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Safety assessment of the substance N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids, for use in food contact materials

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Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) assessed the safety of N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids (SABOFOG F1), FCM No 1081, which is intended to be used as an antistatic and anti-fog agent in all types of polymers at up to 2% w/w. It was requested for use in contact with dry food, acidic foods and alcoholic beverages (represented by simulants E, B and C, respectively) with storage up to 6 months at ambient temperature. The migration data provided did not enable the assessment of the safety of applications intended for contact with foods represented by simulants B and C. In the migrate into simulant E, the non-esterified N,N-bis(2-hydroxyethyl)stearylamine was the main constituent. Its mono- and di-esters migrated to a lower extent. According to the data provided, the Panel concluded that the substance does not raise concern for genotoxicity and accumulation in humans. Based on the results of 28-day oral toxicity study with SABOFOG F1 and on the 90-day oral toxicity study with the read-across substance bis(2-hydroxyethyl)oleylamine, the Panel considered the current SML(T) of 1.2 mg/kg food provided a margin sufficiently large to accommodate the uncertainties related to the read-across approach. Overall, the CEP Panel concluded that N,N-bis(2-hydroxyethyl)stearylamine, of which at least [REDACTED] is partially or fully esterified with saturated C16/C18 fatty acids is not of safety concern for the consumer when used at up to 2% (w/w) in all polymers intended for contact with foods represented by simulant E for up to 6 months at room temperature. Additionally, the migration of the sum of N,N-bis(2-hydroxyethyl)stearylamine and its mono- and di-ester, calculated as N,N-bis(2-hydroxyethyl)stearylamine, should not exceed 1.2 mg/kg, i.e. the SML(T) for FCM substances 19 and 20, in which also the migration of the mono- and di-ester of N,N-bis(2-hydroxyethyl)stearylamine should be included.

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Keywords: FCM substance No 1081, antistatic, antifog, dry foods, plastic, food contact materials, safety assessment

Requestor: Ministero della Salute – Direzione generale per l'igiene e la sicurezza degli alimenti e la nutrizione, Italy

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Competing interests: Roland Franz declared that Fraunhofer institute at which he was employed provides advisory services to private business operators active in the sector on food contact materials. In line with EFSA's Policy on Independence** and the Decision of the Executive Director on Competing Interest Management,*** a waiver was granted to Roland Franz regarding his participation to the EFSA's Working Group on Food Contact Materials (FCM WG) in accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management. Pursuant to Article 21(6) of the above-mentioned Decision, the involvement of Roland Franz is authorised as member in the FCM WG, allowing him to take part in the discussions and in the drafting phase of the scientific output, but he is not allowed to be, or act as, a chairman, a vice-chairman or rapporteur of the working group.

Note: The full opinion will be published in accordance with Article 10(6) of Regulation (EC) No 1935/2004 once the decision on confidentiality, in line with Article 20(3) of the Regulation, will be received from the European Commission. The following information has been provided under confidentiality and it is redacted awaiting the decision of the Commission: manufacturing process; composition of main constituents; results of migration tests; detailed results of *in vitro* mammalian chromosomal aberration test and 90-day oral toxicity study.

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** http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf

*** http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf

Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Non-toxicological data.....	5
3.1.1. Identity of the substance.....	5
3.1.2. Physical and chemical properties.....	6
3.1.3. Specific migration.....	7
3.2. Toxicological data.....	7
3.2.1. Genotoxicity.....	7
3.2.1.1. Bacterial reverse mutation test.....	7
3.2.1.2. <i>In vitro</i> mammalian cell gene mutation test.....	8
3.2.1.3. <i>In vitro</i> mammalian chromosomal aberration test.....	8
3.2.1.4. <i>In vivo</i> mammalian erythrocyte micronucleus test.....	9
3.2.1.5. <i>In vivo</i> mammalian erythrocyte micronucleus test.....	9
3.2.1.6. Conclusions on genotoxicity.....	9
3.2.2. General toxicity studies.....	9
3.2.2.1. 4-week toxicity study in rats.....	9
3.2.2.2. 90-day toxicity study in rats.....	10
3.2.2.3. Read-across assessment.....	11
3.2.3. Accumulation in human.....	11
4. Discussion.....	12
5. Conclusions.....	12
6. Recommendations.....	13
Documentation provided to EFSA.....	13
References.....	13
Abbreviations.....	13

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Before a substance is authorised to be used in food contact materials (FCM) and is included in a positive list EFSA's opinion on its safety is required. This procedure has been established in Articles 8, 9 and 10 of Regulation (EC) No 1935/2004¹ of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

According to this procedure, the industry submits applications to the Member States' competent authorities which transmit the applications to the European Food Safety Authority (EFSA) for their evaluation.

In this case, EFSA received an application from the Ministero della Salute, Direzione generale per l'igiene e la sicurezza degli alimenti e la nutrizione, Italy, requesting the evaluation of the substance 'reaction product of stearyl-diethanol-amine with C18 saturated fatty acids', and with the FCM substance No 1081. The dossier was submitted by Sabo S.p.A.

According to Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food, EFSA is asked to carry out an assessment of the risks related to the intended use of the substance and to deliver a scientific opinion.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of their application for the authorisation of the reaction product of stearyl-diethanol-amine with C18 saturated fatty acids (renamed by the Panel as 'N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids') to be used in FCM.

Additional information was provided by the applicant during the assessment process in response to requests from EFSA sent on 29 March 2017, 31 October 2017 and 24 May 2019 (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 31 October 2017, the applicant requested a clarification teleconference, which was held on 5 December 2017.

Following the request by the working group (WG), a technical hearing was held with the applicant on 7 May 2019.²

Data submitted and used for the evaluation are:

Non-toxicological data and information

- Chemical identity
- Description of manufacturing process of substance/FCM
- Physical and chemical properties
- Intended use
- Existing authorisation(s)
- Migration of the substance

Toxicological data

- Bacterial gene mutation test
- *In vitro* mammalian cell gene mutation test
- *In vitro* mammalian chromosomal aberration test
- *In vivo* mouse bone marrow micronucleus test
- 28-day oral toxicity study in rats
- 28-day oral toxicokinetic study in rats
- 90-day oral toxicity study in rats

¹ Regulation (EC) No 1935/2004 of the European parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ L 338, 13.11.2004, p. 4–17.

² <http://www.efsa.europa.eu/sites/default/files/wgs/food-ingredients-and-packaging-fcmwg-wg-m.pdf>

2.2. Methodologies

The assessment was conducted in line with the principles laid down in Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food. This Regulation underlines that applicants may consult the Guidelines of the Scientific Committee on Food (SCF) for the presentation of an application for safety assessment of a substance to be used in FCM prior to its authorisation (European Commission, 2001), including the corresponding data requirements. The dossier that the applicant submitted for evaluation was in line with the SCF guidelines (European Commission, 2001).

The methodology is based on the characterisation of the substance(s) that is/are the subject of the request for safety assessment prior to authorisation, its impurities and reaction and degradation products, the evaluation of the exposure to those substances through migration and the definition of minimum sets of toxicity data required for safety assessment.

To establish the safety from ingestion of migrating substances, the toxicological data indicating the potential hazard and the likely human exposure data need to be combined. Exposure is estimated from studies on migration into food or food simulants and considering that a person may consume daily up to 1 kg of food in contact with the relevant FCM.

As a general rule, the greater the exposure through migration, the more toxicological data are required for the safety assessment of a substance. Currently, there are three tiers with different thresholds triggering the need for more toxicological information as follows:

- a) In case of high migration (i.e. 5–60 mg/kg food), an extensive data set is needed.
- b) In case of migration between 0.05 and 5 mg/kg food, a reduced data set may suffice.
- c) In case of low migration (i.e. < 0.05 mg/kg food), only a limited data set is needed.

More detailed information on the required data is available in the SCF guidelines (European Commission, 2001).

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009) and considering the relevant guidance from the EFSA Scientific Committee.

3. Assessment

According to the applicant, the substance 'reaction product of stearyl-diethanol-amine with C18 saturated fatty acids' (SABOFOG F1), renamed by the Panel as 'N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids', is intended to be used as an antistatic and anti-fog agent in all types of polymers in the range of 0.1–2% w/w. The intended uses include dry foods, acidic foods and alcoholic beverages, stored at room temperature for up to 6 months. The substance is manufactured from [REDACTED] reacted with [REDACTED] and [REDACTED].

The use of [REDACTED] is authorised under Regulation (EU) No 10/2011 ([REDACTED]) as monomer for which migration should not be detectable and a maximum concentration of 1 mg/kg can be present in the final product.

Alkyl diethanolamines are listed in the Regulation (EU) No 10/2011 under N,N-bis(2-hydroxyethyl)alkyl(C8–C18)amine (FCM No 19), with a total specific migration limit (SML(T)) of 1.2 mg/kg together with FCM No 20, expressed as tertiary amine. Saturated C16/C18 fatty acids are listed as FCM No 105/FCM No 106, respectively, without restriction.

3.1. Non-toxicological data

3.1.1. Identity of the substance³

SABOFOG F1 is a mixture determined by the two steps of manufacture:

- a) [REDACTED] is reacted with [REDACTED]. According to the applicant, ethoxylation resulted in around [REDACTED] N,N-bis(2-hydroxyethyl)stearylamine (Figure 1), around [REDACTED] and about [REDACTED].

³ Technical dossier/Section 1; Appendix B – Technical dossier/Additional data July 2017/Annex A.

- b) This mixture is partially esterified with a mixture of [REDACTED] stearic acid and [REDACTED] palmitic acid. The main component of the mixture reported by the applicant is the mono-ester 2-[(2-hydroxyethyl)octadecylamino]ethyl stearate (CAS No 52497-24-2; [REDACTED] see Figure 3). The di-ester, octadecanoic acid, 1,1'-[(octadecylimino)di-2,1-ethanediyl] ester (CAS No 94945-28-5; Figure 4), made up [REDACTED] and the non-esterified N,N-bis(2-hydroxyethyl)stearylamine (CAS No 10213-78-2; Figure 1) [REDACTED]

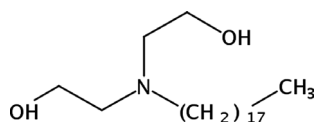


Figure 1: N,N-bis(2-hydroxyethyl)stearylamine (non-esterified)

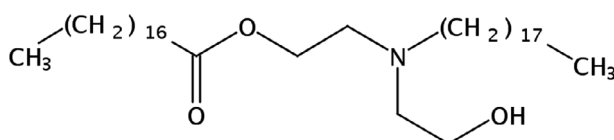


Figure 3: 2-[(2-Hydroxyethyl)octadecylamino]ethyl stearate (mono-ester)

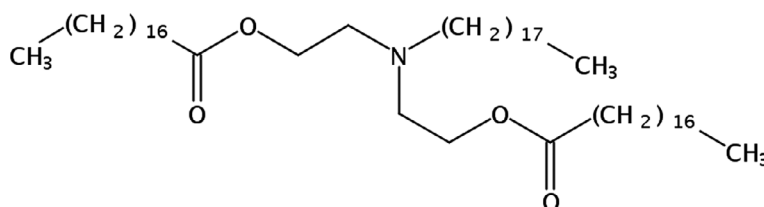


Figure 4: (Octadecanoic acid, 1,1'-[(octadecylimino)di-2,1-ethanediyl] ester) (di-ester)

The applicant also provided data on a product esterified with a higher proportion of [REDACTED] (SABOSTAT A 300), which was used in some of the migration and toxicological studies. The Panel noted that this product contains the same components as SABOFOG F1, though in different proportions.

No data on other minor products or impurities were provided by the applicant. However, the Panel noted that the intermediate substance N,N-bis(2-hydroxyethyl)stearylamine and the saturated C16 and C18 fatty acids used for the esterification are authorised in Regulation (EU) No 10/2011 under FCM substances No 19, 105 and 106, respectively.

3.1.2. Physical and chemical properties⁴

According to the applicant, SABOFOG F1 has a melting point ranging from 38 to 44°C. It is virtually insoluble in water. SABOSTAT A 300 starts to degrade at temperatures above 320°C. It is virtually insoluble in water, but soluble in apolar media and organic solvents, such as acetone and chloroform. The Log Po/w calculated for the main components ranged from 6.9 to 24.2.

⁴ Technical dossier/section 2; Appendix B – Technical dossier/Annexes 4–7.

3.1.3. Specific migration⁵

A migration test was performed on a 100 µm polypropylene (PP) film containing 0.5% w/w SABOFOG F1 with food simulant E (poly(2,6-diphenyl-p-phenylene oxide)) during 10 days at 50°C. The migration of the main components was [REDACTED] for the non-esterified N,N-bis(2-hydroxyethyl) stearylamine, [REDACTED] for its monoester and [REDACTED] for its diester.

Migration tests were performed also using SABOSTAT A 300 at 0.5% in a 100 µm PP film. This film was exposed during 10 days at 60°C to the simulants for low-alcoholic (20% v/v ethanol, simulant C), acidic (3% acetic acid w/v, simulant B) and dry foods (simulant E). Only the migration of the major component (mono-ester) was determined: [REDACTED] into 20% ethanol, up to [REDACTED] into 3% acetic acid and up to [REDACTED] into food simulant E.

The Panel noted that, based on considerations of molecular size and polarity, the highest migration into aqueous simulants is expected for the non-esterified species, principally N,N-bis(2-hydroxyethyl) stearylamine. Upon request to supply the missing migration data, the applicant calculated the total transfer.⁶ Calculated migration would exceed the SML(T) of 1.2 mg/kg for N,N-bis(2-hydroxyethyl)alkyl (C8-C18)amine (FCM No 19) when films containing 2% of the substance exceed a thickness of 10 µm. Hence, the available data did not allow the Panel to conclude on the safety of applications in contact with low-alcoholic and acidic foods (simulated by food simulants C and B, respectively).

3.2. Toxicological data

The following test items were used in the toxicological studies (Table 1) that were considered for the assessment.

Table 1: Overview of toxicological studies and test items

Toxicological data	Test item
Bacterial reverse mutation test	SABOSTAT A 300
<i>In vitro</i> mammalian cell gene mutation test	SABOSTAT A 300
<i>In vitro</i> mammalian chromosomal aberration test	SABOSTAT A 300
First <i>in vivo</i> mammalian erythrocyte micronucleus test	SABOSTAT A 300
Second <i>in vivo</i> mammalian erythrocyte micronucleus test	tallow bis(2-hydroxyethyl)amine (C16-C18)
28-day toxicity study in rats	SABOFOG F1
90-day oral toxicity study in rats	bis(2-hydroxyethyl)oleylamine
28-day oral toxicokinetic study in rats	SABOFOG F1

In addition to the data listed above, the applicant provided the following information that were not considered in this assessment due to the unavailability of the corresponding original study reports:

- Data on a subchronic toxicity study in rats and mice of an antistatic agent of polyolefinic resins (Yanagimoto et al., 1975).
- A memorandum from the Office of prevention, pesticides and toxic substances from the United States Environmental Protection Agency (US-EPA) focusing on the Alkyl Amine Polyalkoxylates (US-EPA, 2009).
- An appendix for the safety assessment of PEGs Cocamine summarising data from toxicological studies (US-EPA, 2003).

3.2.1. Genotoxicity

3.2.1.1. Bacterial reverse mutation test⁷

A bacterial reverse mutation assay was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP) in five strains of *Salmonella Typhimurium* (TA97a, TA98, TA100, TA102 and TA1535) in the presence or absence of metabolic activation. SABOSTAT A 300 (dissolved in DMSO) was tested in two separate experiments both in the absence and in the presence of metabolic activation (rat liver S9 fraction) using the plate incorporation and the

⁵ Technical dossier/section 5; Appendix B – Technical dossier/Annexes 8.1, 8.2.

⁶ Technical dossier/section 5; Appendix B – Technical dossier/Additional data July 2017/Annex B.

⁷ Technical dossier/section 8; Appendix B - Technical dossier/Annex 9.

pre-incubation methods. The following concentrations were applied in the plate incorporation assay 50, 150, 501, 1,500 and 5,000 µg/plate, and at the following concentrations were tested in the pre-incubation assay: 78, 156, 313, 625, 1,250, 2,500 and 5,000 µg/plate. No cytotoxicity was observed and the number of revertant colonies was not increased in comparison with that observed in the negative control assays.

The Panel concluded that SABOSTAT A 300 was not mutagenic in bacteria under the conditions employed in this study.

3.2.1.2. *In vitro* mammalian cell gene mutation test⁸

An *in vitro* mammalian cell gene mutation test using the thymidine kinase gene was performed in accordance with GLP and following OECD test guideline 476 (OECD, 1997b). SABOSTAT A 300 (the pure test item was melted at 50°C and handled as a liquid then diluted in DMSO) was tested for its ability to induce mutations in L5178 TK^{+/−} mouse lymphoma cells in the absence and in the presence of metabolic activation (rat liver S9 fraction). Three tests conditions were applied, one experiment was performed after a 4-h treatment in the presence of metabolic activation, one experiment was performed after a 4-h treatment in the absence of metabolic activation and the last experiment was performed after a 24-h treatment in the absence of metabolic activation. The concentrations of the substance (expressed as volume of pure substance added to the culture medium) ranged from 0.02 to 5 µL/mL. With regard to mutation frequencies, no statistically significant increase was observed at any dose level or treatment time, and with or without metabolic activation, compared with the negative control.

The Panel concluded that SABOSTAT A 300 did not induce gene mutations under the conditions used in this study.

3.2.1.3. *In vitro* mammalian chromosomal aberration test⁹

SABOSTAT A 300 (the pure test item was melted at 50°C and handled as a liquid then diluted in DMSO) was tested for its ability to induce chromosomal aberrations in human lymphocytes after *in vitro* treatment. This assay was performed following OECD test guideline 473 (OECD, 2014a) and according to GLP. Two separate experiments were performed. In the first experiment, lymphocytes were exposed 4 h followed by 19 h recovery period to the following concentrations of the test item (expressed as volume of pure substance added to the culture medium): 0.04, 0.08, 0.16, 0.32, 0.63, 1.25, 2.5 and 5 µL/mL, in the presence and in the absence of metabolic activation (rat liver S9 fraction). In the second experiment, lymphocytes were exposed 24 h followed by 19 h recovery period to different concentrations of the test item in the absence of metabolic activation at the following doses (expressed as volume of pure substance added to the culture medium): 0.02, 0.04, 0.08, 0.16, 0.32, 0.63 and 5 µL/mL.

In the first experiment, a statistically significant increase of structural chromosomal aberrations was observed after the treatment at the lowest concentration tested (0.04 µL/mL) in the presence of metabolic activation. In the second experiment, the three highest evaluated concentrations of the test item showed statistically significant increases of structural chromosomal aberrations with respect to the concurrent control data. No dose-response was observed. The amount of the historical control data was considered insufficient in order to evaluate the results of this experiment.

The Panel noted that the criteria of the OECD guidelines to consider a compound as clearly positive were not completely fulfilled and considered the study as inconclusive and of limited relevance in this risk assessment.

⁸ Technical dossier/section 8; Appendix B - Technical dossier/Annex 10.

⁹ Technical dossier/section 8; Appendix B - Technical dossier/Annex 11.

3.2.1.4. First *in vivo* mammalian erythrocyte micronucleus test¹⁰

SABOSTAT A 300 was tested for its ability to induce chromosomal aberrations in a micronucleus test in mouse bone marrow cells. This assay was performed following OECD test guideline 474 (OECD, 2014b) and according to GLP. The test substance (dispersed in propylene glycol) was administered by oral gavage twice at 24 h interval to five male mice per dose group at the following doses: 1,000 mg/kg body weight (bw) and 2,000 mg/kg bw. As regards the animals treated with SABOSTAT A 300, no increase in the mean frequency of micronucleated polychromatic erythrocytes was observed compared with that observed in the control group. However, due to the unchanged polychromatic to normochromatic erythrocyte ratio, that could indicate that the compound was not able to reach the bone marrow.

Therefore, no conclusion could be drawn by the Panel.

3.2.1.5. Second *in vivo* mammalian erythrocyte micronucleus test¹¹

The report of an *in vivo* micronucleus assay performed in mice with tallow bis(2-hydroxyethyl)amine (C16-C18) was provided by the applicant. This compound was postulated as being representative of the metabolites of the substance 'N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids'. This assay was performed following OECD test guideline 474 (OECD, 1983) and according to GLP. The test substance was administered once by oral gavage to 15 male and 15 female mice at a dose of 10,860 mg/kg bw (corresponding to LD10). Five male and five female mice were sacrificed 24, 48 and 72 h after administration and bone marrow samples were collected and the bone marrow smears prepared. No increase in the number of micronucleated polychromatic erythrocytes was observed in the bone marrow of the animals sacrificed 48 or 72 h after administration. In animals sacrificed 24 h after the administration of the test substance, a statistically significant increase in the number of micronucleated polychromatic erythrocytes was observed. However, this increase was within the historical negative control range. A statistically significant decrease of the polychromatic to normochromatic erythrocyte ratio was observed, confirming the exposure of the bone marrow. The result of the study is clearly negative with regard to the induction of chromosomal aberrations even with the limitation that a single dose was applied.

The Panel concluded, based on these results, that the substance tallow bis(2-hydroxyethyl)amine (C16-C18) did not trigger chromosomal aberrations in the tested conditions. Additionally, the Panel considered that the substance tested in this assay is representative of the metabolites of the substance under evaluation, i.e. 'N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids'.

3.2.1.6. Conclusions on genotoxicity

The Panel considered the substance used in the first four genotoxicity assays (i.e. SABOSTAT A 300; paragraphs 3.2.1.1 to 3.2.1.4) appropriate for the assessment of the genotoxicity of the substance under evaluation, i.e. SABOFOG F1. Overall, considering all the above-reported genotoxicity studies, also including the study on the tallow bis(2-hydroxyethyl)amine (C16-C18), representative of the constituents of SABOFOG F1 and representative of the hydrolysis products of this substance, the Panel concluded that the substance under evaluation, i.e. N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids (SABOFOG F1), does not raise concern for genotoxicity.

3.2.2. General toxicity studies

3.2.2.1. 28-day oral toxicity study in rats¹²

A 28-day oral toxicity study was performed in accordance with OECD test guideline 407 (OECD, 2008) and following GLP. SABOFOG F1 (dispersed in 1% aqueous carboxymethyl cellulose) was administered by oral gavage to three groups (five males and five females per group) of Wistar rats at the following doses: 100, 300 and 1,000 mg/kg bw per day. One additional group received the vehicle only. During the course of the study, one male rat from the 1,000 mg/kg bw per day group died on Day 25. This death was considered as treatment-related. In the high-dose group, piloerection was observed in males and females as well as swollen and/or hardened abdomen, lethargy and flat gait. Males of the high-dose group showed a decreased hind grip strength. These findings were considered as treatment-related. Clinical

¹⁰ Technical dossier/Section 8; Appendix B - Technical dossier/Annex 12.

¹¹ Technical dossier/Section 8; Appendix B – Technical dossier/Additional data December 2019.

¹² Technical dossier/Section 8; Appendix B – Technical dossier/Additional data January 2019/Annex III.

pathology showed treatment-related increased reticulocyte and total bilirubin in parallel with decreased mean corpuscular haemoglobin in females. In males, minor similar effects were observed. These findings, being not correlated with histopathological observations, were considered as not toxicologically relevant. Histopathological findings were observed in the jejunum and its draining lymph node. Accumulation of foamy macrophages within the *lamina propria* of the villi was observed in the small intestine mucosa and in the sinusoid of the mesenteric lymph nodes. According to the applicant, this suggested an increased phagocytotic activity by macrophages in order to process poorly digestible test substance. These findings were dose-related and observed in the 300 as well as 1,000 mg/kg bw per day dose groups. In two females of the high-dose group, the uterus was inactive and the vaginal epithelium was slightly mucified (differentiation of epithelial cells into mucus-secreting cells). This observation not fitting with the normal oestrus cycle in rats, it was considered as toxicologically relevant.

The Panel noted that this type of study is not part of the set of tests that are required in the context of an application dossier for plastic FCM (as outlined in the applicable guidelines for FCM; European Commission, 2001). However, the Panel considered the results of the study supportive of the read-across approach proposed by the applicant (Sections 3.2.2.2 and 3.2.2.3) and therefore included the 28-day oral toxicity study in the assessment.

3.2.2.2. 90-day oral toxicity study in rats¹¹

The toxicity of bis(2-hydroxyethyl)oleylamine (CAS No 25307-17-9) was tested by means of a 90-day oral toxicity study according to OECD test guidelines 408 (adopted in 1998) and following GLP. The test item (purity > 97%, diluted in Arachis oil) was administered daily by gavage to three groups of 20 Wistar rats (10 males and 10 females in each dose group) for 90 consecutive days at the following doses: 5, 30 and 150 mg/kg bw. An additional group of ■ rats (control group) was administered with vehicle only.

No mortality was recorded during the clinical part of the study. Clinical observations revealed increased salivation after dosing in all animals from the three dose groups, the incidence of the observation being dose-dependent, this was considered as being the consequence of the administration of the test item and not being of toxicological relevance. Two males and one female from the high-dose group showed staining around the snout, this being considered by the Panel as a consequence of the hypersalivation.

Functional observations revealed no effect of the treatment at any of the administered doses.

Mean body weight of the males of the high-dose group was statistically significantly lower (■) than that of the control group. This was associated with a lower weekly food consumption.

With regard to haematology, males of the high-dose group showed lower (■) mean cell haemoglobin and mean cell volume (■) than those of the control group but were within the range of historical control values. These findings were considered incidental by the Panel. In the 30 mg/kg bw per day dose group, males had lower platelet count compared to the control (■). In the absence of a similar finding in the high-dose group, this observation was considered by the Panel as being incidental. In females from all dose groups, statistically significant lower mean cell haemoglobin (up to ■ in the high-dose group) and mean cell haemoglobin concentrations (■ in the high-dose group) were observed compared to those of the control group. Additionally, in the females of the high-dose group, a statistically significant lower mean haemoglobin (■) and mean cell volume (■) were observed compared to control values. Individual values of the mean haemoglobin were in majority outside the historical control, however, considering the limited differences observed, the Panel considered them of no toxicological concern. In the high-dose group, females had statistically significant higher number of neutrophils compared to control values (■). This observation being limited to females, the Panel considered this as incidental.

Considering blood chemistry, the following observations were made in males only. Lower levels of total protein including lower blood albumin levels (■) were observed as well as an increase of the albumin to globulin ratio (■) compared with those of the control group in the 150 mg/kg bw per day group. Higher levels of bile acids (■) in the 150 mg/kg bw per day group compared with those of the control group were observed, all individual values being higher than historical controls, however, values from the control groups were also higher than those of historical controls. In the absence of histopathological observations and this increase being most probably due to the high amount of fatty acids administered, the Panel considered it as adaptive.

Mean liver weights relative to body weight were higher in the 150 mg/kg bw per day for both males (■) and females (■) compared to values of the control group and to historical control values. In the absence of histopathological observation in the liver, this observation was considered not

toxicologically relevant by the Panel. Mean kidney weight relative to body weight was statistically significantly higher in the females from the high-dose group compared with that of the control group (████████) and the historical control values. In the absence of histopathological findings confirming the observation, the Panel considered this finding as not toxicologically relevant. In males from the mid-dose group, mean kidney weight relative to body weight was statistically higher than that of the control group (████████). Since such difference was not observed in the high-dose group, the Panel considered this observation as incidental.

Histopathological assessment revealed minimal or mild diffuse epithelial hyperplasia and hyperkeratosis of the forestomach in most animals of the high-dose group (████████). This was associated with focal erosion of the forestomach (████████), submucosal inflammatory cell infiltration (████████), submucosal oedema (████████) or focal dyskeratosis (████████) in the forestomach of a few animals. Additionally, minimal to marked foamy macrophages were present in the *lamina propria* of the small intestine (████████) and in the sinuses of the mesenteric lymph node for all animals. In the mid-dose group, treatment-related histopathological changes were limited to the forestomach where only minimal focal epithelial hyperplasia (████████), minimal focal erosion in 1 female, and submucosal oedema (████████), and minimal or mild submucosal inflammatory cell infiltration (████████) were observed. None of these findings were observed in the low-dose group.

Based on these histopathological findings (hyperplasia of the forestomach observed in the high-dose group and minimal severity and incidence at the mid-dose group), the Panel identified the no observed adverse effect level (NOAEL) at 30 mg/kg bw per day.

3.2.2.3. Read-across assessment

The subchronic study provided by the applicant tested a different compound bis(2-hydroxyethyl) oleylamine, the source compound) than those present in the substance under evaluation, N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids (the target compound). However, the chemical structure of the source compound is very similar to that of N,N-bis(2-hydroxyethyl)stearylamine (Figure 1) that is one of the constituents of the target compound and the postulated metabolite of the other constituents of the target compound (a single central tertiary amine group). Additionally, considering the similar chemical structure of the main constituents of the target compound and those of triglycerides in dietary lipids, it is expected that ester linkages of the target compound are hydrolysed within the gastro-intestinal tract by enzymatic hydrolysis, ultimately releasing fatty acids and N,N-bis(2-hydroxyethyl)stearylamine (Putcha et al., 1993). Consequently, the Panel considered that the same reactivity is expected with both substances. Additionally, the toxicity profiles observed both in the 90-day oral toxicity study after administration of bis(2-hydroxyethyl) oleylamine and in the 28-day toxicity study after administration of the substance under evaluation, i.e. N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids, were similar (histopathological findings in the gastrointestinal tract), although more pronounced in the 90-day oral toxicity study, probably due to the longer exposure time. The Panel concluded that the NOAEL of 30 mg/kg bw per day identified in the 90-day oral toxicity study for bis(2-hydroxyethyl)oleylamine can be the starting point to set the NOAEL for the substance under evaluation, i.e. N,N-bis(2-hydroxyethyl)stearylamine partially esterified with saturated C16/C18 fatty acids.

Based on the results of 28-day toxicological study with SABOFOG F1 and on the 90-day oral toxicity study with the read-across substance bis(2-hydroxyethyl)oleylamine, the Panel considered the current SML(T) of 1.2 mg/kg food provided a margin sufficiently large to accommodate the uncertainties related to the read-across.

3.2.3. Toxicokinetics¹³

Toxicokinetics of one of the components of SABOFOG F1 were investigated by means of a 28-day toxicokinetic study in rats. The study was performed according to OECD principles of GLP. Two groups of animals (3 males and 3 females in each group) received daily administration of the substance (diluted in 1% aqueous carboxymethyl cellulose) by oral gavage for 28 days. The administered doses were 300 and 1,000 mg/kg bw per day. Blood samples were collected on Day 1 and on Day 28 at the following time points: predose, 30 min, 1, 4, 8 and 24 h after dosing. Plasma concentrations of stearate monoester of stearyl-diethanolamine were measured by a LC-MS-QTrap method. The lower

¹³ Technical dossier/Section 8; Appendix B – Technical dossier/Additional data January 2019/Annex II.

limit of quantification (LLOQ) was 200 ng/mL. Results showed that most of the samples collected on Day 1 had stearate monoester of stearyl-diethanolamine concentrations below the LLOQ. On Day 28, only one male had concentrations higher than the LLOQ at all sampling times. All predose samples collected in males of the high-dose group on Day 28 had stearate monoester of stearyl-diethanolamine concentrations slightly higher than the LLOQ.

Overall, the Panel considered that the concern for accumulation in humans can be ruled out.

4. Discussion

The composition of the substance may vary widely. It is essentially determined by the degree of ethoxylation (first step of synthesis), the degree of esterification with fatty acids (second step) and the composition of the fatty acids.

The SABOSTAT A 300 tested for genotoxicity, considered as an appropriate representative of the substance under evaluation, included around [REDACTED]

[REDACTED] No compounds containing only a single ethylene oxide unit (N-2-hydroxyethyl-stearylamine and its esters) were reported. The moderate over-ethoxylation is expected to result in a low proportion of these, if any. However, if these mono-ethoxylated compounds were present, their migration would be expected to be relatively high and the toxicological data provided would not cover them.

As the ester functions are hydrolysed *in vivo*, the degree of esterification is not considered relevant for the safety. However, since hydrolysis liberates N,N-bis(2-hydroxyethyl)stearylamine that is restricted by SML(T) for FCM substance No 19 and 20 (1.2 mg/kg food), this hydrolysis product must be taken into consideration to comply with this restriction.

The degree of esterification influences the migration, since the components of low molecular mass, in particular the non-esterified N,N-bis(2-hydroxyethyl)stearylamine, migrate more than the esters into dry foods (volatility) as well as into low alcoholic beverages and acidic foods (solubility): the lower the degree of esterification, the higher is migration.

Since no data were provided on the migration of non-esterified N,N-bis(2-hydroxyethyl)stearylamine into simulants B and C, no conclusions could be drawn on the safe use of the substance for these types of food contact. The Panel noted that applications involving contact with foods for which simulants D1 and D2 (used for more lipophilic substances) are provided in the Regulation (EU) No 10/2011 were not requested (the expected migration would be high).

Only migration data for PP were available, whereas authorisation was requested for all plastics. The Panel accepted PP as a worst case owing to its low polarity to retain the substance. Furthermore, the experiments only involved addition of 0.5% w/w of the substance, whereas use at up to 2% was requested. The Panel considered that in a limited range, the migration would be proportional to the content, and therefore up to 2% may be acceptable.

The data provided indicate that the migration into foods simulated by simulant E could exceed the SML(T) for FCM substance No 19 (1.2 mg/kg), when the substance is applied at up to 2% in a polymer film of more than 100 µm thickness and/or if the substance contains a higher proportion of N,N-bis(2-hydroxyethyl)stearylamine than specified by the applicant (see Section 3.1.1).

5. Conclusions

Based on the above-mentioned data, the CEP Panel concluded that the substance N,N-bis(2-hydroxyethyl)stearylamine, of which at least [REDACTED] is partially or fully esterified with saturated C16/C18 fatty acids, is not of safety concern for the consumer when used at up to 2% in all polymers intended to contact with dry foods (represented by simulant E¹⁴) stored for up to 6 months at room temperature. Additionally, the migration of the sum of N,N-bis(2-hydroxyethyl)stearylamine as well as its mono- and di-ester, calculated as N,N-bis(2-hydroxyethyl)stearylamine, should not exceed 1.2 mg/kg (SML(T) for FCM substances No 19 and 20, in which also the migration of the mono- and di-ester of N,N-bis(2-hydroxyethyl)stearylamine should be included).

¹⁴ Regulation (EU) No 10/2011 (Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Text with EEA relevance. OJ L 12, 15.1.2011, p. 1–89.

6. Recommendations

The Panel noted the on-going re-evaluation of FCM substances No 19 and 20 (EFSA-Q-2019-00763) and notes that the outcome of this re-evaluation could have a potential influence on the restrictions set out for the substance evaluated here.

Documentation provided to EFSA

- 1) Reaction product of stearyl-diethanol-amine with C18 saturated fatty acids. August 2016. Submitted by Sabo S.p.A.
- 2) Additional data. July 2017. Submitted by Sabo S.p.A.
- 3) Additional data. January 2019. Submitted by Sabo S.p.A.
- 4) Additional data. December 2019. Submitted by Sabo S.p.A.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service

CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP Panel	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
DMSO	Dimethyl sulfoxide
FCM	food contact materials
GLP	good laboratory practice
LLOQ	lower limit of quantification
NOAEL	no observed adverse effect level
Po/w	octanol/water partition coefficient
PP	polypropylene
SCF	Scientific Committee on Food
SML	specific migration limit
SML(T)	total specific migration limit
US-EPA	United States Environmental Protection Agency