SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Bis(4-hydroxy-N-methylanilinium) sulphate

EC No 200-237-1

CAS No 55-55-0

Evaluating Member State: Italy

Dated: 26 July 2021

Evaluating Member State Competent Authority

MSCA Italy National Institute of Health on behalf of Ministry of Health
Viale Regina Elena, 299 - 00161 Rome, Italy in cooperation with Italian National Institute for Environmental Protection and Research (ISPRA). Via Brancati, 48 - 00144 Rome, Italy

Tel.: +390649902061
FAX: +390649902286
Email: leonello.attias@iss.it

Year of evaluation in CoRAP: 2020

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1).

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

bis(4-hydroxy-N-methylanilinium) sulphate was originally selected for substance evaluation in order to clarify concerns about:

- Mutagenicity
- Skin sensitisation
- Other hazard based concern: STOT RE

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Bis(4-hydroxy-N-methylanilinium) sulphate is covered by the Seveso III Directive (Directive 2012/18/EU in the Seveso category E1.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

On the basis of the available information, a harmonised classification of the substance is proposed by the eMSCA, as a follow-up at EU level for the following hazard category: sub-categorisation as Skin sens. 1A – H317, STOT RE 1 – H372 and to add the route of exposure (oral) and the target organ (kidney). Moreover a mutagenicity category 2 - H341 should be proposed based on structure-activity considerations among analogue chemicals of the 'aminophenol group' (see also section 7.2 below).

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

A harmonised classification of the substance is envisaged as a follow-up at EU level for indicated human health.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Prepare an Annex VI dossier according to CLP	To be confirmed	To be confirmed

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Bis(4-hydroxy-N-methylanilinium) sulphate was originally selected for substance evaluation in order to clarify concerns about:

- Mutagenicity
- Skin sensitisation
- Other hazard based concern: STOT RE

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Mutagenicity	The initial finding of the evaluation suggested a strong concern for mutagenicity. No conclusion can be drawn for bis(4-hydroxy-N-methylanilinium) sulphate without a new *in vivo* study to address the mutagenicity concern. Due to the low tonnage and the use at industrial site and by professionals (no consumer use registered) a request of an *in vivo* study is not proportionate. On the other hand, structure activity relationship considerations with analogue substances of 'aminophenol group' point to a reactivity of bis(4-hydroxy-N-methylanilinium) sulphate that could be comparable (if not greater) to 4-aminophenol (CAS RN 123-30-8), which has already a harmonised Mutagen Category 2 classification. A revision of the classification as Mutagen Category 2 - H341 should be performed.
Skin sensitisation	Confirmed: The available data is considered sufficient to conclude that the substance is a strong skin sensitiser. As the substance has already an entry (650-031-00-4) in Annex IV of CLP, C&L process is to be initiated for revise the skin sensitisation classification and propose a sub-categorisation as Skin sens. 1A - H317.
Specific target organ toxicity - repeated exposure	Confirmed: Kidney and blood identified as the most sensitive target organ. Current harmonised classification: STOT RE 2 - H373. An update of this classification is foreseen for the substance as STOT RE 1 – H372 and to add the route of exposure (oral) and the target organ (kidney).

7.2. Procedure

Pursuant to Article 44(2) of REACH, bis(4-hydroxy-N-methylanilinium) sulphate (EC number 200-237-1), was included on the Community rolling action plan (CoRAP) for evaluation in 2020. The Competent Authority of Italy was appointed to carry out the evaluation. The substance evaluation started on 18 March 2020.

Due to structural similarity, the substances, bis(4-hydroxy-N-methylanilinium) sulphate (EC number 200-237-1), 4-aminophenol (EC number 204-616-2), 3-aminophenol (EC number 209-711-2) and 5-amino-o-cresol (EC number 220-618-6) were originally selected to be jointly evaluated during the process.

These substances belong to the aminophenol's chemical class, differing in the relative position of amino and hydroxyl groups on the aromatic ring and on the presence/absence of a methyl substituent (either on the ring or on the amino group).
Aminophenols are potentially reactive chemicals via metabolic pathways involving the formation of electrophilic and/or quinone imines intermediates. The initial evidence induced the eMSCA to perform an evaluation of the substances on human health.

The eMSCA has decided to evaluate the four substances separately, taking into account that these substances are structural analogues according to considerations on structure-activity relationship.

The evaluation was targeted to clarify concerns on mutagenicity, skin sensitisation and specific target organ toxicity. Other endpoints were not evaluated.

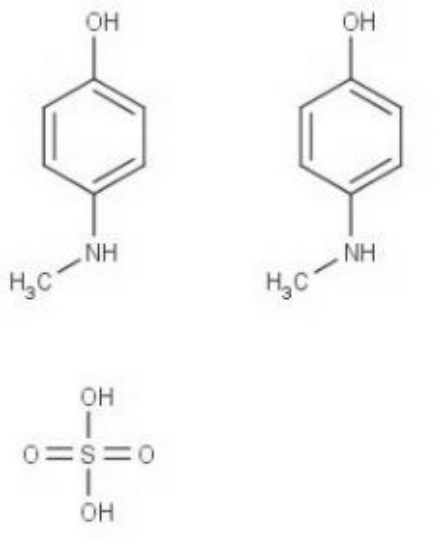
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	bis(4-hydroxy-*N*-methylanilinium) sulphate
EC number:	200-237-1
CAS number:	55-55-0
Index number in Annex VI of the CLP Regulation:	650-031-00-4
Molecular formula:	C14H20N2O6S
Molecular weight range:	
Synonyms:	Sulfate and sulphate are used interchangeably

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Solid
Vapour pressure	0 Pa at 25 °C
Water solubility	81 652 mg/L 25 °C
Partition coefficient n-octanol/water (Log Kow)	0.79 at 25 °C
Flammability	Not classified
Explosive properties	Not classified
Oxidising properties	Non oxidising
Granulometry	The particle size distribution of test item Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) was found to be in the range of 150 micron to 25 micron.
Stability in organic solvents and identity of relevant degradation products	---
Dissociation constant	---

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☒ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

This substance is used by professional workers (widespread uses) and at industrial sites.

In the Registration dossier no consumer uses are declaredand on this basis eMSCA conducted the evaluation on the registered uses. However, eMSCA is of the opinion that the declared uses should be clarified, as bis(4-hydroxy-N-methylanilinium) sulphate is reported as a cosmetic ingredient, together with 4-methylaminophenol (CAS RN 150-75-4, free base) and p-(methylamino)phenol sulphate (CAS RN 1936-57-8, sulfate) in the SCCP evaluation. For sake of completeness the eMSCA reports that in ECHA's infocard the substance is linked to p-methylaminophenol sulphate listed in Annex III – Restricted Substances of the Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.

The maximum threshold for the use of p-methylaminophenol sulphate in consumer products (i.e. hair dye substance in oxidative hair dye products) is 0.68% as sulfate with a maximum of nitrosamine content of 50 µg/kg.

Table 7

USES	Use(s)
Uses as intermediate	---
Formulation	---
Uses at industrial sites	This substance is used in photo-chemicals
Uses by professional workers	This substance is used in photo-chemicals
Consumer Uses	---
Article service life	---

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

The substance is currently listed on Annex VI of CLP Regulation ((EC) No 1272/2008).

Table 8

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
650-031-00-4	bis(4-hydroxy-N-methylanilinium) sulphate	200-237-1	55-55-0	Acute Tox. 4 * Skin Sens. 1 STOT RE 2* Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H373** H400 H410	H302 H317 H373** H410	

7.6.2. Self-classification

- In the registration(s):

Acute Tox. 4 H302
Acute Tox. 4 H332
STOT RE 2 H373

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Eye Irrit. 2 H319
Skin Sens. 1B H317

7.7. Environmental fate properties

Not evaluated.

7.8. Environmental hazard assessment

Not evaluated.

7.9. Human Health hazard assessment

Due to the low tonnage, a CSR is not available for bis(4-hydroxy-N-methylanilinium) sulphate (CAS 55-55-0). Then, eMSCA evaluated the robust study summaries reported in the IUCLID dossier. Moreover, an SCCP report, is also available on the substance "p-methylaminophenol sulphate", including the following substances:

CAS:	150-75-4 (free base)
	55-55-0 (hemisulfate)
	1936-57-8 (sulfate)
EINECS:	205-768-2 (free base)
	200-237-1 (hemisulfate)
	217-706-1 (sulfate)

These chemicals are the parent (the base: CAS RN 150-75-4) and its salts (sulfate and hemisulfate), the latter being the subject of the present evaluation.

7.9.1. Toxicokinetics

Not evaluated.

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3. Sensitisation

The concern for skin sensitisation was based on the available *in vivo* animal and human data on Bis(4-hydroxy-N-methylanilinium) sulphate submitted in the registration dossier.

7.9.3.1 Animal studies.

A positive LLNA was conducted on male and female of mice (Basketter and Scholes, 1992) following criteria equivalent or similar to OECD TG 429. The study is well described in the registration dossier and it can be used for evaluation classification purposes.
Bis(4-hydroxy-N-methylanilinium) sulphate is soluble in DMF that was used as vehicle. The dose volume of 25 µl was applied to the dorsal surface of both ears at concentrations of 0.5%, 1%, and 2.5% once daily for 3 consecutive days. The EC3 value (the theoretical concentration resulting in an SI value of 3) was determined as well the ratio of test to control lymphocyte proliferation (T/C). The values reported were 2.5, 3.4 and 6.7. As the two first SI data point lying immediately below and above the SI value of 3, eMSCA applied the following equation [EC3 = c + [(3 - d)/(b - d)](a - c)] (Basketter et al, 2007) to derive the EC3 value corresponding to a SI of 3. The resulting EC3 is calculated to be 0.77%.
According to the Guidance on the Application of the CLP Criteria Version 5.0 – July 2017, the EC3 value of 0.77% warrant the sub-categorisation as Skin sens. 1A for Bis(4-hydroxy-N-methylanilinium).

These findings are confirmed in a study conducted to evalutate the skin sensitisation property of Bis(4-hydroxy-N-methylanilinium) sulphate with the Guinea pig maximisation test. The study is well described and it can be used for evaluation and classification purposes.
In the intradermal induction was given a concentration of 0.5% the test material. The subsequent epicoutaneous induction was carried out using 25% concentration. In the epicoutaneous occlusive (patch) challenge phase, 10-14 days later, test substance in 5 % concentration was applied. Challenge sites were scored for erythema (Scale 0-3) and

oedema 24 and 48 hr after removal of the patch. 90% animals were observed positive for skin sensitizing reaction after challenge application.
According to the Guidance on the Application of the CLP Criteria Version 5.0 – July 2017, these data warrant the sub-categorisation as Skin sens. 1A for Bis(4-hydroxy-N-methylanilinium) being the concentration of the intradermal induction between >0.1 <[X]≤ 1.0 and the number of animals showing sensitizing reactions ≥60%

7.9.3.2 Human studies.

Even if in the public registration dossier no consumer uses are reported, the following human studies conducted with hair dyes and/or perming solutions are presented by the registrant and was evaluated by eMSCA.
In a first study 195 patients were investigated for suspected allergic contact dermatitis caused by hair dyes or perming solutions containing Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) which at 0.5% and 1% concentration in petrolatum was applied as patch for 2 days exposure by using Finn Chambers. Subsequently, readings were performed on day 2, 3 and 7 after application. 41 patients were positive for skin sensitisation from total 195 tested i.e 21%.
In the second study 23 subjects in treated group and 200 subjects in control group were investigated. In induction phase Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) in 5% in petrolatum was applied as patch for 48 hr exposure by using Finn-Chamber while 2-3 weeks later challenge application 1% concentration in liquid was applied for 48hr and evaluated after 72hr for skin reaction.
6 were positive for skin sensitization from total 23 tested i.e. 26%, in treated group while 1 subject from control group indicated positive skin sensitisation.
Taken together these human experimental studies confirm the characteristics of strong sensitiser of bis(4-hydroxy-N-methylanilinium).

The eMSCA concludes that, according to the Guidance on the Application of the CLP Criteria Version 5.0 – July 2017, the EC3 value of 0.77% from the LLNA study as well as resulting data from the Guinea Pig maximisation test (being the concentration of the intradermal induction between >0.1 <[X]≤ 1.0 and the number of animals showing sensitising reactions ≥60%), warrant the sub-categorisation as Skin sens. 1A H317 for bis(4-hydroxy-N-methylanilinium) sulphate.

7.9.4. Repeated dose toxicity

7.9.4.1 Oral studies

A first repeated dose oral toxicity study (unpublished but well described report presented in the registration dossier) was performed to determine the toxic nature of Elon (other identifier for Bis(4-hydroxy-N-methylanilinium) sulphate- CAS RN 55-55-0) upon repeated exposure for 91-94 days with male and female rats.
Animals were administered with the test material by intubation five days a week with a dose of 0, 30, 100 or 300 mg/kg bw/day. Compound and dose-related effects occurred primarily in the 300 and 100 mg/kg bw/day groups and sporadically in the 30 mg/kg bw/day group.

Hematotoxicity effects.
The major effects were degeneration of hemoglobin in circulating erythrocytes, hemolytic anemia, hemoglobinuric nephrosis and death. Compound-related morphologic effects in erythrocytes were slight (100 mg/kg bw/day group) to moderate (300 mg/kg bw/day group) and included polychromasia, macrocytosis, anisocytosis, siderocytosis and incidence of Howell-Jolly bodies. In addition, minimal to minor hypochromasia, microcytosis, spherocytosis and incidence of target cells occurred in 3 of 22 rats observed in the 100 mg/kg group and in 8 of 20 observed in the 300 mg/kg bw/day group. These effects are indicative of damage to the spleen.

Urinary effects.
Compound and dose-related effects in urine included decreased urine volume and pH, increased specific gravity, increased concentration of protein, increased incidence of red and white blood cells per high power microscopic field of urine sediment, green to brown color and a hazy appearance. Effects in the 100 mg/kg bw/day group were minimal to minor and those in the 300 mg/kg bw/day group were moderate.

Hepatic effects.
Hepatic extramedullary hematopoiesis and hypertrophy of hepatocytes; renal tubular pigmentation, hemoglobinuric nephrosis and regeneration; and splenic extramedullary hematopoiesis, hemosiderosis and congestion were observed. Hemoglobinuric nephrosis occurred in 36 of 40 rats in the 300 mg/kg bw/day group and in 33 of the 40 in the 100 mg/kg group. Renal tubular necrosis occurred in the 27 rats that died or became moribund during the exposure period. Regenerative tubular epithelial cells in the distal convoluted tubules occurred in 37 of the 40 rats in the 300 mg/kg bw/day group, in 36 of the 40 in the 100 mg/kg bw/day group and in 18 of the 20 males in the 30 mg/kg bw/day group. These effects were minor to moderate in the 100 mg/kg bw/day and 300 mg/kg bw/day groups, minimal to minor in the males in the 30 mg/kg group and absent in the females in the 30 mg/kg bw/day group and in both males and females in the 0 mg/kg bw/day group. Extramedullary hematopoiesis occurred in the liver and/or spleen of 25 (16 male, 9 female) of 39 rats in the 300 mg/kg group and in 21 (13 male, 8 female) of 40 in the 100 mg/kg bw/day group. They did not occur in the 30 or in the 0 mg/kg bw/day groups. Hemosiderosis occurred in the liver and/or spleen in 35 of 40 rats in the 300 mg/kg group, in 35 of 39 in the 100 mg/kg bw/day group, in 17 of 20 males in the 30 mg/kg bw/day group and in 9 of 20 males in the 0 mg/kg bw/day group. Hemosiderosis did not occur in the females in the 30 and 0 mg/kg bw/day groups. Hypertrophy of hepatocytes occurred in 13 of 20 female and 3 of 20 male rats in the 300 mg/kg group and in 4 of 20 female and 1 of 20 male rats in the 100 mg/kg bw/day group.
Registrants therefore consider a NOAEL of 30 mg/kg bw in male and female rats. eMSCA agreed with this Registrants conclusion.

A second repeated dose oral toxicity study was performed to determine the toxic nature of Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) for 92 days. This study has also been evaluated by SCCP in its opinion SCCP/0963/05 published in 2006.
Ten male and 10 female Sprague Dawley rats per dose were administered the test chemical at dose levels of 0, 3, 10 or 30 mg/kg bw/day by oral gavage route of exposure for 13 weeks followed by a 4-week treatment-free period. Six additional animals per sex for the high dose plus control group (which were kept for a 4-week treatment free period) and 6 animals per sex in the satellite groups (3, 10 and 30 mg/kg bw/day) were also included in the study.

Hematology, clinical chemistry and urinanalysis
These examination was followed by gross and histopathology. The test item was clinically well tolerated at all dose-levels and did not cause any change in haematological or blood biochemical parameters. Only a higher urinary volume and a lower specific gravity were noted in some males given 30 mg/kg/day. No effects on organ weights and no macroscopic findings were noted.

Microscopic examination
Microscopic examination revealed tubular epithelial degeneration/single cell necrosis in the kidneys of animals given 30 mg/kg bw/day, and complete reversibility of these changes was noted at the end of the treatment-free period.

Therefore, Registrants considered a NOAEL of 10 mg/kg bw/day when male and female Sprague-Dawley rats were orally exposed for 92 days. eMSCA agreed with this Registrants conclusion.

7.9.4.2 Dermal studies

A first repeated dose dermal toxicity study submitted by the Registrant(s) was performed to determine the dermal toxic nature of Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0).

The study was performed using only female rats applying 2 mL/Kg of two dye formulations 7404 and P-26 containing 1% and 0.05% corresponding to 11.47 mg/kg bw/day of Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) for formulation 7404 and 0.5735 mg/kg bw/day for formulation P26. The application was made during the gestation days 1, 4, 7, 10, 13, 16 and 19. The animals were observed for clinical signs, body weight changes, dermal irritation if any and food consumption. No dye formulation related toxicity was noted. Changes in female body weights and food consumption were similar for rats in the untreated controls and all dye-treated groups. No irritation or other changes in appearance were noted except for changes in skin and hair color at the site of topical application of the dye formulation. Based on the observations made, the NOAEL for N-methyl-p-aminophenol sulfate in female Charles River CD rats is considered by the Registrants to be 11.47 mg/kg bw/day for formulation 7404 and 0.5735 mg/kg bw/day for formulation P26.

Another dermal study submitted by the Registrants was performed on male and female New Zealand White rabbits, where 1 ml/kg dye of formulation 7404 and P-26 containing 1% (5.735 mg/kg bw/day) and 0.05% (0.2867 mg/kg bw/day) Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) respectively, applied to the back and dorso-lateral aspects of the thoracic-lumbar area. The dye formulations contained other active ingredients in an aqueous solution and were mixed with an equal volume of 6% hydrogen peroxide prior to application. The application was made twice weekly for 13 weeks. The animals were observed for mortality, body weight changes, hematological, clinical chemistry, urine analysis, and gross and histopathology. No dye formulation-related toxicity was noted. Body weight gain of all test groups was at least equal to that of the controls. Scattered statistically significant differences in the hematologic and clinical chemistry values were noted between test and control groups at the various sampling intervals. These differences were not considered by the Registrants to be of any toxicological significance because of either the direction or continuity of the differences or the fact that they fell within the range of historical control values. No remarkable urinalysis data was noted. No dye discoloration of the urine was seen at any time during the test. Statistically significant differences in relative organ weights between a test group and the combined controls were observed where the differences were not significant when the group was compared with each control group separately. No gross and microscopic lesions were noted that were judged to be due to the administration of the hairdye formulations containing the test compound. Therefore, NOAEL for Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) is considered by the Registrants to be 5.735 mg/kg of formulation 7404 and 0.2867 mg/kg of formulation P26 in male and female New Zealand White rabbits.

The eMSCA considers that these dermal studies are not relevant, nor reliable and not sufficient for classification and labeling purposes due to lack of consistency to the guidelines and the paucity of the available information reported. Thus no conclusion on repeated dose toxicity by dermal route could be drawn on Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) on the basis of the available information.

Conclusion on repeated dose toxicity

The eMSCA concludes that, according to the Guidance on the Application of the CLP Criteria Version 5.0 – July 2017, the NOAEL of 10 mg/kg/day from the oral repeated study on rats and the hazard profile resulting based on the evaluation of the submitted data, warrant the classification of Bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) as STOT RE Cat. 1 H372 (May cause damage to organs (kidney) through prolonged or repeated oral exposure).

7.9.5. Mutagenicity

The data available in the robust study summaries in the IUCLID dossier are on bis(4-hydroxy-N-methylanilinium) sulphate (CAS RN 55-55-0) and on p-methylaminophenol sulphate. This last one was also evaluated in the SCCP opinion SCCP/0963/05 published in 2006. It is important to note that, in the SCCP opinion "p-methylaminophenol sulphate" is used to indicate the following substances:

CAS:	150-75-4 (free base)
	55-55-0 (hemisulfate)
	1936-57-8 (sulfate)
EINECS:	205-768-2 (free base)
	200-237-1 (hemisulfate)
	217-706-1 (sulfate)

These chemicals are the parent (the base: CAS RN 150-75-4) and its salts (the hemisulfate CAS 55-55-0 and the sulfate CAS RN 1936-57-8). Therefore, the studies reported in the SCCP opinion, and to which we refer for the genotoxicity evaluation, have been performed on an unidentified p-methylaminophenol sulphate, considering both the salts and the base form of the substance.
Since the parent chemical, p-methylaminophenol (CAS RN 150-75-4) and its hemisulfate (CAS RN 55-55-0) are expected to have similar toxicity profiles, we used the results reported in the SCCP to assess the substance under evaluation, on a "read across" basis, i.e., we have relied on their parent relationship.

The original reports of the studies were not provided to eMSCA, therefore in the following evaluation we refer to the robust study summaries reported in the IUCLID dossier and to the SCCP opinion (where no indication on realiability are reported). Unless otherwise specified, in the genotoxicity evaluation that follows, the name "p-methylaminophenol sulphate" is intended as defined in the SCCP report, i.e. composed of chemicals: p-methylaminophenol (CAS RN 150-75-4), bis(4-hydroxy-N-methylanilinium) (CAS RN 55-55-0) sulphate and p-methylaminophenol sulphate (CAS RN 1936-57-8).

Genotoxicity in vitro

p-Methylaminophenol sulphate has been investigated for the induction of gene mutations in *Salmonella typhimurium*, in a study conducted according to OECD TG 471 at the following concentration: 0.064-1000 μg/plate without S9 mix and 0.064-2000 μg/plate with S9 mix. p-Methylaminophenol sulphate induced gene mutations in *S. typhimurium* in some of the five strains (TA 100 in the presence and absence of metabolic activation; TA 98 and TA 1537 in the presence of metabolic activation only). p-Methylaminophenol sulphate is mutagenic in the bacterial gene mutation assay. This study is reported by the SCCP without indication about realiability (Unpublished report , 2005). These results are also confirmed in the publically available study of Zeiger et al. conducted in 1987.

p-Methylaminophenol sulphate has been investigated for induction of chromosomal aberrations in cultured human lymphocytes according to OECD TG 473 at the following concentration 11.26 – 27.49 μg/ml with and without metabolic activation. After 3 hours of treatment (in the absence and presence of metabolic activation) and at harvest time of 20 hours, metaphases were analysed for chromosomal aberrations. The test substance induced a significant increase in the frequency of chromosome aberrations in experiments with and without S9 mix. Then, under the experimental conditions used, p-Methylaminophenol sulphate was clastogenic in mammalian cells (human lymphocytes) in vitro (Unpublished report, 2005).

p-Methylaminophenol sulphate has been investigated for induction of gene mutations at the TK locus in L5178Y mouse lymphoma cells according to OECD TG 476 at the following concentration 0.1–3.0 μg/ml without metabolic activation and 1.0–38 μg/ml with metabolic activation for 3 hours of exposure. The test substance induced significant increases in the mutant frequencies after the 3-hour treatment in the presence of S9 mix. Under the experimental conditions used, p-Methylaminophenol sulphate was mutagenic in

mammalian cells in vitro. No information is available on the mechanism of mutation induction (i. e. induction of point mutations or chromosomal effects), because the colony sizing was not performed (Unpublished report, 1993).

p-Methylaminophenol sulphate has been investigated for induction of gene mutations at the HPRT-locus in L5178Y mouse lymphoma cells according to OECD TG 476 at the following concentration 0.5– 20 µg/ml without metabolic activation and 2.5–60 µg/ml with metabolic activation after 3 hours of exposure. The test substance did not induce significant and / or reproducible increases in the mutant frequencies after the 3-hour treatment in the presence or absence of S9 mix. Under the experimental conditions used, p-Methylaminophenol sulphate was not mutagenic in the in vitro HPRT gene mutation test with L5178Y mouse lymphoma cells (Unpublished report, 2005).

Genotoxicity *in vivo*

p-Methylaminophenol sulphate has been investigated for induction of micronuclei in the bone marrow cells of rats *in vivo*, according to OECD TG 474 at the following doses 100, 200 and 400 mg/kg bw (once by gavage). Since in the preliminary range-finding study mortality was observed at 500 mg/kg, 400 mg/kg was tested as the top dose-level. No direct indication of bone marrow toxicity was observed (i.e. PCE/NCE ratio) although signs of oral bioavailability was assumed by the systemic clinical signs and the death of one animal treated with 400 mg/kg reported in the study. The mean MNPCE frequencies were not significantly increased in any of the groups treated with the test substance. p-Methylaminophenol sulphate did not induce chromosome aberrations or damage to the mitotic apparatus in bone marrow cells of rats after oral treatment under the appropriate test conditions used (Unpublished report, 2005).

p-Methylaminophenol sulphate has been investigated for induction of unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro following in vivo dosing according to OECD TG 486 at 50 and 500 mg/kg bw doses administered by gavage. The top dose level was selected on the basis of a preliminary toxicity study. Animals were sacrificed after 16 hours and for an additional high dose group after 2 hours. In none of the groups treated with the test substance there was a significant induction of UDS compared to the control group. There were no differences in the viability of hepatocytes isolated from rats of different dose groups. The results met all the pre-defined criteria for a negative response. The negative test result indicates that p-Methylaminophenol sulphate does not induce DNA damage that is detectable with the UDS test (Unpublished report, 1997).

A negative prediction for bis(4-hydroxy-N-methylanilinium) sulphate on genetic toxicity in vivo (chromosomal aberrations), performed with OECD QSAR Toolbox v.3.3, was reported by the Registrants. However, eMSCA considers the prediction unreliable as it is based on a read across with 5 chemicals which are structurally very different from the target chemical (CAS RN 55-55-0). The structural differences and their consequences on the genotoxicity of the chemicals were neither discussed nor justified by the Registrants.

Conclusion

The data reported in the robust study summaries of the IUCLID dossier refer to the studies evaluated in the SCCP report, performed on p-methylaminophenol sulphate. This chemical name is used in the report to indicate the free base, CAS RN 150-75-4, the hemisulfate, CAS RN 55-55-0 and the sulfate, CAS RN 1936-57-8. Given that the parent chemical (the base) and its salts are expected to have similar toxicity profiles, we used the results reported in the SCCP to assess the substance under evaluation (the hemisulfate), on a "read across" basis.
From the analysis of the aforementioned evidence, eMSCA is of the opinion that no firm conclusion can be drawn for genotoxicity of bis(4-hydroxy-N-methylanilinium) sulphate without new studies.
Moreover, structure activity relationships considerations with respect to the chemicals in the 'aminophenols' group, suggest a strong concern for mutagenicity of bis(4-hydroxy-N-methylanilinium) sulphate. In particular, it cannot be excluded that a comparable reactivity (if not greater) of bis(4-hydroxy-N-methylanilinium) sulphate occurs, with respect to the structural analogue 4-aminophenol (CAS 123-30-8), which has already an harmonised

classification as Mutagen Category 2. The two chemicals differ in structure due to the presence, in bis(4-hydroxy-N-methylanilinium) sulphate, of a methyl group on the amino nitrogen. This factor can give the substance greater genotoxic potential, because the methyl substituent, by inductive effect, could enhance the stability of the nitrenium ion, the reactive metabolite putative responsible of DNA interaction and damage.

Due to the low tonnage band and the use at industrial site and by professionals (no consumer use) a request of an *in vivo* study might not be proportionate. eMSCA suggests to apply Muta 2 classification for bis(4-hydroxy-N-methylanilinium) sulphate, in line with the present harmonised classification of 4-aminophenol (CAS RN 123-30-8).

7.9.6. Carcinogenicity

Not evaluated.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Not evaluated.

7.9.8. Hazard assessment of physico-chemical properties

None impacting human health.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not applicable.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

On the basis of the available information, a harmonised classification of the substance is proposed by eMSCA, as a follow-up at EU level for the following hazard category: sub-categorisation as Skin sens. 1A H317, STOT RE 1 H372 and to add the route of exposure (oral) and the target organ (kidney). Moreover a Mutagenicity category 2 should be proposed based on structure activity considerations on the group of analogues chemicals, i.e. the 'aminophenol group', as reported in paragraph 7.2.

7.10. Assessment of endocrine disrupting (ED) properties

Not evaluated.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

Since the tonnage of the substance is 1-10 t/y, exposure scenarios and exposure assessment have not been presented.

7.13. Risk characterisation

Not applicable.

7.14. References

Basketter D.A., Gerberick G.F., Dearman R.J. and Kimber I. The Local lymph node assay ant the assessment of relative potency: status of validation. Contact Dermatitis 57: 70-75, 2007.

Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017

Registration dossier for Bis(4-hydroxy-N-methylanilinium) sulphate: European Chemicals Agency http://echa.europa.eu/

Scientific Committee on Consumer Products on p-METHYLAMINOPHENOL sulphate, SCCP/0963/05, Adopted the 28th March 2006

7.15. Abbreviations

CAS Chemical abstracts service

C&L Classification and labelling

CLP Classification, labelling and packaging (Regulation (EC) No 1272/2008)

EC3 value (the theoretical concentration resulting in an SI value of 3);

eMSCA Evaluating Member State Competent Authority

LLNA - Local lymph node assay

NOAEL - No observed adverse effect level

OECD - Organisation for Economic Co-operation and Development

QSAR – Quantitative Structure-Activity Relationship

SCCP - Scientific Committee on Consumer Products