data publicly available in literature and the opinion 0978 of Scientific Committee on Consumer Products (SCCP) published in 2006 (SCCP/0978/06 opinion) were considered in the evaluation.

Genotoxicity *in vitro*

The available *in vitro* genotoxicity studies for the analysis of gene mutation or clastogenic/aneugenic effects both in bacteria and/or mammalian cells for the Substance are the following:

- An unpublished report for the Substance performed according to OECD TG 471 is reported in the CSR ([redacted], 2005a). The following five strains of bacteria *Salmonella typhimurium* were tested: TA 1535, TA 1537, TA 98, TA 100 and TA 102. Each strain was exposed to five concentration-levels of the test item (three plates/ concentration-level). All strains were treated with 0, 625, 1250, 2500, 3750 and 5000 mg/plate both with and without S9 mix. The test material did not induce any noteworthy increase in the number of revertants, in any of the five tested strains in the absence of S9. A moderate toxicity was noted at concentration-levels ≥ 2500 µg/plate in the TA 98 strain, without S9 mix. A reproducible, dose-dependent increase in the number of revertants was found for TA98 in the presence of S9 metabolic activation.
- Other studies on gene mutation in bacteria available in literature and reported in the CSR and in the IUCLID registration dossier also support these data. All the studies confirmed the negative results for strains TA 1535, TA 1537, TA 100 and TA 102 and *E. coli* WP2 both with and without S9 mix, while mixed results are reported for the TA 98 strain.
- The Substance was also tested for its ability to induce gene mutation in mammalian cells in a study conducted in mouse lymphoma L5178Y cells, performed according to OECD TG 476 ([redacted], 2005). Cells were treated for 3 hrs followed by an expression period of 7 days to fix the DNA damage into a stable *tk* mutation. When tested up to

10 mM, a statistically significant increase in the mutant frequency at the *tk* locus was not observed following treatment with the Substance at any dose level tested in the absence or presence of S9 in all three experiments. Therefore, in the conditions of this test, the Substance is not able to induce gene mutation at the *hprt* locus of mouse lymphoma cells.

In your comments to the draft decision you stated that: "*The Substance can be considered non-mutagenic in mammalian cells as it has tested negative for gene mutation at the hprt locus in mouse lymphoma L5178Y cells.*".

The evaluating MSCA agrees that the Substance does not appear to induce gene mutations. However, the potential of the Substance to induce clastogenicity *in vitro* is demonstrated (see below).

- The Substance was also tested for chromosomal aberration (CA) in human lymphocytes of two healthy donors (male and female) performed according to OECD TG 473 ([redacted], 2005b). In two independent experiments (duplicate cultures), the cells were treated for 3 hours, both with and without S9 mix, using at least five dose-levels of the test item (0, 0.63, 1.25, 2.5, 5, 7.5 or 10 mM) and harvest after 20 hours. Toxicity was monitored using mitotic index.

  Without S9 mix, in the first experiment a statistically significant increase in the frequency of cells with CA was only found at 10 mM whereas in the second experiment a statistical significant and dose-related increase in the number of cells with CA was observed.

  With S9 mix in both experiments a statistically significant and dose-related increase in the number of cells with CA was noted. Under the experimental conditions the Substance induced CA in cultured human lymphocytes, both in the presence and absence of metabolic activation.
- The ability of the Substance to induce CA was also tested in Chinese hamster lung (CHL/IU) cells according to OECD TG 473 ([redacted], 2012). The experiment was conducted at the following concentration: -S9 mix (short-term treatment) at 0, 0.28, 0.55, 1.1 mg/mL; +S9 mix (short-term treatment) at 0, 0.015, 0.030, 0.060 mg/mL; -S9 mix (continuous treatment for 24 hours) at 0, 0.034, 0.069, 0.14 mg/mL. The Substance did not induce CA in CHL/IU cells, with S9 mix. Cells with structural CA increased dose dependently with continuous treatment for 24 hours, without metabolic activation (frequency: 8.0-21.0%). Therefore the Substance is able to induce CA also in CHL/IU cells.
- An *in vitro* mammalian micronucleus assay (MN) performed in human lymphocytes of 4 healthy, non-smoking, male volunteers according to OECD TG 487, is reported in the CSR ([redacted], 2005). Four independent experiments were conducted with and without S9 mix. Treatment periods were 20 hours without S9 mix and 3 hours with S9 mix. Harvest times were 72 hours in the experiment 1 and 96 hours in the experiment 2 and 4 hours after beginning of the culture, respectively. During the final 24 hours of incubation, cells were treated with cytochalasin B.

  In experiment 1 without S9 mix (704 - 1100 µg/ml) the Substance did not induce an increase in the frequency of micronuclei compared to concurrent vehicle controls. Statistical significant increases in the MN-frequency were found in the presence of S9 (564.7 - 1100 µg/ml). However, there was no clear dose-response relationship.
  In experiments 2 and 3 (performed at the similar dosage 794.8 - 1100 µg/ml) in the absence of S9 but with an extended Phytohaemagglutinin (PHA) stimulation, statistically significant increases in micronucleus induction were observed. Again a dose-response relationship was not apparent. Since data observed in experiment 2 were confirmed in experiment 3, these results were considered as biologically relevant.
  In the presence of S9 and an extended PHA treatment only in experiment 3, a

statistically significant increase in micronucleus induction (without dose response relationship) was observed. In both other experiments (experiments 3 and 4) MN frequencies similar to those of concurrent control cultures were found. The biological relevance of the positive results in the presence of S9 are considered questionable. In conclusion, the study showed evidence of genotoxic effect in human lymphocytes *in vitro*. This study is also reported in the SCCP/0978/06 opinion with the same conclusion.

The evaluating MSCA considers that the potential of the Substance to induce clastogenicity *in vitro* is demonstrated: the Substance is able to induce chromosome aberrations (CA) and micronuclei (MN - clastogenicity/aneugenicity) both in human lymphocytes and in CHL/IU cells. Therefore this finding needs to be followed up.

Genotoxicity *in vivo*

- In the IUCLID dossier you reported only one literature study (Hossack and Richardson, 1977) of an *in vivo* MN assay in Sprague-Dawley rats by gavage: the test was performed on twelve substances by oral administration in rats and negative results are reported for all tested substances, including the Substance. No indication on target exposure or toxicity is reported. Also this study was performed before the adoption of OECD TG 474 and is considered inconclusive.
- In the SCCP/0978/06 report a micronucleus assay in bone marrow of rats according to OECD TG 474 was reported. The Registrant(s) provided the original report to the eMSCA for the evaluation during the Registrant(s)' commenting phase. The study was conducted at 0, 375, 750 and 1500 mg/kg bw doses by gavage ([redacted], 2005): 24 hr or 48 hr (highest dose and concurrent vehicle control only) after dosing bone marrow cells were collected. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE). Bone marrow preparations were stained and examined microscopically for the PCE/NCE ratio and MN. The results of the assay reported indicated the following: the Substance induced mortality in male (2/16) and female (9/16) rats treated with the highest dose of 1500 mg/kg bw and in one female (1/16) treated with 1000 mg/kg bw. Clinical signs indicating systemic toxicity were observed in all treated animals. Decreases in the PCE/NCE ratio were not observed at doses up to 1000 mg/kg bw. A satellite group of 3 rats/sex treated with 1000 mg/kg bw was also included for possible determination of plasma concentrations of the test chemical at 1 and 4 hours post-dosing. The mean values measured in the circulation in both male and female rats were 100 ± 26 µg/ml and 11 ± 8 µg/ml at 1 and 4 hours, after treatment with 1000 mg/kg bw, respectively. These values are two orders of magnitude lower than the concentrations found to be positive in the *in vitro* CA assay (100 ± 26 µg/ml and 11 ± 8 µg/ml at 1 and 4 hours, after treatment with 1000 mg/kg bw, respectively, the top dose used *in vitro* CA study being 1090 µg/ml).

  In the experimental conditions, no genotoxic effect was reported in the bone marrow. As reported in the OECD TG 474 the assay is considered conclusively negative provided that the bone marrow exposure to the Substance is demonstrated. Evidence of bone marrow exposure may derive from reduction in the PCE/NCE ratio or measurements of plasma levels. In the MN assay no reduction in PCE/NCE ratio was observed and the plama levels reported did not demonstrate a sufficient exposure of bone marrow to the substance. Therefore, the negative result in the bone marrow MN test is not conclusive because possible effect at the first site of contact cannot be ruled out. A new MN assay in bone marrow would not add relevant information, as the available study was conducted up to the maximum tolerated dose (MTD).

Therefore, the initially requested micronucleus test (OECD TG 474) was removed from the current decision.

In your comments to the draft decision you stated that: "*The Substance can be considered non-clastogenic in vivo as it has tested negative for micronuclei induction in PCEs obtained from rat bone marrows*". Nonetheless the evaluating MSCA considers the *in vivo* MN study inconclusive for the reasons reported in details above.

In conclusion, the *in vitro* experimental data showed a clear genotoxic effect (clastogenic and/or aneugenic) of the Substance (positive CA and MN in human lymphocytes) while its ability to induce gene mutation is less clear (slight mutagenic activity was observed in presence of S9 only in TA 98 strain, negative results were reported in mouse lymphoma L5178Y cells). The clastogenic effect observed *in vitro* seems not to be confirmed in the *in vivo* study: no direct (i.e. PCE/NCE) evidence of target exposure was reported, but the study was performed according to OECD TG 474 and it was considered conclusive negative as cited in the SCCP/0978/06 report. The eMSCA considers the *in vivo* MN inconclusive based on the considerations reported above.
Therefore, the eMSCA deems a genotoxicity assay able to reveal site-of-contact effects (such as the Comet assay *in vivo*) is needed, in order to conclude on the genotoxicity of the Substance.

In your comments to the draft decision you also draw the attention to: "*the absence of carcinogenicity risk*", because "*the Substance is not N-oxidized in the presence of hepatic microsomes*".
The evaluating MSCA considers that the *in vitro* metabolism studies you provided (Skare et al., 2009; Zeller and Pfuller, 2014; Manwaring et al. 2015) can be taken into account in the overall assessment of the Substance. Nonetheless, in the presence of an *in vitro* positive outcome in a genotoxicity assay, an *in vivo* follow-up is anyway required under REACH.

Moreover, the studies you provided (in particular Skare et al., 2009) addressed the N-oxidation by the hepatic CYP enzymes in a similar substance to the Substance and were not performed with the Substance itself.
However, the evaluating MSCA notes that the studies you referred to in your comments during the commenting phase were not included in the CSR nor were they present in the SCCP/0978/06 report. Therefore the CSR should be updated.

In addition, the Substance gave positive results *in vitro* also in the absence of metabolic activation, which is indicating that the hepatic metabolism does not appear to be the only pathway of genotoxicity.

The current information is not sufficient to draw a conclusion on mutagenicity of the Substance. Consequently, further information is needed on clastogenicity of the Substance.
It is appropriate to perform a comet assay *in vivo* in order to address the potential *in vivo* clastogenicity of the Substance: an *in vivo* mammalian alkaline comet assay (OECD TG 489) in liver, gastro-intestinal tract (glandular stomach and duodenum) and urinary bladder performed in rats via oral route is requested.

### 1.2 Potential exposure

According to the information you submitted in the 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 and professional workers in cosmetics and personal care products, and in indoor use (e.g. [redacted]);
- by industrial workers in textile treatment products and dyes and leather treatment products, in manufacture of another substance (use of intermediates), and as processing aid.

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 cause 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* genotocity 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 in somatic cells and possibly in germ cells.

**b) Specification of the requested study**

*Test material*: the Substance

*Route of exposure*

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

In your comments to the draft decision, you argued that "[the Substance is] *mostly used as a component in cosmetics, personal and home care products by consumers; therefore, the dermal route should be considered the most relevant human exposure route."* The evaluating MSCA acknowledges this comment. However, in the CSR and SCCP/0978/06 report no mutagenicity assay was performed through the dermal route for the Substance. Doubts on the appropriate dosage and the ability of the Substance to reach distal sites, in case of negative results, could still remain and invalidate the results of the assay. Moreover, in the SCCS/0978/06 report it is shown that only about 2% of the applied dose is absorbed by the dermal route. In the absence of ADME data for dermal absorption, it is doubtful that a relevant experimental study to verify the genotoxic hazard of the Substance could be conducted using dermal exposure because the adequate exposure of the target tissue(s) is not ensured. Furthermore, the oral route ensures a higher systemic exposure to the substance. Hence the request was not amended.

*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 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.
The urinary bladder is requested because it represents a distal site where the Substance or its metabolites can accumulate before their elimination. Moreover, urinary bladder was evaluated in the comet *in vivo* with 5-amino-o-cresol (CAS RN 2835-95-2) which is also part of the group of 'aminophenol'. As the evaluating MSCA intends to compare the results, you are therefore requested to also analyse this tissue.

- Gonadal cells

You may consider collecting the male gonadal cells from the seminiferous tubules at the same time as the other tissues, as it would 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 (OECD TG 489) is:

- appropriate, because it will provide information which will clarify the 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 genotoxicity (both gene mutation and chromosomal aberration) 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.

In the comments to the draft decision, you indicated that "*the use of non-animal test methods and testing strategies shall be preferred […] to generate information on intrinsic properties and risk assessment of the Substance to meet the REACH regulation requirement*." You further proposed an alternative testing strategy were non-animal test methods will be used to address the mutagenicity concern. The proposed tests are:

1) 3D reconstructed human skin comet assay and
2) Analysis of the metabolite profile of a substance in human hepatocytes *in vitro*.

The evaluating MSCA cannot support the use of the proposed strategy.
The appropriate *in vivo* follow-up for clastogenic effect observed *in vitro* is the *in vivo* mammalian alkaline comet assay (OECD TG 489). The test can be applied in many tissues including the site-of-contact tissues, metabolic organs and distal sites, while the *in vitro* 3D comet assay provides only information at the site of contact, which is not sufficient, because for example the metabolism in the skin could be different from the liver

metabolism. Moreover, the *in vitro* assay is not able to deliver conclusions on the suspected hazard and cannot lead to classification/ clarification of the concern.
On the other hand, the *in vivo* assay will enable the evaluating MSCA to conclude on potential classification for mutagenicity, enabling to investigate the site of first contact, the liver (where oxidative metabolism may occur) and the urinary bladder (the potential carcinogenic site for aromatic amines).

At present the recommended test under REACH for *in vitro* positive substances is an appropriate *in vivo* test such as MN or Comet. The requested assay is the least onerous measure amongst the *in vivo* tests to obtain the information that would clarify the potential mutagenicity hazard.

In addition, you proposed to perform the analysis of the metabolite profile of a substance in human hepatocytes *in vitro*. The eMSCA does not consider this approach useful for clarification of the mutagenicity concern. In fact, the Substance gave positive results *in vitro* also in the absence of metabolic activation, indicating that the hepatic metabolism does not appear to be the only pathway involved in the genotoxicity. In this case an *in vivo* genotoxicity test, where all the metabolic pathways are active, is most suitable.

Consequently there is no other alternative test which will generate the necessary information and which does not need to use vertebrate animals.

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

Opinion of *Scientific Committee on* Consumer Safety (SCCS/0978/06) on 3-aminophenol published in 2006, available at:
https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_088.pdf

Opinion of *Scientific Committee on* Consumer Safety (SCCS, 2021) on 'THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION, 11TH REVISION'

[redacted]: Mutation at the hprt locus of L5178Y Mouse Lymphoma Cells using the MicrotitreR Fluctuation Technique (study report), [redacted]Report no: 413/106-D6173.

[redacted], 2005a: Bacterial Reverse Mutation Test (study report).

[redacted], 2005b: *In vitro* mammalian chromosome abberation test in cultured human lymphocytes (study report) no[redacted].

[redacted], 2012: *In Vitro* Chromosomal Aberration Test of 3-Aminophenol on Cultured Chinese Hamster Cells (study report).

[redacted], 2005: Induction of micronuclei in cultured human peripheral blood lymphocytes, [redacted] study number no: [redacted].

Hossack, D.J.N., Richardson, J.C. Examination of the potential mutagenicity of hair dye constituents using the micronucleus test. Experientia 33, 377–378 (1977). https://doi.org/10.1007/BF02002837

[redacted], 2005: *In Vivo* Rat Micronucleus Assay in m-Aminophenol (A015). [redacted] Study No. [redacted].

Manwaring J., Rothe H. , Obringer C., Foltz D.J. ,Baker T.R., Troutmana J.A., Hewitt N.J., Goebel C., Extrapolation of systemic bioavailability assessing skin absorption and epidermal and hepatic metabolism of aromatic amine hair dyes in vitro Toxicology and Applied Pharmacology 287 (2015) 139–148

Skare J. A., Hewitt N. J., Doyle E., Powrie R. & Elcombe C. (2009) Metabolite screening of aromatic amine hair dyes using in vitro hepatic models, Xenobiotica, 39:11, 811-825, DOI:10.3109/00498250903134443

Zeller A. and Pfuhler S. (2014), N-acetylation of three aromatic amine hair dye precursor molecules eliminates their genotoxic potential; Mutagenesis vol. 29 no. 1 pp. 37–48; doi:10.1093/mutage/get053

## 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 for other hazard-based concern, the Member State Committee agreed to include the Substance (EC No 209-711-2, CAS RN 591-27-5) 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

Four registrants commented that the concerns identified in the Decision are not relevant to their specific strictly controlled conditions of use:

- three of the registrants provided appropriate information and were removed as addressees of the Decision;
- the fourth registrant subsequently informed ECHA that they cease manufacture in accordance with Article 50(3). Consequently they are no longer addressed in this 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