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SCIENTIFIC OPINION

Update of the safety assessment of N,N-bis(2-hydroxyethyl) alkyl(C8-C18)amines (FCM No 19) and N,N-bis(2-hydroxyethyl) alkyl(C8-C18)amine hydrochlorides (FCM No 20) for their use in plastic materials and articles intended to come into contact with food

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The European Commission asked EFSA to review whether the authorisation of N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine (FCM No 19) and N,N-bis(2-hydroxyethyl) alkyl(C8-C18)amine hydrochlorides (FCM No 20) is still in accordance with Regulation (EC) No 1935/2004, as provided for in Article 12(3). The FCM Panel concluded that some uses of the substance N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine (FCM No 19) are not in accordance with this Regulation, since the migration is likely to exceed the current SML(T) of 1.2 mg/kg food under certain conditions of use. Based on the provided data, the FCM Panel concluded that the FCM substance No 19, N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine, is not of safety concern for the consumer if (i) the substance is used at up to 0.1% w/w as polymer production aid and as processing aid to manufacture polyolefin materials and articles of thickness up to 1 mm that are intended for contact with all types of food except infant foods. This exception for infant foods and the restriction for maximum thickness do not apply to caps of bottles; (ii) the migration does not exceed 5 mg/kg food; (iii) the source of the alkyl group is either from hydrogenated vegetable oil or synthetic from ethylene oligomers with a high degree of linear structure and (iv) the impurities do not exceed 5% w/w. As they bear unsaturation, PFAEO-coco, PFAEO-oleyl, PFAEO-HT, PFAEO-T and PFAO-C18 do not fall within the scope of the FCM substance No 19. The information related to these substances was only considered supportive for FCM substance No 19. If they were intended to be used to manufacture FCMs, a proper application following the EFSA Guidance documents should be submitted. No uses of the FCM substance No 20, N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides, were claimed and no information was provided to support that the current authorisation is in accordance with the Regulation (EC) No 1935/2004.

KEYWORDS

evaluation, FCM substance No 19, FCM substance No 20, food contact materials, N,N-bis(2-hydroxyethyl)alkylamine, plastic, polymer production aid, processing aid, safety assessment

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1 | INTRODUCTION

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

1.1.1 | Background from the mandate letter

"The chemically and structurally similar substances N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine (FCM No 19), and N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides (FCM No 20) are currently authorised as additives for the manufacture of plastic food contact materials (FCM), and are listed in Annex I of Regulation (EU) No 10/2011, with a Group Specific Migration Limit (SML (T)) of 1.2 mg/kg food and additional restrictions.

A business operator notified EFSA of scientific evidence on FCM 20 that indicates this substance may cause previously unknown adverse effects to health. These effects were shown in an OECD 414 developmental toxicity study that was conducted as part of additional testing required under REACH. The evidence should however have been submitted to the Commission under Article 11(5) of Regulation (EU) No 1935/2004, allowing the Commission to decide whether to consult EFSA. EFSA therefore rejected the application on 14 December 2015 and pointed that the new evidence should be submitted to the Commission.

The submitted evidence was nevertheless considered by the EFSA CEF FCM working group (WG) which noted that the study could indeed indicate possible health effects following a single high exposure from either of the two substances. The study should therefore be examined in more detail, although the SML already in place would reduce the urgency thereof.

The current authorisation is based on a 1997 opinion of the Scientific Committee on Food (SCF, 1997). The SCF established a temporary TDI (t-TDI), which currently functions as the basis for the present SML. In a subsequent 2001 opinion on the 13th additional list of monomers and additives for food contact materials (SCF, 2001b), the SCF requested three in vitro mutagenicity tests, which were subsequently submitted by a consortium of companies and evaluated by EFSA in 2006. In this evaluation, the substances were listed on SCF list 7, which concerns "substances for which some toxicological data exist, but for which an ADI or a TDI could not be established and additional genotoxicity information should be furnished".

Given the potential concerns regarding these two substances based on the evidence submitted by the business operator and the t-TDI on which the present SML is based, the Commission requests that the initial assessments made by the SCF and EFSA should be reviewed and if necessary be updated, as provided for in Article 12(3) of Regulation (EC) No 1935/2004.

To evaluate whether its existing opinion on FCM No 19 and 20 is still in accordance with the Regulation, EFSA should make use of the available information on the safety of the substance from the dossier supporting the original application, old SCF files and evaluations, the data submitted in 2006, and the data submitted in 2014, as well as any additional information that may be available to the applicant, to other users of the substances and in the public domain. To this end, where necessary, EFSA may consult the applicant, as set out in Article 12(3) and consider organising a call for information to ensure that the most complete data package is used for the elaboration of its opinion. Once the needed data package becomes available, EFSA should deliver its opinion in line with the timelines set out in Article 10 of Regulation (EU) No 1935/2004."

1.1.2 | Terms of Reference as provided by the European Commission

In accordance with Article 12(3) of Regulation (EC) No 1935/2004,[1] the European Commission asks EFSA to review whether the authorisation of N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine (FCM No 19), and N,N-bis(2-hydroxyethyl)alkyl(C8-C18) amine hydrochlorides (FCM No 20) is still in accordance with Regulation (EC) No 1935/2004, as provided for in Article 12(3).

The substances are currently authorised as additive or polymer production aid for the manufacture of plastic food contact materials (FCM), and listed in Annex I of Regulation (EU) No 10/2011, with a SML(T) of 1.2 mg/kg food and additional restrictions.

EFSA should evaluate whether the authorisation of FCM 19 and FCM 20 is still in accordance with Regulation (EC) No 1935/2004, as provided for in Article 12(3). To evaluate whether its existing opinion on FCM no 19 and 20 is still in accordance with the Regulation, EFSA should make use of the available information on the safety of the substance from the dossier supporting the original application, old SCF files and evaluations, the data submitted in 2006, and the data submitted in 2014, as well as any additional information that may be available to the applicant, to other users of the substances and in the public domain. To this end, where necessary, EFSA may consult the applicant, as set out in Article 12(3) and consider organising a call for information to ensure that the most complete data package is used for the elaboration of its opinion. Once the needed data package becomes available, EFSA should deliver its opinion in line with the timelines set out in Article 10 of Regulation (EU) No 1935/2004.

[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.

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1.2 | Interpretation of Terms of Reference

The European Commission asked EFSA to evaluate whether the authorisations of FCM substance No 19 (N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine) and FCM substance No 20 (N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochloride) are still in accordance with the Regulation (EC) No 1935/2004. In addition to the information available in the existing dossiers supporting the related original applications, EFSA considered it necessary to launch a call for information and to request additional information to ensure that the most complete and fit for purpose data package is used for the elaboration of its opinion.

2 | DATA AND METHODOLOGIES

2.1 | Data

The FCM Panel based its assessment on the available information on the substances from the dossiers supporting the original application, SCF files and evaluations, the data submitted to EFSA in 2006 and 2014, as well as additional information available to the original applicant, and to other business operator of the substances. To this end, on 6th December 2019, EFSA published a call for data[2] to ensure that the most complete data package is used for the elaboration of its opinion. The deadline for submitting data was 20 April 2020. Upon requests from business operators, it was extended until 30 June 2022, at which date EFSA considered necessary to start with the assessment, acknowledging that additional studies were aimed to be launched.

In the call, information was sought on the characterisation and the safety of the substances in relation to:

- specification of the substances, if any;
- description of the manufacturing process(es), purity of the substances and impurities related to the manufacturing process(es), including the manufacturing process(s);
- physical and chemical properties of the substances;
- uses of the substances, including uses in plastic articles expected to be in contact with food for infant;
- migration of the substances, their impurities and related reaction and degradation products;
- residual content of the substances in the plastic food contact articles;
- potential toxicity, including its potential to accumulate in human;
- any other data relevant for the safety assessment.

All data potentially relevant for the toxicological assessment of the substances were requested to be submitted, i.e. not limited to the minimum data requirement triggered by the estimated exposure/migration level and tiers as per the Guidelines of the SCF for the presentation of an application for safety assessment of a substance to be used in FCM prior to its authorisation (European Commission, 2001a) and the EFSA Note for Guidance for the preparation of an application for the safety assessment of a substance to be used in plastic food contact materials (EFSA, 2008).

In response to the call, four business operators submitted technical dossiers following the EFSA Note for Guidance structure: Croda, Clariant, Nouryon[3] and LyondellBasell. In addition, the Dutch National Institute for Public Health and the Environment (RIVM) submitted original SCF files.

Additional information was provided by the business operators LyondellBasell, Clariant, Nouryon and Croda/Cargill[4] during the assessment process in response to the requests from EFSA sent on 10 November 2022 (see Documentation provided to EFSA).

Data submitted and used for the evaluation are:

Non-toxicological data and information

Croda/Cargill *N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine*	Chemical identity Description of manufacturing process of the substance/FCM Purity and impurities Physical and chemical properties Intended uses Existing authorisation(s) Migration of the substance

[2]Call for technical and toxicological data on the substances N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine (FCM No 19) and N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine hydrochlorides (FCM No 20) for their use in plastic materials and articles intended to come into contact with food, including in food for infants. https://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-substances-nn-bis2.

[3]Nouryon on behalf of the Primary Fatty Amine Ethoxylates (PFAEO) Consortium, expressed "Nouryon" hereafter. List of interested parties: Nouryon AB, Nouryon Chemicals BV, Arkema, Clariant Produkte (Deutschland) GmbH.

[4]The additional data requested to Croda were submitted by Cargill as it acquired the rights to Atmer 163 and all supporting data as part of Cargill's acquisition of Performance Technology from Croda in 2022.

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Clariant *N,N-bis-(2-hydroxyethyl)alkyl(C16/18)amine*	• Chemical identity • Description of manufacturing process of the substance/FCM • Purity and impurities • Physical and chemical properties • Intended uses • Existing authorisation(s) • Migration data • Residual content in the plastic after migration experiment
Clariant *N,N-bis(2-hydroxyethyl)alkyl(C8)amine*	• Chemical identity • Description of manufacturing process of the substance • Purity and impurities • Physical and chemical properties • Intended uses • Existing authorisation(s)
Clariant *N,N-bis(2-hydroxyethyl)alkyl(C18)amine*	• Chemical identity • Purity • Intended uses
LyondellBasell *N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine*	• Migration data • Residual content in the plastics samples tested for migration
Nouryon *Primary Fatty Amine Ethoxylates (PFAEO) incl.: PFAEO-coco, oleyl, tallow, hydrogenated tallow, C18*	• Chemical identity • Purity and impurities • Physical and chemical properties

Toxicological data

The toxicological data submitted by the four Business Operators are reported in detail in Appendix A.

2.2 | Methodologies

The assessment of application is 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 SCF Guidelines (European Commission, 2001a), including the corresponding data requirements.

The methodology is based on the characterisation of the substance that is the subject of the request for safety assessment, its impurities as well as 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, 2001a) and in the EFSA Note for Guidance (EFSA, 2008).

In this case, in the call, business operators and other interested parties were requested to submit data following the recommendation of the SCF Guidelines and EFSA Note for Guidance with the exception of the non-applicability of the tiered approach for data requirement. In other words, all the data relevant to the toxicological potential of the substances were requested to be submitted and not to be limited to the minimum data requirement triggered by the estimated exposure/migration level.

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

EU authorisation

The substances N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine and N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides are listed for use as additives or polymer production aids in the Regulation (EU) 10/2011 (FCM Substances No 19 and 20, respectively), with an SML(T) for the sum of the two substances of 1.2 mg/kg food, expressed as tertiary amine.

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Past assessments

The authorisation is based on the safety assessment by the SCF, which classified the two substances in the SCF-List 2[5] with a group temporary tolerable daily intake (t-TDI) of 0.02 mg/kg body weight (bw) per day (European Commission, 1997). In 2001, the SCF reiterated the need for additional data and with the remark that 'on the basis of the available studies it remains included in the group t-TDI pending the results of the required mutagenicity studies. If negative it will be classified in L3 with a restriction of 5 mg/kg of food.' (European Commission, 2001b).

The substances were re-evaluated by the EFSA FCM WG in 2006 and in 2014. In the 2006 application, a consortium submitted the three requested mutagenicity tests. The assessment ended with a request for additional data on identity and migration.[6] In 2014, Croda applied for re-evaluation, provided the requested data and informed about the results of a developmental toxicity test carried out as part of a REACH registration. The mandate was rejected following a preparatory assessment and discussion with the European Commission.

Uses

In the applications submitted to EFSA in 2006 and 2014, the substance N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine (FCM No 19) was reported to be used in polyolefins (PO; up to 0.15%, typically 0.1% w/w), polystyrene (PS; up to 2%) and acrylonitrile/butadiene/styrene (ABS) copolymers (up to 1% w/w) as antistatic agent, and as a processing aid in polypropylene (PP) production at up to 0.15% w/w. Intended applications of the final articles included all types of food without restrictions in terms of contact temperature and time.

As listed in the Annex I of the Regulation (EU) 10/2011, the substance can be used in all plastic types in contact with all food types without restriction (e.g. compositional) other than the SML(T) of 1.2 mg/kg food, expressed as tertiary amine.

In the call for data and the subsequent responses to a request for additional data from EFSA, the uses were updated as follows:

- Croda/Cargill reported using N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine only as a processing aid (up to 0.1%) in PO production for food contact. The intended conditions of contacts are with all types of food and without restrictions of time and temperature. A large proportion of food contact applications are for films of 20–200 μm thickness. For PP, there are some uses in moulded and thermoformed applications with thicknesses around 500 μm and the maximum thickness, for instance, in the case of bottles caps is around 1 mm. The maximum use level in these applications as stated is 1000 mg/kg, but more commonly < 500 mg/kg and typically 100–300 mg/kg.
- LyondellBasell indicated using the N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine as an in-process antistatic agent (up to 0.1%) in the manufacturing of PO.
- Clariant reported the use of N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine as an antistatic agent at up to 0.1% in high-density polyethylene (HDPE), low-density polyethylene (LDPE) and biaxially oriented polypropylene (BOPP) in contact with all types of food at frozen to ambient temperature. Clariant also submitted supporting data on N,N-bis(2-hydroxyethyl)alkyl(C8)amine (Genamin 3920) and N,N-bis(2-hydroxyethyl)alkyl(C18)amine, indicating that both were not to be used in FCM.
- Nouryon did not provide specific information on the uses of the Primary Fatty Amine Ethoxylates (PFAEO), for which "toxicological data have been generated under REACH for the FCM substance No 19". From the submitted information, it remained unclear whether they are used to manufacture FCM or merely submitted as supporting information. All five PFAEO bear unsaturation, hence are, in principle, not covered by the entry FCM No 19. Therefore, the data provided by Nouryon were considered supportive information. If those substances were intended to be used to manufacture plastic FCM, a proper application following the EFSA Guidance documents should be submitted.
- RIVM submitted a summary datasheet of the SCF assessment of N,N-bis(2-hydroxyethyl)lauramide (FCM No 923 N,N-bis(2-hydroxyethyl)dodecanamide). The information provided was reviewed. However, being an amide, it does not support the assessment of the substances FCM No 19 and 20 and is not reported in the present assessment.
- No data was provided to support the assessment of the substance N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides (FCM No 20) either during the call or in response to the EFSA request for additional data. All four business operators declared not using the substance.

The reported uses with regard to the source of the information, substances, composition, function and plastic types are summarised in the Table 1 below.

[5]Reports of the Scientific Committee for food (Thirty-third series); https://aei.pitt.edu/40842/1/33rd_food.pdf.

[6]The request for additional data was not followed up and the application was finally not accepted.

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TABLE 1 Uses, source of the information, substances, composition, function and plastic types.

BO	Substance(s)	Max. use level (% w/w)	Function	Plastic types	Food contact (food types, t, T)
Croda (2014)	N,N-bis(2-hydroxyethyl) alkyl(C8-C18)amine represented by N,N-bis(2-hydroxyethyl)alkyl(C13/C15) amine	0.15 2 1 0.15	Antistatic agent Processing aid	PO PS ABS PP	All types of food, no restrictions in time and temperature
Croda (2022) Cargill (2024)	N,N-bis(2-hydroxyethyl) alkyl(C8-C18)amine represented by N,N-bis(2-hydroxyethyl)alkyl (C13/C15) amine	0.1	Processing aid	PO	All types of food, no restrictions in time and temperature
LyondellBasell (2022)	N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine	0.1	Antistatic agent (Polymer Production Aid)	PO	NA
Clariant (2022)	N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine	0.1	Antistatic agent	HDPE, LDPE and BOPP	All types of food from frozen to ambient storage
	N,N-bis(2-hydroxyethyl)alkyl(C8) amine	NA	Neutralising agent and co-emulsifier	Not food contact metal working fluids	Submitted as supporting information
	N,N-bis(2-hydroxyethyl)alkyl(C18) amine	NA	NA	NA	Submitted as supporting information
Nouryon (2022)	PFAEO-Coco (ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives), PFAEO-Oleyl (2,2′-(octadec-9-enylimino)diethanol, PFAEO-T (2,2′-(C16/C18, C18 unsaturated)alkylimino) diethanol), PFAEO-HT (2,2′-(C16/C18 alkylimino) diethanol), PFAEO-C18 (2,2′-(octadecylimino) diethanol) see Table 2 for more details	NA	NA	NA	NA

Abbreviation: NA, not available.

3.1 | Non-toxicological data

3.1.1 | Identity and specifications of the listed substance

The substance listed in Regulation 10/2011 under the FCM No 19, N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine is an aliphatic tertiary amine. Its structure includes an alkyl group R of 8–18 carbon atoms, which may be linear or branched and of natural as well as synthetic source since these are not specified in the entry of the Union list. According to Croda/Cargill, the substance is an undefined mixture with no specific single CAS number and is commercialised under 21 trade names.[7]

The molecular mass of N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine, aliphatic, linear, would range from 217 to 357 Da (Figure 1).

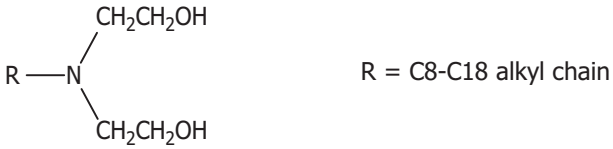


FIGURE 1 Generic structural formula of N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine.

[7]Atmer 163, Atmer 163LM, Synprolam 35X2, Hostastat FA 14, Hostastat FA 18, Hostastat FA 38, NOROPLAST 3000, NOROPLAST 2002, NOROPLAST 2000, NOROPLAST 8500, ELEC EA-1, ELEC EA-5, AMIET CED/14.2, AMIET CTD/14.2, AMIET TD/14, Armostat 700, Armostat 600, Armostat 400, Armostat 300, Armostat 1800, Armostat 310.

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Table 2 below lists the individual or mixed substances used to support the authorised substance and discussed in this assessment, as well as their data sources. In this table, the substances were renamed by the FCM Panel to align between themselves and with the EU Union list. However, as there are variations in the chemical names used for commercial products and as presented by the different data providers of the company BOs, a clarification of the chemical names is given in Appendix B.1. It is recommended to consult this Appendix before further reading.

TABLE 2 Individual or mixed substances used and reported by the business operators discussed in this section as well as their data sources. The descriptions below reported by the business operators should be considered as indicative and for the main constituents. Better description is provided in the respective sections below.

Business operator	Substance	CAS no	Trade name	C-range	Linear/ branched	Source of alkylamines
Croda/Cargill	N,N-bis(2-hydroxyethyl) alkyl(C13/C15)amine	97925-95-6 70955-14-5	Atmer 163, Atmer 163-LQ-(AP) –	C13/C15	1:1	Synthetic
LyondellBasell	N,N-bis(2-hydroxyethyl) alkyl(C13/C15)amine	97925-95-6	Atmer 163	C13/C15	1:1	Synthetic
Clariant	N,N-bis(2-hydroxyethyl) alkyl(C16/C18)amine	1218787-30-4	Hostastat FA 68 V	C16/C18	Linear	[redacted]
	N,N-bis(2-hydroxyethyl) alkyl(C8)amine*	15520-05-5	Genamin 3920	C8	Linear	[redacted]
	Tallow amine ethoxylate: N,N-bis(2-hydroxyethyl) alkyl(C18)amine*	NA	[redacted]	C18, saturated	NA	[redacted]
Nouryon	PFAEO-coco: N,N-bis(2-hydroxyethyl) alkyl(C12-C18)amine*	71786-60-2 61791-31-9[8]	[redacted]	C12-C18 (mainly C12-C14)	Linear	[redacted]
	PFAEO-oleyl: N,N-bis(2-hydroxyethyl) oleyl(C18:9)amine*	25307-17-9 [redacted] –	[redacted] Cecajel 210 R [redacted]	C18:1 n-9	Linear	NA
	PFAEO-T: N,N-bis(2-hydroxyethyl)alkyl(C16/C18, C18 unsaturated) amine*	1218787-32-6 61791-44-4 – –	Ethomeen T/12 [redacted] [redacted] [redacted]	C16/C18, C18 Unsaturated	Linear	[redacted]
	PFAEO-HT: N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine*	1218787-30-4 – 90367-28-5	Ethomeen HT/12, [redacted] [redacted]	C16/C18	Linear	[redacted]
	PFAEO-C18: N,N-bis(2-hydroxyethyl)alkyl(C18) amine*	– 10213-78-2	[redacted]	C18	Linear	[redacted]

Abbreviation: NA, information not available.

*Submitted as supporting information.

3.1.2 | N,N-bis(2-hydroxyethyl)alkyl(C13/15)amine (Croda/Cargill, LyondellBasell)[9]

Two data sources were provided on the substance N,N-bis(2-hydroxyethyl)alkyl(C13/15)amine. Croda/Cargill provided a comprehensive data set. LyondellBasell provided a report on new migration data only. If not otherwise mentioned in the text, the following data and discussion stem from and refer to the Croda/Cargill dossier.

3.1.2.1 | *Identification*

N,N-Bis(2-hydroxyethyl)alkyl(C13/15)amine is a synthetic substance that has alkyl groups reported to be [redacted]% linear n-C13/C15 [redacted] and [redacted]% iso-C13/15 [redacted].

The identity of the substance was confirmed by ultraviolet/visible spectroscopy (UV/VIS), gas chromatography/mass spectrometry (GC–MS) of trimethylsilyl (TMS) derivatives, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, infra-red spectroscopy (IR) as well as high-resolution liquid chromatography-mass spectrometry (LC–MS). The molecular formula is $C_{17}H_{37}NO_2$/$C_{19}H_{41}NO_2$ and the molecular weight is 287/315 Da.

[8]A mixture of substances [redacted] falls under this CAS No 61791-31-9. It includes alkyl diethanol amines and fatty acid diethanolamides (and possibly others). There is no chemical structure depicted and from all information including Sci-Finder and the ECHA website, the identity is unclear.

[9]Call for data/Croda/technical dossier/sections 1, 2, 5 and related annexes; Additional data/Croda-Cargill/technical dossier/sections 1, 2, 5 and related annexes; Additional data/LyondellBasell.

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3.1.2.2 | *Manufacturing process, (im)purities and specification*

Ethylene oxide is reacted with [redacted] alkylamines [redacted]

According to Croda (2022), the purity of the product N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine (Atmer 163), determined by ^{13}C NMR spectroscopy, is [redacted]%[redacted]

The Panel noted that the potential level of impurities[redacted] specified by the business operator with data provided in 2022 was not fully investigated. This leaves uncertainty as to which extent impurities such as mono-ethoxylated species (secondary amines), over-reacted (more than two ethoxy) or unreacted species (primary amines), [redacted] could migrate into food. In 2024, new and more detailed analytical data were provided by Cargill on the purity of Atmer 163 [redacted]. High-resolution LC–MS and GC–MS analyses showed purities of 99% and [redacted]%, respectively. Impurities and reaction products were analysed, characterised and largely identified and thus the presence of such species as already tentatively found before was confirmed. Details on impurities and reaction products are given in Section 3.1.7.

[redacted]

3.1.2.3 | *Physical and chemical properties*

The substance has a melting point of approximately 2°C and a boiling point above 200°C. Thermal stability measured by thermogravimetry showed decomposition at around 300°C, which is above the usual melt and processing temperatures of PO, PS and ABS.

The substance is soluble in water (80.8 mg/L at 20°C) and solvents such as methanol, isopropanol, acetone and toluene. No log octanol/water partition coefficient (Po/w) value was provided, because its determination was technically not possible due the surface-active properties of the substance. No calculated log Po/w value was provided, since computer-based calculations would not take into account surfactant properties and, thus, would significantly over- or underestimate the partition coefficient. This justification was considered acceptable by the Panel.

3.1.2.4 | *Residual content and migration*

The residual content of the substance in the plastic samples tested for migration (Croda) was not determined.

The nominal (added) content was specified to be 0.1% (w/w). Specific migration tests were carried out in the total immersion mode using PP plaques of 2.5 mm and 2 mm thickness in contact with olive oil (simulant D2) and 3% acetic acid (simulant B), respectively. For the test with olive oil, PP homopolymer and PP block copolymer (ethylene–propylene–rubber copolymer with 25% rubber) were used. For the test with 3% acetic acid, cast PP plaques were used. The plaques were exposed to olive oil during 30 min at 100°C followed by 10 days at 40°C and to 3% acetic acid for 2 h at 100°C (in an autoclave) followed by 10 days at 40°C.

Migration into olive oil was determined photometrically (of a coloured amine salt derivative) time-resolved after the 100°C contact and during the 10 days contact phase at three time points (24, 96 and 240 h). Migrations from PP homopolymer and copolymer were up to 2.0 and 2.9 mg/kg, respectively. The equilibrium at this maximum value was already reached after the first time point at 40°C (following the 100°C contact). Migration into 3% acetic acid, measured by LC–MS analysis, was up to 10 mg/kg. The Panel noted that the applied migration contact conditions were not as severe as those legally prescribed for the intended food contact applications reported in Table 1 that require 10 days at 60°C. At the high thickness of the tested PP sample, it is expected that the short period of contact at 100°C does not compensate for the required 60°C instead of 40°C. Therefore, the measured migration may not have covered the highest possible migration into fatty and acidic foods. In addition, another uncertainty is that the substance, due to its chemical properties, is likely to be enriched or bloomed out at the surface of a PO FCM which might explain the observed early equilibrium due to wash-off from the surface.

According to additional information from Croda/Cargill, (i) the maximum use level in FCM applications is 1000 mg/kg, but more commonly < 500 mg/kg and typically 100–300 mg/kg, and (ii) a large proportion are films of 20–200 μm. Cargill provided also an overview of usual food contact applications which include: (i) PO films, such as polyethylene (PE) (50–100 μm) for frozen food (e.g. French fries), PE (30–50 μm) for sliced bread bags for storage at ambient T for 7 days, PP (30–50 μm) for prepared salad packaging for storage at refrigerated T for 7 days, PP or PE for dry food (e.g. beans, rice) packaging for storage at ambient T for months/year, and (ii) for trays, boxes and pots (PP 500 μm) for e.g. cook/chill meals, raw meat, yoghurts, butter, for storage at freezing T or refrigerated T for 7 days. Concerning thickness, the exception is closures (e.g. for water polyethylene terephthalate (PET) bottles) with thickness said to be around 1 mm but, expected to be up to 1.5 mm.

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This, however, with very limited food contact. Taking this information into account, the Panel noted that due to possible exhaustive migration from thin films in such FCM applications, the migration values under those conditions may be significantly lower than those tested at worst-case conditions. This was confirmed by the results from the migration testing of films of 80 μm in Section 3.1.3.4 (N,N-bis(2-hydroxyethyl)alkyl(C16/18)amine (Clariant)).

Specific migration was also measured from eight PP and two HDPE samples (plaques of 2 mm thickness) in one-sided (test cell) contact with 95% ethanol after 10 days at 60°C by LC–MS (LyondellBasell Report). The amount of the substance added to the test samples ranged from 58 to 950 mg/kg polymer (0.0058%–0.095%). LyondellBasell indicated that these migration tests were not representative of food contact applications but were conducted to evaluate the effect of the physical properties (i.e. variable crystallinity) of the samples on migration. However, no information was provided on the requested maximum intended amounts used to manufacture food contact applications. Migration ranged between 0.7 and 41 mg/kg, depending on the type of polymer and the added content of the substance in the test samples. The two highest migration values, 41 and 27.3 mg/kg, correlated with the two highest amounts of 950 and 642 mg substance/kg polymer (low crystallinity PP samples), whereas the lowest content of 58 mg substance/kg polymer (PP sample) showed a migration of up to 1.7 mg/kg. In conclusion, roughly an indicative correlation between level in the polymer and migration was supported by the data, but it was not clear to which extent potential blooming was contributing to migration. Migration exceeded the current SML of 1.2 mg/kg food in 8 out of the 10 samples and was above 5 mg/kg food in three samples. The Panel noted that the use of 95% ethanol as alternative fatty food simulant at the applied (and legally specified) contact conditions represents exaggerated test conditions because ethanol is more aggressive than olive oil under these contact conditions. Therefore, the obtained migration values are an over-indication of the expectable worst-case migration levels rather than a realistic indication of migration in many, if not most, food contact applications for fatty foods as indicated by Croda/Cargill (see above).

Taking the data from Croda/Cargill and LyondellBasell together, the Panel noted that, under the applied test conditions, the migration of the substance exceeds the current SML of 1.2 mg/kg food in most cases and has the potential to largely exceed 5 mg/kg, depending on the combination of the three factors: (i) the use level, (ii) the FCM thickness and (iii) the type of food contact. However, a thin film of e.g. 50–100 μm thickness may not exceed 5 mg/kg even at the maximum intended level of 0.1% and at elevated/high temperature contact. Also, a thicker FCM (e.g. 0.5 mm trays or bottle closures with higher thicknesses) may not exceed 5 mg/kg due to the low/ambient temperature contact or due to the limited surface in contact with water or beverages.

3.1.3 | N,N-bis(2-hydroxyethyl)alkyl(C16/18)amine (Clariant)[10]

3.1.3.1 | *Identification*

Clariant provided a dossier on the substance N,N-bis(2-hydroxyethyl)alkyl(C16/18)amine. [redacted]. The identity of the substance was confirmed by GC–MS analysis and further supported by gas chromatography-flame ionisation detection (GC-FID) analysis, ^{13}C-NMR spectroscopy, UV/VIS and Fourier transform infrared (FTIR)-spectroscopy as well as by the expected chemistry from the alkyl amines used for the manufacture of the substance.

The molecular formula is $C_{20}H_{43}NO_2/C_{22}H_{47}NO_2$ and the molecular weight is 329/357 Da.

3.1.3.2 | *Manufacturing process, (im)purities and specification*

[redacted].

The purity of the batch analysed by GC-FID was given as [redacted].

Summed levels of unknowns in three batches were reported to be [redacted]; they should be considered as indicative rather than maximum levels. No further analyses were performed in response to EFSA's request to explore the structures of the unknown fraction, in particular more polar and less volatile ones. Taking the chemistry of the manufacturing process into account as well as the unknown or variable composition, complex reaction products and biological

[10]Call for data/Clariant/Technical dossier (Hostastat FA 68V)/Sections 1,2,5,6 and related annexes; Additional data/Clariant/Answer and related annexes.

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materials (UVCB) nature of the fatty acid starting material, the unknown fraction is likely to contain or consist of analogues of identified impurities with other C-chain lengths, unsaturated chains and under- or over-ethoxylated species.

3.1.3.3 | *Physical and chemical properties*

Differential scanning calorimetry (DSC) of the substance showed a melting point of 44.6°C and thermal stability up to 348.6°C. The boiling point was not measured.

The solubility in water was given as 20 g/L. This high value is not consistent with the solubility of the C13/C15 alkyl amine (80.8 mg/L at 20°C, cf. 3.1.2.3). Due to the longer alkyl chains, the Panel expected the solubility to be lower than that of the C13/C15 compound. It is likely that due to the surface-active properties of the substance, micelles were formed in the solubility test and were misinterpreted as dissolved substance. In fact, the European Chemicals Agency (ECHA) website[11] gives the critical micelle concentration as 3.5 mg/L. No solubility data in other solvents were provided. Log Po/w was not measured and not calculated, which is acceptable due to the surfactant character of the substance (cf. 3.1.2.3).

3.1.3.4 | *Residual content and migration*

Specific migration was determined for polymer films of 80 µm thickness, made from LDPE, HDPE and PP, containing 0.069%, 0.096% and 0.090%, respectively, of the substance. These values were determined by LC–MS analysis and found to be close to the maximum use level of 0.1% for HDPE and PP samples and one-third lower for the LDPE film due to a lower nominal amount used. Migration tests were performed in the one-sided contact mode with the simulants 95% ethanol, iso-octane, 20% ethanol, 3% acetic acid and simulant E (Tenax®). The applied test conditions for iso-octane were 10 days at 20°C, and for all other simulants, they were 10 days at 60°C. Migration was measured by LC–MS. The migration into 20% ethanol was up to 1.6 mg/kg; for all other liquid food simulants, it was comparable being between 3.1 and 4.0 mg/kg food whereby migration into 3% acetic acid (3.4–4.0 mg/kg food) was slightly higher than into 95% ethanol (3.5–3.6 mg/kg) and iso-octane (3.1–3.8 mg/kg). Migration into simulant E ranged between 0.9 and 1.1 mg/kg.

In conclusion, migration into foodstuffs other than dry foods from PO films of 80 µm thickness made with up to ca. 0.1% of the substance was above the SML(T) of 1.2 mg/kg food, and below 5 mg/kg food. It appears that migration from films with thickness up to 100 µm and containing the substance at the maximum use level may not exceed the 5 mg/kg. These data are in agreement with or even confirm the conclusion drawn in Section 3.1.2.4.

3.1.4 | N,N-bis(2-hydroxyethyl)alkyl(C8)amine (Clariant)[12]

3.1.4.1 | *Identification*

Clariant, who submitted the information on N,N-bis(2-hydroxyethyl)alkyl(C8)amine (Genamin 3920), declared not to use the substance for the manufacture of plastic FCM, but in metal working fluid. The information was limited and submitted as supporting information.

The substance has a [redacted] chain. The identity of the substance was supported by GC-FID analysis of the TMS derivate, ^{1}H-NMR, ^{13}C-NMR as well as FTIR and UV/VIS spectroscopy. The molecular formula is $C_{12}H_{27}NO_2$ and the molecular weight is 217 Da.

3.1.4.2 | *Manufacturing process, (im)purities and specification*

[redacted]. The purity was specified to be ≥ 90% and was [redacted]% for a batch analysed by GC-FID.

[redacted]

3.1.4.3 | *Physical and chemical properties*

According to the applicant, the melting point is ≤ –20°C (Organisation for Economic Co-operation and Development Testing Guidelines (OECD TG)) 102 (OECD, 1995a) and the boiling point 319°C (OECD TG 103 (OECD, 1995b)). The substance is soluble in

[11] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14327/4/9.

[12] Call for data/Clariant/Technical dossier (Genamin 3920)/Sections 1,2,5,6 and related annexes; Additional data/Clariant/Answer.

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water (1.4 g/L at 20°C) and miscible in 1-octanol at 23°C (OECD TG 105 (OECD, 1995c)). The log Po/w, which was likely affected by the surfactant character of the substance, was determined to be 2.8 (EU method A.8. of Directive 84/449/EEC).

3.1.4.4 | *Residual content and migration*

Because the substance is used as neutralising agent and co-emulsifier for metal working fluids and not in plastic FCM, Clariant did not provide data on residual content and migration.

3.1.5 | N,N-bis(2-hydroxyethyl)alkyl(C18)amine (Clariant)[13]

Clariant, who submitted the information on N,N-bis(2-hydroxyethyl)alkyl(C18)amine, declared not to use the substance for the manufacture of plastic FCM. The information was submitted as supporting information.

Limited information on the source (tallow), the trade name ([redacted]), the purity ([redacted]%) and the structural formula was provided in the submitted toxicity study (Section 3.2.4). No information was provided on impurities, residual content and migration.

Without characterisation of the substance and information on its manufacture (e.g. whether it was hydrogenated), the identity and composition could not be verified. [redacted]

3.1.6 | PFAEO-coco, PFAEO-oleyl, PFAEO-HT, PFAEO-T, PFAEO-C18 (Nouryon)[14]

Nouryon provided information on five PFAEO substances, namely PFAEO-coco, PFAEO-oleyl, PFAEO-HT, PFAEO-T and PFAEO-C18, corresponding to the different source and/or main component(s). Non-toxicological information was limited and mostly referred to physical–chemical properties. General information was provided on their identity (CAS numbers, EC numbers, sources, range of carbon covered, purity; see Table 2 in 3.1.1 and Table 3 below) without sufficient supporting data. Limited or no information was provided on the impurities, thermal stability and potential migration. It was not informed on which food contact applications the substances are used and it remained unclear whether they are used to manufacture FCM at all.

According to Nouryon, the fatty amines of all five PFAEO originate from natural but not fully specified sources, and have straight alkyl chains. As natural sources would include vegetable and animal sources, the Panel noted that small proportions of branched alkyl chains are expected to be present when coming from animal sources. The length of the alkyl chain varies from C8 to C18 and most likely beyond C18 for minor constituents. From the boundary composition information and certificates of analysis (CoAs) provided, all five PFAEO bear unsaturation (see Table 3 below) with up to three double bonds, hence are—in principle—not covered by the entry FCM No 19. The degree of ethoxylation ranges [redacted] The main ethoxylated constituents are [redacted] Details of the composition of the PFAEO-coco, PFAEO-oleyl, PFAEO-HT, PFAEO-T and PFAEO-C18 as given by Nouryon are reported in Appendix B.2.

In Table 3, a compilation of physico-chemical data from the documentation provided by Nouryon is given. In the absence of the supporting raw data, the reliability of these data could not be properly assessed.

TABLE 3 Overview of the physico-chemical data on the five PFAEO substances.

Substance	Melting point[a]	Boiling point[a]	Water solubility/CMC[b]	Unsaturation
PFAEO-coco	5°C	> 200°C	5 mg/L[c]/5 mg/L	[redacted]
PFAEO-oleyl	< −30°C	> 300°C	5.9 mg/L[c]/5.9 mg/L	[redacted]
PFAEO-HT	44°C	> 300°C	3.5 mg/L[c]/3.5 mg/L	[redacted]
PFAEO-T[d]	Read across between PFAEO-Coco and -Oleyl	Read across to PFAEO-Oleyl	Read across to PFAEO-HT	[redacted]
PFAEO-C18	49°C	> 400°C	0.6 mg/L/NA	[redacted]

Abbreviation: NA, information not available.

[a]Determined by DSC.

[b]Critical micelle concentration.

[c]CMC is taken as water solubility.

[d]No experimental data available.

[e]Calculated.

[13]Call for data/Clariant/Technical dossier (Hostastat FA 68 V)/Section 8 and Annex 10.

[14]Additional data/Nouryon/Technical dossier/Section 1,2 and related annexes.

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3.1.7 | Impurities and degradation/reaction products[15]

When purity data were provided, they were determined as 100% minus the other substances detected, in general based on GC peak areas, and in one case by LC–MS. The Panel noted that these are upper bound purities, since other impurities may have not been observed in the chromatogram, e.g. because of low volatility or high polarity, or not ionisable compounds. Also, there is not always a clear distinction between isomers of the substance and impurities.

Croda/Cargill provided a detailed screening analysis and semi-quantification of the components in three different batches of Atmer 163 (N,N-Bis(2-hydroxyethyl)alkyl(C13-C15)amine) using LC-MS and GC-MS. Determined purities were [redacted]% and [redacted]%, respectively.

- With LC-MS, in total, [redacted] **components** were tentatively identified as impurities and were grouped based on their structural similarities:

[redacted]

- With GC-MS, [redacted] components were detected and tentatively identified, from which [redacted] **impurities** differed from those detected by LC-MS. [redacted] All impurity levels in the product ranged between [redacted]% and [redacted]%.
- Based **on the provided calculation for a total mass transfer from a PP packaging material of 1-mm thickness containing 0.1% Atmer 163**, the summed migration of all impurities measured by LC-MS ([redacted]%) and GC-MS ([redacted]%) would be 0.56 and 0.9 mg/kg food, respectively. For all individual impurities, either found by LC–MS or GC–MS, maximum migration levels were calculated based on this total mass transfer scenario. [redacted]
- The Panel noted that a total mass transfer assumption is exaggerated, and also considered **pro-rata migration calculation** of the impurities, relative to a potential 5 mg/kg SML; as their migration will be indirectly controlled via the maximum allowed migration of the substance. The chemical similarity of most impurities with the substance was considered to justify this pro-rata approach. Pro-rata migration was calculated for the individual impurity levels/percentages measured in Atmer 163. For impurities falling within the same structural groups, their pro-rata migration was summed up from the individual group members. All pro-rata migration values are presented in Table 4. They are below 50 μg/kg food except for [redacted]

When assuming pro-rata migration of the total impurities level, the [redacted]% impurities (GC–MS determined) would be expected to migrate at 80 μg/kg food for a possible 5 mg/kg food restriction. A 5% total impurity level as specified by Croda in 2022 would be expected to trigger pro-rata migration at 250 μg/kg food for a possible 5 mg/kg food migration. When considering the 12 individuals and groups of impurities from Table 4 and their distribution, their individual migration is still expected to be below 50 μg/kg food [redacted] and in the range of 50 μg/kg food for [redacted] (group).

[15]Additional data/Croda-Cargill/technical dossier/section 1 and related annexes; Call for data/Clariant/Technical dossier (Hostastat FA 68V)/Section 1 and related annexes; Call for data/Clariant/Technical dossier (Genamin 3920)/Section 1 and related annexes; Additional data/Nouryon/Technical dossier/Section 1 and related CoAs.

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TABLE 4 Estimation of the potential migration of the impurities identified from N,N-bis(2-hydroxyethyl)alkyl(C13-C15)amine) (Atmer 163) pro-rata to a 5-mg/kg migration into food (calculated from the percentage of impurities in the substance).

Compound/group	Pro rata migration to 5 mg/kg food
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
SUM	0.110

[redacted]

The business operators Clariant and Nouryon[16] did not carry out the analysis requested by EFSA to better characterise the impurities. Thus, not all peaks were identified [redacted]. In addition to the [redacted][17] impurities described above (LC-MS and GC-MS), the following [redacted] **impurities** were identified: [redacted][18]

3.2 | Toxicological data

All data received with the Mandate from the European Commission, assessed by EFSA in the previous application (in 2006 and 2014) and submitted by the four business operators Croda/Cargill (original applicant), Clariant, Nouryon and LyondellBasell in response to the call and to the additional data requested were assessed. The data submitted by RIVM on N,N-bis(2-hydroxyethyl)lauramide (FCM No. 923) were reviewed. However, being an amide, it does not support the assessment of the substances FCM No. 19 and 20 and was not reported in the present assessment.

To derive a starting point from the critical dose–response toxicological data, a benchmark dose (BMD) approach was applied, following the criteria reported in the 2022 EFSA guidance on BMD modelling (EFSA Scientific Committee, 2022). In case the data sets were not suitable for BMD modelling, i.e. a clear dose–response trend was not observed and significant responses were only observed at the high dose, the traditional no observed adverse effect level (NOAEL) approach was applied. The standard benchmark response (BMR) of 10% for quantal data was considered as default, in specific cases, a different BMR was selected and justified.

3.2.1 | N,N-Bis(2-hydroxyethyl)alkyl(C13-C15)amine[19]

In 2006, the EFSA FCM WG of the Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) evaluated three in vitro genotoxicity studies with N,N-bis(2-hydroxyethyl)alkyl(C13-C15)amine (Atmer 163). Based on the results obtained in the bacterial reverse mutation test (Ames test), in a gene mutation assay in mouse

[16] LyondellBasell too, however, they use the same Atmer 163 substance that is covered by the data submitted by Croda/Cargil.

[17] [redacted]

[18] [redacted].

[19] Call for data/Croda/Technical dossier/Section 8 and related annexes; Additional data/Croda-Cargill/Technical dossier/Section 8 and related annexes.

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lymphoma L5178Y cells and in a chromosomal aberration test in human lymphocytes, the FCM WG concluded that the results did not indicate a mutagenic or chromosomal damaging potency for the test item. The same studies were reviewed by the EFSA FCM WG of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) in 2014 which concluded that the studies provided convincing evidence of the lack of induction of gene mutations and chromosome aberrations by N,N-bis(2-hydroxyethyl)alkyl(C13-C15)amine, but noted the lack of adequate information on numerical chromosome aberrations. These same studies were resubmitted by Croda and re-assessed under Sections 3.2.1.1, 3.2.1.2 and 3.2.1.3. In addition, a new in vitro micronucleus test (Section 3.2.1.4) was submitted upon request from EFSA to complete the dataset and address the numerical chromosome aberrations.

The subchronic oral toxicity of the substance N,N-Bis(2-hydroxyethyl)alkyl(C13-C15)amine (PM REF No 39090 or 39,120-HCl; Atmer 163) was evaluated by the SCF-FCM WG in 1996 and reviewed by the EFSA FCM WG in 2006 and in 2014. These subchronic oral toxicity studies in dogs and in rats are the same as those provided by Croda, hence reported under paragraphs 3.2.1.5 and 3.2.1.6 of this Opinion.

3.2.1.1 | *Gene mutation in bacteria*

N,N-bis(2-hydroxyethyl)alkyl(C13-C15)amine (Atmer 163, batch No [redacted], purity [redacted]%, CAS No not specified) was tested in a bacterial reverse mutation test (Ames test) with four Salmonella Typhimurium (TA98, TA100, TA1535, TA1537) and one *Escherichia coli* (WP2*uvrA* pKM101) tester strains using the plate incorporation procedure, in the presence and absence of liver S9 obtained from phenobarbital/β-naphtoflavone induced rats. The study was performed in 2005, in compliance with good laboratory practices (GLP) principles and following the OECD TG 471 (OECD, 1997a). [redacted] No dose-related reproducible increase in revertant colonies was observed at any dose and in any strain, either with or without S9 mix.

The Panel considered this study as reliable without restrictions, and the negative results obtained of high relevance.[20]

3.2.1.2 | *In vitro mammalian cell gene mutation test*

N,N-bis(2-hydroxyethyl)alkyl(C13-C15)amine (Atmer 163, batch No [redacted], purity [redacted]%, CAS No not specified) was tested in a gene mutation test in mouse L5178Y lymphoma $TK^{+/-}$ cells, with and without metabolic activation with liver S9 obtained from phenobarbital/β-naphtoflavone induced rats. The study was performed in 2005, in compliance with GLP principles and following the OECD TG 476 (OECD, 1997b). Based on the results obtained in a preliminary experiment, the test item was tested in two main assays in the concentration ranges 1–20 μg/mL in the presence of S9 metabolic activation, and 1–10 μg/mL in the absence of S9 mix. [redacted] Treatments induced dose-related toxicity, with about 10% of survival at the maximum tested doses, with and without S9 mix. No reproducible or statistically significant increases in mutant frequency were observed in cultures treated with the test substance at any dose, either in the presence or in the absence of S9 mix.

The Panel noted that the study protocol did not include an extended treatment in the absence of metabolic activation. This protocol followed the OECD TG in force at the date of the study and was acceptable as based on the more recent dedicated OECD TG 490 (OECD, 2016a), where longer treatment is only considered case by case. Therefore, the study is considered reliable without restrictions, and the results obtained of high relevance.

3.2.1.3 | *In vitro mammalian chromosome aberration test*

N,N-bis(2-hydroxyethyl)alkyl(C13-C15)amine (Atmer 163, batch No [redacted], purity [redacted]%, CAS No not specified) was evaluated for its clastogenic potential in an in vitro chromosomal aberration test using pooled human lymphocytes from two male and two female donors. The study was conducted in 2005, in compliance with GLP principles and following the OECD TG 473 (OECD, 1997c). Two separate experiments were performed both with and without metabolic activation with liver S9 from phenobarbital/β-naphtoflavone-induced rats. [redacted]. A small but statistically significant increase in the percentage of cells with structural chromosome aberrations (breaks and fragments/minutes) was only observed in experiment 2 at

[20]Reliability and relevance of genotoxicity studies evaluated according to the EFSA SC criteria reported in Annex I.

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the highest dose in the absence of metabolic activation. The percentage of aberrant cells (4.5%) was outside the historical control range for experiments in the absence of S9-mix (0.0%–4.0%) but within the historical control range for experiments in the presence of S9-mix (0.0%–5.5%); moreover, the increase was associated with an ~ 55% reduction of mitotic index (the maximum acceptable in OECD TG 473 (OECD, 1997c)) and, according to the TG, positive results observed only at the end of the cytotoxicity range should be interpreted with caution. Overall, the results of the study are evaluated as negative in the presence of S9, and equivocal in the absence of S9.

The study is considered reliable without restrictions and the results of limited relevance for the structural chromosomal aberration. However, the Panel noted that polyploidy and numerical aberrations were not recorded in this study, which therefore did not address the aneugenic potential of the test item.

3.2.1.4 | *In vitro micronucleus test*

N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine (Atmer 163, batch No [redacted], purity > [redacted]%) was tested in the in vitro micronucleus assay in human peripheral blood lymphocytes. The test was carried out in 2024 following the OECD TG 487 (2016 corr. 2023 (OECD, 2023)) and in compliance with GLP principles. [redacted] No increase in binucleated cells containing micronuclei was observed in treated cultures compared to vehicle controls under any experimental condition. A clear-cut and statistically significant response was induced by the positive control substances.

The results of the study were considered negative, i.e. the test item did not induce the formation of micronuclei under the conditions of the study. The Panel considered the study reliable without restrictions and the results of high relevance.

Intermediate conclusion on the genotoxic potential: N,N-bis(2-hydroxyethyl)alkyl (C13/C15)amine (Atmer 163) was tested with negative results in reliable in vitro tests for the induction of gene mutations in bacteria and in mammalian cells. In an in vitro test for the induction of structural chromosomal aberrations, the results were negative in the presence of metabolic activation and equivocal without metabolic activation. Negative results of high relevance were obtained in an in vitro micronucleus test in human lymphocytes. Based on these data, the Panel concluded that N,N-bis(2-hydroxyethyl) alkyl (C13/C15)amine does not raise concern for genotoxicity.

3.2.1.5 | *Repeated dose 90-day oral toxicity study in dogs*

Atmer 163 (batch No, purity and CAS No not stated) was tested in a 13-week subchronic toxicity study in Beagle dogs. The study was performed in 1991, in compliance with GLP principles and following the US Food and Drug Administration (FDA) Toxicological Principles for the Safety Assessment of Direct Food Additives and Colour Additives Used in Food. Animals (four/sex/group) were treated via capsule at the dose levels of 0 (control), 15, 30, and 100 mg/kg bw per day. [redacted]

Based on clinical signs (i.e. salivation and emesis) in both sexes and liver histopathological changes, correlated with increased ALT values in females, starting from 100 mg/kg bw per day, the Panel identified a NOAEL of 30 mg Atmer 163/kg bw per day.

3.2.1.6 | *Repeated dose 90-day oral toxicity study in Sprague Dawley rats*

Atmer 163 (batch No, purity and CAS No not stated) was tested in a 90-day subchronic toxicity study in Sprague Dawley rats. The study was performed in 1991 in compliance with GLP principles and following the FDA Toxicological Principles for the Safety Assessment of Direct Food additives and Colour Additives Used in Food. Animals (20/sex/group) were treated by gavage at the dose levels of 0 (control, deionised water), 15, 30 and 150 mg/kg bw per day. Two males died in the mid-dose group and four males and 1 female died in the high-dose group. [redacted]

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[redacted] Ophthalmological findings, likely referable to the administration of the test material, were noted, starting from the mid-dose with a higher incidence than those observed at the low-dose and related to microscopic cataracts only in the high-dose male and female groups.

[redacted]

Dose-related severe ophthalmological effects, macroscopic and histopathological findings after necropsy, were observed starting from 30 mg/kg bw per day. For ophthalmological effects (i.e. chromodacryorrhoea, retinal atrophy, subcapsular cataract), the lower confidence limit of the benchmark dose (BMDL) corresponding to 10% response ($BMDL_{10}$) estimate in males was 27 mg Atmer 163/kg bw per day. At non-glandular stomach level, for gross findings (thickened mucosa), the $BMDL_{10}$ was estimated at 24 and 22 mg/kg bw per day (males and females, respectively) and for histopathological findings (acanthosis) at 20 and 23 mg/kg bw per day (males and females, respectively).

3.2.1.7 | *Prenatal developmental toxicity study in Han Wistar rats*

The test substance Atmer 163 (CAS No not stated; batch No [redacted]; purity: [redacted]%) was orally administered to pregnant Han Wistar rats (RccHanTM: WIST(SPF)) in a study conducted in 2014 in compliance with GLP principles and following the OECD TG 414 (OECD, 2001). Maternal toxicity and the development of the embryo and fetus were examined. Twenty-two mated females per group were treated by gavage with the test substance at doses of 10, 30 and 90 mg/kg bw per day from day 6 to day 20 post coitum. The dose selection was determined in a dose-range finding prenatal developmental toxicity study. A control group received the vehicle polyethylene glycol (PEG) 300 alone. All females were sacrificed on day 21 p. c. and the fetuses were removed by Caesarean section.

Maternal toxicity: No mortality occurred until the scheduled necropsy and no test substance-related clinical signs were noted. Food consumption of the high-dose group was decreased following the start of dosing until necropsy on day 21 ([redacted] statistically significant from days 12–15 p.c. onwards). This decrease in food consumption was accompanied by a statistically significant decrease in mean body weight and body weight gain in the high-dose group. Corrected body weight gain (corrected for the weight of the gravid uterus) was reduced without statistical significance (mean + 6.6% compared to +10.7% in the control group). A statistically significant increase in post-implantation loss (30.2% of implantation sites compared to 2.6% in the control group) was recorded only in the high-dose group, which resulted in a significant decrease in the mean number of fetuses per dam in comparison to control animals. Macroscopic examination of the females did not reveal any abnormalities.

Developmental toxicity: No test item-related effects on the sex ratio of the fetuses were noted. The mean body weights of the fetuses on the litter basis were not affected, while there was a slight, dose-related reduction of the weights on an individual basis. A higher incidence of fetuses with external abnormalities (5 fetuses in 4 litters) was observed in the high-dose group: All five fetuses were affected with abnormalities of the head (including slightly misshapen head, no skin over the head, missing eyes and nasal opening, cleft lip and clear membrane over part of the head). No abnormal findings were found in the controls and in the mid-dose groups. In the low-dose group, a shortened lower jaw and a closed mouth were observed. As no such findings were reported in higher dose groups, these effects were not considered of toxicological relevance.

Incidences of visceral abnormalities in the fetuses were not considered test substance- related due to only isolated findings (small eye, dilatation of the lateral ventricle of the brain, anal atresia). As a potential variation, the higher incidence of malpositioned testis in the high-dose group was considered by the authors to be a treatment-related effect. A dose-dependent increase in the altered texture of the cut surface of the eye lens was reported (normally considered to be a process artefact). According to the authors, this effect could be considered a possible effect of the test substance due to the especially high incidences in the mid- and high-dose groups.

During skeletal examinations, increased incidence of abnormalities (cervical vertebra, cranial bone) was observed in 1, 2, 3 and 7 litters in controls, the low-, mid- and high-dose group (1 fetus in each affected litter), respectively.

Variations of the ribs (cervical rib and wavy rib) as well as fused costal cartilages were outside the range of the historical control data in the high-dose group. All remaining findings were considered isolated, not observed in a dose-dependent pattern or were within the range of the historical control data. There was a statistically significant increase in the incidence of incomplete ossification in the cranium as well as supernumerary rudimentary ribs in the high-dose group on litter basis. These increased incidences were considered to be test substance-related. Additional cartilage variations included

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a statistically significant increase in the incidence of a long ventral plate in the high-dose group on both a litter and a fetus basis. The incidence was outside the range of the historical control data and was, therefore, considered to be a test substance-related effect.

In conclusion, based on to the apparent decreases observed only in the high-dose group in both the food consumption and body weight gain, and the accompanied increase in post-implantation loss with an associated decrease in the number of fetuses per litter, a NOAEL for maternal toxicity of 30 mg/kg bw per day was identified.

Based on the historical control data on the observed skeletal abnormalities in fetuses (i.e. cervical vertebrae), the Panel considered a BMR of 5% as suitable for BMD modelling of these developmental effects present in the mid- and high-dose groups. The Panel estimated a $BMDL_5$ for developmental toxicity of 12 mg/kg bw per day.

3.2.1.8 | *Prenatal developmental toxicity study in Sprague Dawley rats*

The effects of the test substance Atmer 163-LQ-(AP) (Lot No [redacted]; CAS No not stated; [redacted]) on the pregnant rat and development of the embryo and fetus was examined in a GLP-Guideline study conducted in 2015 according to OECD TG 414 (OECD, 2001). Twenty-five mated female Sprague Dawley (Crl:CD(SD)) rats were randomly assigned to the control and the treatment groups and were fed a dietary formulation containing the test substance at doses of 0, 10, 30 and 90 mg/kg bw per day from day 0 to day 20 post coitum. The dose selection was based on the results of previous studies.

Maternal toxicity: All females survived to the scheduled necropsy on gestational day (GD) 20. Test substance-related increased incidences of red material around the nose and/or mouth were noted at all doses in a dose-related manner compared to the control group. However, these findings were not considered adverse by the Panel.

A test substance-related mean body weight loss and a lower mean food consumption were observed in the high-dose group during GDs 0–3. Mean food consumption in this group remained lower during GDs 3–6 but was generally similar to the control group for the remainder of the exposure period. Mean body weight gains in the high-dose group were similar to the control group during GD 3–6 and 6–9, but lower mean body weight gains were noted during GD 9–12 (–20%). Due to the aforementioned decrements, mean body weight gain [redacted] and food consumption [redacted] in this group were lower than in the control group when the overall exposure period (GD 0–20) was evaluated. As a result, mean body weights in the high-dose group were lower (up to 5.1%) than in the control group during GD 2–20. Mean net body weight and net body weight gain in this group were lower than the control group.

Mean gravid uterine weight in all dose groups was unaffected by test substance exposure. There were no test substance-related effects on mean body weights, body weight gains, food consumption, net body weights, net body weight gains in the low- and mid-dose groups. There were no test substance-related macroscopic findings noted for the dams at any exposure level. [redacted]

Developmental toxicity: An increase in a test substance-related external malformation (fetal oedema; localised to the neck and/or thorax) was noted in the 90 mg/kg bw per day (high dose) group (4 fetuses in 2 litters) compared to controls (1 fetus in 1 litter); the increase in affected litters was not statistically significant. However, the Panel noted that according to the authors *'an increased occurrence of fetal edema/anasarca was noted for fetuses in a previous embryo/fetal development range-finding study at 250 mg/kg/day (providing a mean of 193 mg/kg/day)'*. [redacted]

The Panel considered that, based on the lower mean body weight gains at 90 mg/kg bw per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day. Based on the increase in fetal external malformations at 90 mg/kg bw per day, the NOAEL for embryo/fetal development in this study was 30 mg/kg bw per day.

3.2.1.9 | *Potential for accumulation in humans*

The systemic effects observed in the repeated dose studies show that the substance is absorbed and distributed to various tissues of the body. The Panel considered complete absorption for the substance as the worst case.

No experimental data were provided on the potential for accumulation of N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine (Atmer 163) in humans. Instead, Croda provided in silico model predictions and information of the possible metabolism pathways of the shortest and longest linear alky chain of the FCM substance No 19 (C8 and C18 derivatives; CAS No 15520-05-5 and CAS No 10213-78-2, respectively).

Concerning the metabolism, information on the chemical structure and the results from an in silico model provided [redacted] as well as from a computational model [redacted]

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[redacted]

Based on the in silico predictions provided (albeit with some limitations related to the intrinsic characteristics and precision of the models), the Panel concluded that the entire category of N,N-bis(2-hydroxyethyl)alkylamine can be metabolised into molecules that enter well-known metabolic pathways, thus does not give rise to concern for accumulation in humans.

3.2.2 | N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine[21]

3.2.2.1 | *Gene mutation in bacteria*

N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine (Hostastat FA 68 V, batch No CNP1015848, purity [redacted]%, CAS No 1218787-30-4) was tested in a bacterial reverse mutation test (Ames test) with the Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 *uvrA* pKM101, in the presence and absence of a metabolic activation system (liver S9 from phenobarbital/b-naphtoflavone-induced rats). The study was conducted in 2018, in compliance with GLP and following the OECD TG 471 (OECD, 1997a). Two experiments were performed. The first experiment was conducted using the plate incorporation method, assaying eight concentrations of the test item dissolved in DMSO in the range 3.16–5000 μg/plate (six in the range 3.16–1000 μg/plate for TA1535 and TA1537 without S9). The second experiment was performed with the pre-incubation method, assaying eight concentrations in the ranges 0.316–1000 μg/plate without S9 and 3.16–5000 μg/plate with S9. All concentrations and controls were tested with triplicate plates. Toxicity, as reduction of background lawn and/or decrease in revertant counts, was observed in the first experiment at 100 μg/plate and above without S9 and at 316 μg/plate and above in the presence of S9. In the preincubation experiment, toxicity was observed starting from 3.16 to 100 μg/plate (depending on the strain) without S9 and from 316 μg/plate with S9. In both experiments, no increase in revertant colonies was observed at any dose, either with or without metabolic activation.

The Panel considered the study reliable without restrictions, and the negative results reported highly relevant.

3.2.2.2 | *Repeated dose 90-day oral toxicity study in Wistar rats with 28-day recovery period*

N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine (Hostastat FA 68 V Solid, CAS No 1218787–30-4; batch No CNP1028238; [redacted] purity not reported) was tested in a repeated dose 90-day oral toxicity study with a 28-day recovery period in Wistar rats. The study was performed in 2023 in compliance with GLP principles and following the OECD TG 408 (OECD, 2018a). Three groups of animals (10/sex/group) received the test material by gavage at doses of 40, 160 or 320/240 mg/kg bw per day (the high dose was decreased from day 16, due to a significant reduction in body weight and food consumption). An additional group (five/sex/group) was treated by gavage at the high-dose and sacrificed after a recovery period (28 days). In this dose group, urine collection and quantification of the main metabolite (DEA) were also performed. Similarly constituted control groups received the vehicle (corn oil).

No mortalities were observed during the treatment at all dose levels.

No clinical signs were evidenced in the low-dose group. Slight and transient (few minutes) salivation was observed immediately following the treatment in the mid- and high-dose group. No ocular abnormalities were observed in the ophthalmological examination. In a functional observation battery, some changes in motor activity parameters (i.e. ambulatory time, resting time, distance) were considered incidental, with no changes in the home cage or open field observations. Significantly lower hindlimb grip strength was seen in the high-dose group females.

A transient body weight loss was observed in males and females treated with 320 mg/kg bw per day, fully recovered after lowering the dose to 240 mg/kg bw per day. A reduction in body weight was observed at the mid-dose group in males only at the end of treatment. A reduction in food consumption were observed in all dose groups, not significantly affecting body weights and body weight gains.

Haematological examination revealed a reversible increase in neutrophil count (94%–126%) in the mid- (only females) and high- (males and females) dose groups. A reversible increase in total leucocyte count (27%–38% in females; 13%–16% in males) and in reticulocytes (45% in females and 4%–12% in males) was noticed at doses ≥ 160 mg/kg bw per day.

Biochemical changes in the blood plasma in the mid- and high-dose groups of both sexes included: lower total cholesterol (–13% and –9% in males; –27% and –30% in females), high-density lipoprotein (HDL) cholesterol (–20% and –22% in males; –29% and –35% in females), total protein (–6% and –7% in males; –6% and –13% in females) and globulin (–12% and –15% in males; –12% and –23% in females). An increase (27% and 61%) in males and a decrease (–25% and –28%) in females of low-density lipoprotein (LDL) cholesterol in the mid- and high-dose groups were also reported.

[21]Call for data/Clariant/Technical dossier (Hostastat FA 68V)/Section 8 and related annexes; Additional data/Clariant/Answer and related annexes.

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In males, at 320/240 mg/kg bw per day, a decrease in body weight-adjusted seminal vesicles with coagulating glands weight (–15%) was observed. The lower organ weights in epididymis, heart, kidneys, liver, prostate, thyroid with parathyroid and thymus were related to the decreased body weights. These findings were not associated with microscopic changes in these organs. The absolute kidney, liver and seminal vesicles coagulating gland weights were lower at 160 mg/kg bw per day. An increase in body weight-adjusted liver (9%) and kidney (12%, also noted at the lower dose) weights and a decrease in body weight-adjusted thymus weight (–20%) were reported at 320/240 mg/kg bw per day females without any microscopic changes. All the weight changes (except for kidney in the high-dose group females), including terminal body weights, were reversible at the end of non-dosing period.

In males, macroscopic necropsy revealed non-glandular stomach lesions (thickening) at all doses, which, upon microscopic examination, were associated with non-glandular epithelial hyperplasia, inflammation in submucosa of non-glandular stomach and mucosal erosion.

In females, thickening was observed only at doses starting at 160 mg/kg bw per day, which was associated with epithelial hyperplasia in 1, 7 and 6 animals of 10 of the low-, middle- and high-dose groups, respectively.

Both the gross and microscopic findings were partially reversible in males (1/5 animals affected) and completely reversible in females at the end of non-dosing period.

No alterations in stages of oestrous cycle were recorded at necropsy.

In the mid-dose and above, in both males and females, an increased number of foamy macrophages was observed in the *lamina propria* of small intestinal segments, duodenum, jejunum and ileum. These foamy activated macrophages were also present in mesenteric lymph nodes. Decreased lymphoid cellularity was noted at doses ≥ 160 mg/kg bw per day in males and at the high-dose group females. In lungs, an increased number of alveolar macrophages were noted in both sexes (7 and 4 out of 10 males and females, respectively). These findings were reversible in duodenum and lungs and were not fully reversible in jejunum, ileum and mesenteric lymph nodes.

There were no other test item-related changes at the low dose, except for the local effect at the first site of contact, in the non-glandular stomach (epithelial hyperplasia and in males only submucosal inflammation of minimal and mild degree), mainly related to the irritant properties of the test item. For the endpoints thickening (focal, multifocal) and epithelial hyperplasia, only the data in females allowed to perform a BMD analysis. For these two endpoints, $BMDL_{10}$ estimates were 44 and 20 mg/kg bw per day, respectively.

3.2.2.3 | *Urinary excretion of the metabolite diethanolamine after repeated 90-day treatment by oral in Wistar rats and bioaccumulation potential*

In the same repeated dose 90-day oral toxicity study with a 28-day recovery period, urine samples were collected during 12 h from the control and high-dose recovery groups (320/240 mg/kg bw per day; males and females) on day 90 (last day of treatment) and 2 and 5 days, 1 week, 2 and 4 weeks after last day of treatment. The metabolite DEA was extracted from urine samples by protein precipitation and measured by LC–MS/MS (limit of detection (LOD) = 0.1 mg/L).

The highest levels of DEA (17 and 9 mg/L, in males and females, respectively) were measured in urine samples of the last day of treatment (day 90). A decrease was observed after termination of the treatment, down to values around the LOD after 5 and 27 days of the recovery period in females and males, respectively. The results indicated that DEA was completely cleared during the 4-week recovery period, with a faster elimination in females than males.

Based on two other studies (Kaling, 2017; Lee et al., 2017) provided by Clariant on rats orally exposed up to 150 mg/kg bw per day for 7 days to the structurally related substance N,N-bis(2-hydroxylethyl)alkyl(C12-C18)amine, the in vivo formation of monoethanolamine (MEA) and DEA was described as a potential metabolic pathway also for the substance N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine. In these studies, urinary DEA concentrations on study day 9 (i.e. 2 days after the end of treatment) were 12 and 2.5 mg/L in males and females, respectively, which supports the observation from the 90-day study.

A steady state of systemic bioavailability of DEA and urinary elimination within 8 weeks and an elimination half-life of 1 week have been reported in the literature, in rats after daily oral exposure to DEA for 8 weeks (Mathews et al., 1997).

Although other oxidative metabolic pathways with the formation of additional metabolites (e.g. urea) can be foreseen for fatty amine ethoxylates, the Panel considered the cleavage of the C-N bond as one of the relevant in vivo metabolic reactions. Indeed, the substance may undergo dealkylation to form DEA and a primary alcohol (further metabolised to a fatty acid); then DEA may be further metabolised to MEA.

Overall, based on these findings, it is assumed that during the time course of the 90-day repeated treatment with the substance N,N-bis(2-hydroxyethyl)alkyl(C16-C18)amine, a steady state for DEA formation/elimination was reached. DEA is almost completely eliminated (> 90%) during the 4-week recovery period, indicating that no new DEA is formed from the potentially bioaccumulated parental substance.

Because of the limited available information on the metabolic pathway of the substance, a robust conclusion on the fate of other potential metabolites could not be reached. However, the Panel considered the accumulation of the parent substance N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine in humans to be unlikely.

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3.2.3 | N,N-bis(2-hydroxyethyl)alkyl(C8)amine[22]

3.2.3.1 | *Gene mutation in bacteria*

2,2′-(Octylimino)diethanol, i.e. N,N-bis(2-hydroxyethyl)octylamine (Genamin 3920, batch No [redacted], purity [redacted], CAS No 15520-05-5) was tested in a bacterial reverse mutation test (Ames test) with the Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102, in the presence and absence of a metabolic activation system (liver S9 from phenobarbital/β-naphtoflavone induced rats). The study was conducted in 2012, in compliance with GLP principles and following the OECD TG 471 (OECD, 1997a). Two experiments were performed. The first experiment was conducted using the plate incorporation method, assaying eight concentrations of the test item dissolved in DMSO in the range 3.16–5000 μg/plate. The second experiment was performed with the pre-incubation method, assaying eight (nine with S9 mix) concentrations in the ranges of 1–2500 μg/plate without S9 and of 1–5000 μg/plate with S9 mix. All concentrations and controls were tested with triplicate plates. Toxicity, assessed as reduction of background lawn and/or decrease in revertant counts, was observed in the first experiment at 1000 μg/plate and above without S9 and at 2500 μg/plate and above in the presence of S9 mix. In the preincubation experiment, toxicity was observed starting from 31.6 to 316 μg/plate (depending on the strain) without S9 and from 1000 μg/plate with S9. In both experiments, no increase in revertant colonies was observed at any dose, either with or without metabolic activation.

The Panel considered the study reliable without restrictions, and the negative results reported highly relevant.

3.2.3.2 | In vitro *mammalian cell gene mutation test*

2,2′-(Octylimino)diethanol, i.e. N,N-bis(2-hydroxyethyl)octylamine (Genamin 3920, batch No [redacted], purity [redacted], CAS No 15520-05-5) was tested in an in vitro mammalian cell gene mutation assay at the *hprt* locus in V79 Chinese hamster lung fibroblasts, with and without metabolic activation (liver S9 from phenobarbital/β-naphtoflavone induced rats). The study was conducted in 2012, in compliance with GLP principles and following the OECD TG 476 (OECD, 1997b). The substance was dissolved in culture medium (MEM + 0% FBS 4h treatment; MEM + 10% FBS 20h treatment) and tested over a dose range determined based on the indications of a preliminary toxicity test, in order to reach the target toxicity level (10%–20% relative survival according to TG 473). In the first experiment, with 4 h exposure, 10 concentrations in the range 0.025–2.5 mM and 0.05–1.75 mM were tested with and without S9, respectively. In the second experiment, 10 concentrations in the range 0.3–4.0 mM (limit of solubility) with 4 h exposure in the presence of S9, and 10 concentrations in the range 0.05–0.7 mM for 20 h in the absence of S9. Treatments resulted in dose-related growth inhibition, up to ~80% in the first experiment and ~ 90% in the second assay, both with and without S9. In both experiments, there was no statistically significant or dose-related increase in mutant frequency.

The Panel considered the study reliable without restrictions, and the negative results reported highly relevant.

3.2.3.3 | *In vivo micronucleus test*

2,2′-(Octylimino)diethanol, i.e. N,N-bis(2-hydroxyethyl)octylamine (Genamin 3920, batch No [redacted], purity [redacted], CAS No 15520-05-5) was tested in an in vivo micronucleus test in mouse erythropoietic cells. The study was conducted in 2013, in compliance with GLP and following the OECD TG 474 (OECD, 1997d). The substance was dissolved in cottonseed oil and administered by single intraperitoneal injection to groups of five male and five female NMRI mice. Negative control animals received the vehicle only (10 mL/kg bw). Animals were sampled for peripheral blood 44 h (all doses) and 68 h (high dose only) after administration, together with the corresponding control groups. The dose range applied included the maximum tolerated dose (MTD, 100 mg/kg bw), determined in a preliminary toxicity test, and two lower doses corresponding to 0.5 and 0.2 MTD (50 and 20 mg/kg bw). The incidence of micronucleated polychromatic erythrocytes (PCE) in blood was determined by flow cytometry, analysing 10,000 PCE per animal. In the main experiment, administration of the MTD was associated with signs of systemic toxicity such as reduction of spontaneous activity, constricted abdomen, half eyelid closure and tremor in both sexes. Slight and/or no signs of systemic toxicity were observed at the lower doses. The Panel noted that the clear signs of systemic toxicity elicited by the high-dose treatment can be considered as a line of evidence of systemic exposure to the test agent (EFSA Scientific Committee, 2017). The relative PCE ratio (proportion of PCE among total erythrocytes) showed some variations compared to negative control animals, but such differences were not statistically significant and within the historical control ranges. The incidences of micronucleated PCE in male and female mice treated with 0.2 and 0.5 MTD, and females treated with 1 MTD, were within the range of the corresponding negative controls at both sampling times. In male mice treated with the MTD, the mean incidence of micronucleated PCE was slightly elevated at 44 h (0.27 ± 0.06 vs. 0.19 ± 0.09), but the difference was not statistically significant and within the range of the corresponding negative control values; at the 68 h harvest time, the incidence of micronucleated PCE in treated mice was statistically significantly higher ($p < 0.05$, Mann–Whitney test) than in the corresponding negative controls (0.28 ± 0.04 vs. 0.19 ± 0.05), even though within the spontaneous historical control range (0.08%–0.34%). According to the evaluation criteria for the micronucleus test stipulated in the OECD TG 474 (OECD, 2016b), the results obtained in the

[22]Call for data/Clariant/Technical dossier (Genamin 3920)/Section 8 and related annexes; Additional data/Clariant/Answer and related annexes.

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high-dose males are evaluated as equivocal. However, the Panel noted that a parallel increase in micronucleated PCE was not observed in the group of female mice: based on the lack of gender specific susceptibility highlighted in the toxicity experiment, this group could be considered as a replicate of the experimental group of males, indicating that the effect observed in the high-dose male group is not reproducible and incidental.

The Panel also noted that the intraperitoneal route of administration used in the study is not recommended in the more recent OECD TG 474 (OECD, 2016b) and considered the study reliable with restrictions and the results of limited relevance.

3.2.3.4 | *Repeated dose 28-day oral toxicity study in Wistar rats, including 14-day recovery period*

Genamin 3920 (batch No [redacted]; [redacted]; CAS No 15520-05-5 as in the dose Formulation Analysis) was tested in a 28-day subacute oral toxicity study in Wistar rats. The study was performed in 2015, in compliance with GLP principles and following the OECD TG 407 (OECD, 2008). Animals (10/sex/group) received the test item, dissolved in water, by gavage at the dose levels of 0 (control), 20, 100 and 500 mg/kg bw per day. Five additional animals per sex of the control and high-dose groups were subjected to necropsy 14 days after the last administration.

In the high-dose group, 5 of 10 female rats died. Mild to moderate clinical signs, including behaviour of moving the nose through the bedding, salivation and piloerection (mid- and high-dose group), were noted in a dose-dependent manner, likely due to the irritant properties of the test item. Slight, but not statistically significant changes in body weight, body weight gain and daily food consumption were observed in the mid- and high-dose groups, reversible after the 14-day recovery period. No ophthalmological findings were noted. Results from the functional observational battery (body temperature, rearing supported, rearing not supported, urination, defecation) did not indicate any effects of neurotoxicological relevance.

Haematological, biochemistry and urinary parameters were not affected by the treatment. [redacted]

In males, a slight decrease in absolute thymus weight was noted in the mid-group [redacted], which reached statistical significance in the high-dose group [redacted]. In females, relative uterus weight was moderately, but not statistically significantly decreased at 500 mg/kg bw per day at the end of the treatment [redacted] and of the recovery period [redacted]. Due to the low number of surviving animals, a relation to the test item cannot be excluded. [redacted]

For the five female rats (high-dose group) found dead, local necrotic lesions in glandular stomach and oesophagus were reported. This was also observed in trachea and lungs, and is likely related to accidental regurgitation/aspiration of the test item.

Necrotising inflammation in the non-glandular stomach was recorded. Atrophy of spleen and thymus and adrenocortical diffuse hypertrophy were observed at the high-dose level (males and females). These lesions showed a tendency to recover after 14 days.

Based on mortality, clinical and histopathological findings after necropsy observed in the high-dose group, mainly related to the irritant properties of the test item, the Panel identified a NOAEL of 100 mg/kg bw per day after 28-day repeated oral administration of Genamin 3920 in Wistar rats.

3.2.3.5 | *Reproduction/developmental toxicity screening test in Wistar rats*

To evaluate the effects of the test substance, a reproduction/developmental toxicity screening test was performed in Wistar rats. The study was conducted in 2014 in compliance with GLP principles and following the OECD TG 421 (OECD, 1995d).

The test substance (Genamin 3920, batch No [redacted]; 2,2′-(octylimino)diethanol, i.e. N,N-bis(2-hydroxyethyl)octylamine (CAS No 15520–05-5); [redacted]) was administered daily by gavage in doses of 0, 20, 80 and 320 mg/kg bw per day for 54 days, i.e. during 14 days of pre-mating and a maximum 14 days of mating in both males and females, during the gestation period and up to post-natal day 3 in females. Males were dosed after the mating period until the minimum total dosing period of 28 days was completed. Water for injection was used as vehicle in this study. The test substance formulation was prepared freshly on each day of administration. The four groups comprised 10 male and 10 female Wistar rats (Crl: WI(Han)).

No treatment-related mortality occurred during the study. However, one female treated at 20 mg/kg bw per day was found dead prematurely on premating day 5 (considered to be due to accidental influx (regurgitation or aspiration) into the respiratory tract). There were clinical signs including salivation, piloerection and slight to severe abnormal breathing in most animals in the high-dose group. These clinical signs were considered to be due to discomfort or caused by the local irritative effects due to the physicochemical character of the test substance. Decreases in body weight [redacted]

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[redacted], body weight gain [redacted] and food consumption [redacted]) were considered treatment-related. However, the statistical significance of these effects was only observed transiently.

There were no statistically significant changes between the control and dose groups for litter data, including total number of pups born, still birth and runts on post-natal day 0 and number of live pups, number of male and female pups and sex ratio on post-natal day 0 and post-natal day 4. There were also no statistically significant treatment-related changes for litter weight, including pup mean weight, total litter weight and male and female litter weight on post-natal day 0 and 4. In addition, no treatment-related changes were observed for the duration of precoital and GDs in the treated groups. No adverse changes were reported for pre- and post-natal data, including number of corpora lutea, number of implantation sites and number of live pups (post-natal day 0 and 4). It was noted that there were dose-dependent increases in pre-implantation [redacted] and post-implantation [redacted]. Although rather large, these increases were not statistically significant and the mean values were within the historical control data range. No statistically significant treatment-related changes were observed for reproductive and viability indices.

There was no effect of toxicological relevance on the survival of the pups from post-natal day 0 to post-natal day 4 in any treatment group. The few incidences of external findings in pups (e.g. injuries on head or abdomen) were considered to be spontaneous and not treatment-related.

Macroscopic findings in males and females did not show treatment-related effects.

[redacted] All microscopic findings recorded in the survivors were within the range of normal background lesions that may be recorded in animals of this strain and age. There were no adverse histological findings in the organs and tissues of the reproductive system, i.e. testes, epididymides, prostate, seminal vesicles, coagulating glands, ovaries, uterus and cervix and vagina. There were no treatment-related effects on the testicular histomorphology, including spermatogenesis and interstitial cell structure.

No treatment-related changes were observed in the reproductive organs of one female (mid-dose group) which was not pregnant.

In conclusion, the Panel considered that the tendency towards a reduced body weight gain and food intake in males and females of the high-dose groups was minimal or transient and, therefore, not adverse. In the absence of clear treatment-related adverse effects for reproductive toxicity parameters, the NOAEL for both systemic toxicity in adult animals and the reproductive/developmental toxicity was considered to be 320 mg/kg bw per day.

3.2.4 | N,N-bis(2-hydroxyethyl)alkyl(tallow C18)amine[23]

3.2.4.1 | In vivo *micronucleus test*

A sample (E-2352.01) of a 15% (C18) tallow amine ethoxylate solution in water [redacted] and orthophosphoric acid [redacted] ([redacted], purity [redacted]%, CAS No and batch No not specified) was tested in an in vivo micronucleus test in mouse bone marrow. The study was conducted in 1984 following the OECD TG 474 (OECD, 1983); a statement of compliance with the GLP criteria set forth in Titre 21 of US Federal Regulation was provided. The test substance was diluted in water and administered by single intragastric administration to groups of five male and five female CD-1 mice at the limit dose of 10,860 mg/kg bw, corresponding to the lethal dose 10% (LD_{10}) as determined in a preliminary lethality test. Negative control animals received the vehicle alone (distilled water). Animals were sacrificed 24, 48 and 72 h after administration, together with the corresponding control groups. The incidence of micronucleated PCE and normochromatic erythrocytes (NCE) in bone marrow was determined by scoring 1000 PCE and 1000 NCE per animal. In the main experiment, the administration of the test substance produced signs of bone marrow toxicity, with a significant reduction of the PCE/NCE ratio at 24 and 72 h ($p < 0.05$) and 48 h ($p < 0.001$). The incidence of micronucleated PCE in treated animals (males + females) was not statistically significantly different from untreated controls at 48 and 72 h. A slight, statistically significant ($p < 0.05$) increase of micronucleated PCE was observed at 24 h [redacted] No increase in micronucleated NCE was observed at any sampling time. The higher incidence of micronucleated PCE observed at the 24 h sampling time, although statistically significant, fell within the historical control range [redacted], with no individual animal exceeding the historical range (0–7 micronucleated PCE per thousand). Moreover, the Panel noted that a parallel significant increase in micronucleated PCE was not observed in the group of female mice [redacted]; based on the lack of gender-specific susceptibility highlighted in the toxicity experiment, this group could be considered as a replicate of the experimental group of males, indicating that the effect observed in the males was not reproducible and incidental. The Panel concluded that the study results did not indicate a biologically relevant clastogenic or aneugenic activity of the

[23]Call for data/Clariant/Technical dossier (Hostastat FA 68 V)/Section 8 and Annex 10.

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test material. The Panel also noted the low purity of the test material but considered that, under the experimental conditions of the study, a sufficiently high dose of [redacted] was administered (approx. 1170 mg/kg bw), eliciting systemic and bone marrow toxicity.

Overall, the Panel considered the study reliable with restrictions and the results of limited relevance.

Intermediate conclusion on the genotoxic potential of the assessed Clariant substances (N,N-bis(2-hydroxyethyl) alkyl(C16/C18, C8, C18)amine): N,N-bis(2-hydroxyethyl)alkyl(C8)amine (Genamin 3920) was negative in reliable in vitro assays for the induction of gene mutations in bacteria and in mammalian cells. Negative results were also obtained in a reliable gene mutation assay in bacteria with the C16/C18 diethanolamine (Hostatat FA 68 V). In vivo overall negative results were obtained with Genamin 3920 and the saturated C18 (tallow) diethanolamine in micronucleus assays with limited reliability. The Panel concluded that the available data on N,N-bis(2-hydroxyethyl)alkylamines with even C numbers (C16/C18 and C8) do not indicate genotoxic potential.

3.2.5 | PFAEO-coco[24]

3.2.5.1 | *Gene mutation in bacteria*

N,N-bis(2-hydroxyethyl)alkyl(coco)amine (CAS No 61791–31-9, [redacted] batch No [redacted], purity [redacted]%) was tested in a bacterial reverse mutation test (Ames test) with the Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 *uvrA*$^{-}$, in the presence and absence of a metabolic activation system (liver S9 from phenobarbital/β-naphtoflavone induced rats). The study was conducted in 2009, in compliance with GLP principles and following the OECD TG 471 (OECD, 1997a). Two experiments were performed with triplicate plates, assaying a dose range intended to cover at least four not toxic doses and the toxicity limit, as determined in a preliminary toxicity test. The first experiment was conducted using the plate incorporation method with 0.5, 1.5, 5, 15, 50, 150 and 500 μg/plate without metabolic activation, and 1.5, 5, 15, 50, 150, 500 and 1500 μg/plate with metabolic activation. The second experiment was performed using the pre-incubation method with 0.15, 0.5, 1.5, 5, 15, 50 and 150 μg/plate without metabolic activation, and 0.5, 1.5, 5, 15, 50, 150 and 500 μg/plate with metabolic activation. DMSO was used as solvent and vehicle control. Toxicity, as reduction of background lawn and/or decrease in revertant counts, was observed in the first experiment at 150–500 μg/plate, depending on the strain, and at 50 μg/plate and above in the second experiment. In both experiments, no increase in revertant colonies was observed at any dose, either with or without metabolic activation.

The Panel considered the study reliable without restrictions, and the negative results reported highly relevant.

3.2.5.2 | *In vitro mammalian cell gene mutation test*

N,N-bis(2-hydroxyethyl)alkyl(coco)amine (CAS No 61791-31-9, [redacted] batch No [redacted], purity [redacted]%) was tested in a gene mutation assay in mouse L5178Y lymphoma TK+/– cells in a fluctuation assay using microtitre plates, with and without metabolic activation with liver S9 obtained from phenobarbital/β-naphtoflavone-induced rats. The study was conducted in 2010, in compliance with GLP principles and following the OECD TG 476 (OECD, 1997b). The test item was tested in two assays, with 4 h exposure (in the first experiment with 2% S9 and without S9, and in the second experiment with 1% S9) and 20 h exposure (in the second experiment without S9). Six concentrations in following dose ranges were evaluated: in the first experiment 0.63–10 μg/mL without S9 and 2.5–25 μg/mL with S9; in the second experiment, five concentrations were tested in the ranges 0.31–5 μg/mL without S9 and 2.5–17.5 μg/mL with S9. Duplicate cultures were used for each dose and for solvent (DMSO) and positive controls. Treatments induced a steep increase in toxicity in a narrow concentration interval: relative total growth at the highest analysable concentration were [redacted]% and [redacted]% without S9 (in the first and second experiment, respectively) and [redacted]% and [redacted]% with S9, with the subsequent concentration, spaced less than a 1.5-fold factor, completely toxic. In both experiments, no increase in mutant frequency which was reproducible, or statistically significant, or above the Global Evaluation Factor, was observed, either in the presence or in the absence of S9-mix.

The Panel noted that the target toxicity level (10%–20% relative total growth was not reached in the first experiment, due to the steep onset of toxicity, but this was achieved in the repeat experiment. Overall, the study is considered reliable without restrictions, and the results obtained of high relevance.

3.2.5.3 | In vitro *mammalian chromosome aberration test*

N,N-bis(2-hydroxyethyl)alkyl(coco)amine (CAS No 61791–31-9, [redacted] batch [redacted], purity [redacted]%) was tested in a chromosome aberration test in human lymphocytes in vitro, with and without metabolic activation with liver S9 from phenobarbital/β-naphtoflavone-induced rats. The study was conducted in 2010 in compliance with GLP principles and following the OECD TG 473 (OECD, 1997c). The test item was tested in two assays, with 4 h exposure (in the first experiment with 2% S9 and without S9, and in the second experiment with 1% S9) and 24 h exposure (in the second experiment without S9). All cultures were harvested 24 h after treatment initiation. Based on the observed reduction in mitotic index, the

[24]Additional data/Nouryon/Technical dossier/Section 7 and related annexes.

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following doses were tested using duplicate cultures: in the first experiment, [redacted] µg/mL in the presence of S9 and [redacted] µg/mL in the absence of S9; in the second experiment, [redacted] µg/mL with S9, and [redacted] µg/mL in the absence of S9. For each dose and vehicle (DMSO) controls, [redacted]. Treatments induced a dose-related reduction in mitotic activity in both experiments: [redacted]% with S9 and [redacted]% without S9 in first experiment, [redacted]% and [redacted]% with and without S9, respectively, in the second experiment. No statistically significant increase in the percentage of cells with structural chromosome aberrations or polyploidy was observed in any experiment, either with or without metabolic activation.

The Panel considered this study reliable without restrictions, and the results obtained of high relevance.

3.2.5.4 | *Repeated dose 90-day oral toxicity study in Wistar rats*

Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives, i.e. 2,2′-(C12-18 alkylimino)diethanol ([redacted] CAS No 71786-60-2; batch No [redacted]; [redacted]; purity not stated) was tested in a 13-week subchronic toxicity study in RccHan™ WIST rats. The study was performed in 2018, in compliance with GLP principles and following the OECD TG 408 (OECD, 1998). Three groups of animals (10/sex/group) received the test material by gavage at doses of 10, 30 or 125 mg/kg bw per day and a similarly constituted control group received the vehicle (arachis oil).

No deaths occurred during the study.

No clinical signs were evidenced in both the low- and mid-dose groups. No effects on body weight and body weight gain or on food and water consumption were observed. Opaque eyes were observed in three males and two females receiving 125 mg/kg bw per day. This, first observed in week 9 as unilateral finding, rapidly became bilateral as the treatment period progressed. The ophthalmic examination revealed the presence of opacification/cataract in the lens of males and females [redacted] of the high-dose group that was mainly only on the posterior capsule, but in two males and two females, the effect was a total nuclear cataract. No treatment-related ophthalmological findings were noted at the two lower doses.

Very slight decreases in clinical haematology parameters, including haematocrit (–[redacted]%), haemoglobin concentration (–[redacted]%) and mean cell volume (–[redacted]%), were evidenced in the high-dose group in females. In the males of the high-dose group, minimal alterations included increased red blood cells (+[redacted]%), decreased mean corpuscular haemoglobin (–[redacted]%) and mean cell volume (–[redacted]%). Significant increases in white blood cell count were also observed in males and females receiving 125 mg/kg bw per day ([redacted]% and [redacted]%, respectively). Treatment-related alterations in leucocyte numbers included increased neutrophil count in males at the mid-dose ([redacted]%), and in males and females from the high-dose group ([redacted] and [redacted]%, respectively). Increased lymphocyte counts in females ([redacted]%) and eosinophil count in males ([redacted]%), basophil and large unstained cell count in females ([redacted]%) and monocyte count in both sexes receiving 125 mg/kg bw per day ([redacted]% and [redacted]%) were also noted.

Biochemical changes in the plasma in the high-dose group included: high urea concentrations in both sexes ([redacted]% and [redacted]% for males and females, respectively); low creatinine (–[redacted]%) and high total cholesterol concentrations in females ([redacted]%) with no effect on triglyceride concentration; high glucose concentrations in males ([redacted]%); minimal increase in calcium concentrations in females ([redacted]%); high total protein concentration in females ([redacted]%), with a slight increase in both the albumin ([redacted]%) and globulin fractions and a decrease of the albumin to globulin ratio (–[redacted]%, observed in a minor extent (–[redacted]%) also at 30 mg/kg bw per day).

Significant increases in body weight-adjusted organ weights in the high-dose group, including adrenal gland in males ([redacted]%), kidney in males and females ([redacted]% and [redacted]%, respectively) and liver weight in males ([redacted]%) and females ([redacted]% observed in a lesser extent at 30 mg/kg bw per day ([redacted]%)) were reported.

Macroscopic necropsy revealed thickening of the non-glandular mucosa of the stomach at 30 and 125 mg/kg bw per day (only one female was affected at 10 mg/kg bw per day) and unilateral or bilateral lens opacity were reported in three males and two females treated at 125 mg/kg bw per day.

Treatment-related histopathological findings were observed in the non-glandular stomach as epithelial hyperplasia (acanthosis) and dose-related hyperkeratosis at 10 (one female, minimal), 30 and 125 mg/kg bw per day; in the liver as increased hepatocyte rarefaction (particularly females), in thyroid glands (follicular cell) hypertrophy and in eyes (lenticular degeneration in four and three males and females, respectively) at the high dose.

No adverse effects were recorded at the macroscopic and microscopic examination of the male and female reproductive organs.

Treatment-related macroscopic (thickening) and histopathological findings (epithelial hyperplasia and hyperkeratosis) at the first site of contact (non-glandular stomach) were observed, starting from 30 mg/kg bw per day, mainly related to the irritant properties of the test item. For macroscopic findings (thickening) in the non-glandular stomach, the $BMDL_{10}$ estimate in females was 5 mg ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives/kg bw per day. For histopathological findings in the non-glandular stomach, $BMDL_{10}$ estimates were for acanthosis, 8 and 5 (for males and females, respectively), and for hyperkeratosis, 8 and 6 (for males and females, respectively) mg ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives/kg bw per day.

3.2.5.5 | *Oral combined repeated dose toxicity study with reproduction/developmental toxicity screening test in Wistar rats*

A combined repeat dose toxicity study with reproduction/developmental toxicity screening test in the rat was performed with 2,2′-(C12-18 alkylimino)diethanol (CAS No 61791-31-9; [redacted] batch No [redacted]; purity not stated; vehicle: arachis oil British Pharmacopoeia) in rats. The study was conducted in 2010 in compliance with GLP and following the OECD

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TG 422 (OECD, 1996). The test substance was administered by gavage to three groups of 10 male and 10 female Wistar rats (HanTM:HsCCHanTM:WIST) per group for up to 45 consecutive days (including a 2-week maturation phase, pairing, gestation and early lactation for females) at dose levels of 10, 30 and 125 mg/kg bw per day. The dose levels were selected considering the results of a 14-day repeated dose oral range-finding toxicity study. A control group of 10 males and 10 females received the vehicle arachis oil British Pharmacopoeia. Two recovery groups, each of five males, were treated with the high dose (125 mg/kg bw per day) or the vehicle alone for 42 days and then maintained without treatment for a further 14 days.

[redacted]

In conclusion, the Panel considered the marked effects on body weight gain in high-dose females during lactation, on post-implantation loss as well as on litter sizes and weights to be adverse. In addition, a higher incidence of missing/dead offspring was observed in the high- and mid-dose group. The panel identified a NOAEL of 30 mg/kg bw per day for the parental toxicity and a NOAEL of 10 mg/kg body weight per day for the developmental toxicity.

3.2.5.6 | *Prenatal developmental toxicity study in Han Wistar rats by oral administration*

Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives, i.e. 2,2′-(C12-18 alkylimino)diethanol ([redacted] CAS No 71786-60-2 and 61791-31-9; batch No [redacted]; [redacted]; vehicle: arachis oil) was tested in a prenatal developmental toxicity study with Han Wistar rats (RccHan™:WIST) in a GLP-Guideline study conducted in 2018 according to the OECD TG 414 (date not indicated). Maternal toxicity and the development of the embryo and fetus were examined. Three groups of 20 females received the test substance at doses of 10, 30 or 125 mg/kg bw per day by gavage from GDs 6 to 19. A control group received the vehicle, arachis oil, at the same volume dose as the treated groups. Animals were killed on day 20 after mating for reproductive assessment and fetal examination.

With regard to **maternal effects**, no adverse clinical signs were observed and body weights were similar to controls in all dose groups. In the high-dose group (125 mg/kg bw per day), minor maternal toxicity was observed such as statistically significant reductions in mean body weight gain (–[redacted]% compared to controls) and in the adjusted body weight gain (–31% compared to controls) during GDs 6–20. A statistically significantly lower mean food consumption (–[redacted]% compared to controls) was also reported during GDs 6–18 of gestation. No macroscopic findings were observed in females at GD 20 – except for one female which was found not pregnant. No adverse effects of maternal treatment were seen in numbers of implantations, late resorptions, the extent of pre-implantation loss, number of live pups and sex ratio. However, in the high-dose group, twofold increases were reported for post-implantation loss (statistically significant) and for total resorptions (statistically non-significant). Placental, litter and fetal weights were all considered to be unaffected by treatment – except for a statistically significant increase in male fetal weights ([redacted]%). Based on the reported findings, the Panel identified a maternal NOAEL of 30 mg/kg bw per day.

Fetal development was severely affected in the high-dose groups. There were eight litters with similar major abnormalities, the majority affecting the head, eye and vertebral column. The authors stated that the combination of the observed abnormalities and the severities are rare in rats and the majority were outside of the historical control data range. In the mid-dose group, there was an incidence of microphthalmia (1 fetus/1 litter), which was also observed in the high-dose group (2 fetuses/2 litters) as well as anophthalmia. [redacted] Based on the historical control data for microphthalmia/anophthalmia (no fetus affected in 142 litters), the Panel considered these findings as extremely rare events

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and, therefore, used a BMR of 1% for BMD modelling of these effects. The Panel estimated a developmental $BMDL_1$ of 18 mg/kg bw per day.

3.2.5.7 | *Prenatal developmental toxicity study in New Zealand white rabbits by oral administration*

A prenatal developmental toxicity study was performed in New Zealand White Rabbits with ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives, i.e. 2,2′-(C12-18 alkylimino)diethanol ([redacted] CAS No 71786–60-2; batch No [redacted]; [redacted]; vehicle: 0.5% methylcellulose) conducted in 2018 according to GLP and the OECD TG 414 (date not indicated) guidelines.

Based on results from two dose-range finding studies, the authors concluded that dose levels of 20, 60 and 175 mg/kg bw per day would be suitable for this prenatal development study. Three groups of 22 females received the test substance by oral (gavage) administration from day 6 to 28 after mating. A similarly constituted control group received the vehicle, 0.5% methylcellulose. Animals were killed on day 29 after mating for reproductive assessment and fetal examination. Clinical observations, body weight and food consumption were recorded. Adult females were examined macroscopically at necropsy and the gravid uterus weight recorded. All fetuses were examined macroscopically at necropsy and subsequently by detailed internal visceral examination of the head or skeletal examination.

Two females of the high-dose group were killed for welfare reasons in late gestation. Both were pregnant, and all fetuses appeared grossly normal. Maternal toxicity in all treated groups was also reflected by body weight losses and overall reduced body weight gains. These effects reached statistical significance in the high-dose females (for body weight gain on GDs 8–28: –[redacted]%). Also, the food consumption was statistically significantly lower in this group during GDs 6–20. No treatment-related effects were observed on GD 29. Two low-dose females, three mid-dose and two high-dose females were not pregnant. No treatment-related effects were reported for the litter data (the mean numbers of implantations, early, late and total resorptions, the total number of live young and the sex ratio). There were statistically significant reductions in placental weight (–[redacted]%), male fetal weight (–[redacted]%) and overall fetal weight (–[redacted]%) in the high-dose groups compared to the control group. Regarding fetal pathology in the high-dose group, there was an increased incidence of fetuses/litters with incompletely ossified cervical vertebrae and metacarpals/phalanges forepaws when compared to concurrent control, and this incidence was outside the historical control range. These skeletal effects were considered treatment-related. Other pathological findings in fetuses/litters were not treatment-related.

Based on overt maternal toxicity (body weight loss, low food intake, clinical signs) and reductions in fetal weights along with skeletal effects in the high-dose group (175 mg/kg bw per day), the Panel considered the maternal and the developmental NOAEL to be 60 mg/kg bw per day.

3.2.5.8 | *Extended one-generation reproductive toxicity study in Wistar rats by oral administration*

Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives, i.e. 2,2′-(C12-18 alkylimino)diethanol ([redacted] CAS No 71786-60-2; batch No [redacted]; purity [redacted]%; vehicle: arachis oil) was tested in an Extended One-Generation Reproductive (EOGRT) study in RccHan™ WIST rats following gavage administration. The study was conducted in 2023 in compliance with GLP principles and following the OECD TG 443 (OECD, 2018b).

Methods: The doses for this study were chosen based on the results of a dose-range finding study with eight pregnant females dosed at 60 or 125 mg/kg bw per day (GD 6 until weaning) and F1 offspring (treated from weaning until maturation): One female was found not pregnant, three females failed to litter (with implantations sites) and one female offspring died on day 1 post-partum (and had five implantation sites); F1 animals showed minor differences in body weight and food consumption.

Based on the information from earlier OECD TG 422 and 414 studies and the results from the dose range finding study, the following doses were chosen for the OECD TG 443 (OECD, 2018b) study: 10, 30 and 100 mg/kg bw per day.

F0 rats (25 rats/sex/group) were treated 2 weeks before pairing until termination after litters were weaned. The following parameters were studied: clinical observations, body weight, food, consumption, oestrous cycles, mating performance and fertility, gestation length and parturition observations as well as reproductive performance; clinical pathology (haematology, blood chemistry and urinalysis) and thyroid-related hormones, sperm assessment, organ weight, macroscopic and microscopic pathology investigations.

F1 offsprings were treated from weaning until termination. The following parameters were studied: clinical condition, litter size and survival, sex ratio, body weight, anogenital distance, organ weights and macropathology as well as nipple counts and thyroid-related hormones.

F1 Cohort 1A (20 rats/sex/group) was treated for 13 weeks. Clinical condition, body weight, food consumption, ophthalmic examination, sexual maturation and oestrous cycles as well as clinical pathology including thyroid-related hormones, sperm assessment, ovarian follicle and corpora lutea counts, organ weights, macroscopic and microscopic pathology were investigated as well as immunophenotyping investigations (relative proportions (%), cell numbers/spleen).

F1 Cohort 1B (20 rats/sex/group) was treated for approximately 20 weeks. Clinical condition, body weight, food consumption, ophthalmic examination, sexual maturation and oestrous cycles, mating performance, fertility, gestation length and gestation index were studied as well as organ weights and macroscopic pathology. F1 males and females were mated and F2 offsprings were studied for clinical condition, litter size and survival, sex ratio, body weight, anogenital distance, organ weights and macroscopic pathology as well as nipple counts on male offspring (day 13 of age).

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F1 Cohort 1C (10 rats/sex/group) was treated until 6–8 weeks of age and assessed for clinical condition, body weight and food consumption, sexual maturation and macroscopic pathology.

F1 Cohort 2A (10 rats/sex/group) was treated until approximately post-natal day 75 and assessed for clinical condition, body weight and food consumption. Neurobehavioural screening as well as investigations of brain weight, macroscopic and microscopic neurohistopathology were performed.

F1 Cohort 2B (10 rats/sex/group) was 'directly' treated after weaning. Rats were dispatched to necropsy at weaning for microscopic pathology investigations of the brain.

Results

No test item-related mortalities were observed in this study. Three males in the F0 generation were euthanised: one male from the low-dose group due to poor clinical condition and two males from mid-dose group due to mis-dosing. Five female decedents in different dose groups of the F1 generation were found dead or euthanised due to mis-dosing or poor clinical condition.

Lower thyroxine (T4) and higher thyroid-stimulating hormone (TSH) levels were observed in F0 males from the high-dose group, but no statistically significant increase in absolute or relative thyroid/parathyroid weights and no macroscopic or microscopic findings in the thyroids were reported. No toxicologically relevant effects were observed on T4 or TSH levels in F0 females, F1 adult animals or F1/F2 offspring.

Statistically significant effects in the F0 generation included the following findings in treated animals compared to controls:

During week 1, body weight gain in high-dose groups was very low in males (22% of controls), but high in females (180% of controls). Food consumption was lower in males and females.

Some changes in haematology/red blood cell parameters were observed in high-dose males (slightly higher red blood cell counts, lower mean corpuscular haemoglobin and mean corpuscular volume) and in females (lower haematocrit, higher red cell distribution width). Lower plasma glucose and higher potassium concentrations were noted in high-dose males; in females, a dose-related increase in the cholesterol concentration was observed (up to +54%; values were above the historical control range). Urinalysis revealed higher protein concentrations in the high-dose groups of males (+87%) and females (+75%).

Lower sperm and spermatid counts were reported along with an increase in abnormal sperms (3.8% in the high-dose males vs. 1.8% in controls; outside the historical control range), while there was no treatment-related effect on the sperm motility. Histology of the testes showed a normal progression of the spermatogenic cycle.

Slightly higher absolute and relative organ weights were observed in kidneys of mid- and high-dose males, in liver and adrenal glands of high-dose females. A dose-related thickening of the non-glandular stomach was observed along with dose-related histological changes (incidences, severity), i.e. hyperkeratosis and epithelial hyperplasia in all dose groups of males and in the mid- and high-dose females.

Compared to controls, F1 litter responses included the following findings:

The number of litters with a reduced birth index (< 100%) was higher in the high-dose group (6 litters vs. 1 litter in controls). Lower body weights on day 1 (–[redacted]%) were observed in the high-dose groups of males and females along with a lower body weight gain on post-natal day 1–21 (–[redacted]%).

Statistically significant effects in F1 offspring included the following findings in treated animals compared to controls:

F1 males of the high-dose group had a lower body weight on post-natal day 127 (–[redacted]%) and lower bw gain on post-natal day 1–127 (–[redacted]%). The ophthalmologic examination of Cohort 1A and 1B animals revealed a dose-dependent increase in lens opacities/cataracts in the mid- and high-dose groups – with high incidences predominantly in the nuclear region ([redacted]).

In females of Cohort 1A, the white blood cell counts were increased in the mid- (+[redacted]%) and high-dose groups (+[redacted]%), with the monocytes reaching statistical significance at all doses. Blood chemistry changes of Cohort 1A animals in the high-dose groups included high gamma-glutamyl transferase (GGT) and urea levels; increased potassium levels were recorded in all treated animals. Urinalysis revealed a lower volume, higher specific gravity and urinary potassium concentration (outside historical control) in high-dose males of Cohort 1A.

Higher relative kidney weights were observed in males (up to +[redacted]%) and females (up to +[redacted]%) of the Cohort 1A in the mid- and high-dose groups. High-dose females had higher absolute and relative weights of liver (+[redacted]% and + [redacted]%, respectively), adrenal glands (+[redacted]% and + [redacted]%, respectively) and thyroid glands (+[redacted]% and + [redacted]%, respectively). The relative weight of thyroid glands was also increased in high-dose males (+[redacted]%). Absolute and relative ovary weights were increased in mid- and high-dose females.

Dose-related increases of incidences of thickened non-glandular stomach were observed in Cohort 1A and 1B animals. Histologically dose-related increases of incidences and severity of hyperkeratosis and epithelial hyperplasia were found in all dose groups of males and females of Cohort 1A. In addition, histology of the vas deferens revealed dose-dependent increases in the vacuolisation in all dose groups and degeneration in the high-dose groups of males in Cohort 1A and 1B.

Sexual maturation of F1 males (balano-preputial separation) and females (vaginal opening) in the high-dose groups was delayed (3 days), all treated groups exceeded the historical control data. The conception rate and the fertility index

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in Cohort 1B were in all dose groups (85, 89, 80%) below the concurrent controls (95%) and the historical control data (95%–100%).

Neurobehavioural investigations of Cohort 2A revealed low values for forelimb grip strength in males and females of the high-dose groups. In addition, brain morphology measurements showed a lower hippocampus thickness in high-dose males and females in Cohort 2A and 2B. Both independent findings were considered by the study authors to represent adverse effects.

Spleen cell immunotyping using Cohort 1A showed no treatment-related effects – except for an increase of neutrophils in males but without dose–response. In the absence of histological signs of inflammation and considering that the percentages of changes are limited together with the broad inter-animal variability, these changes do not seem to be of biological significance. Therefore, the Panel considered that no further assessment of immunotoxicity was needed.

Compared to controls, F2 litter responses included the following findings:

Several F1 females failed to litter (1 in controls, 3 at low-dose, 3 at mid-dose, 4 at high-dose: out of 20 females in each group). The mean post-implantation survival index was lower in the high-dose group (87%) compared to that of concurrent controls (95.4%) and the historical control range (92%–97%) and resulted in a low litter size at post-natal day 1 and a lower viability index at post-natal day 4.

The body weight gain on post-natal day 1–21 of F2 male and female offspring was lower in the high-dose groups ([redacted]% and [redacted]%, respectively).

In summary, based on the above findings in the EOGRT study, for the different endpoints, the Panel considered the following NOAELs and BMDL of ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives as relevant:

The NOAEL for systemic toxicity in F0 was considered to be 30 mg/kg bw per day due to increased liver weights along with higher cholesterol levels at 100 mg/kg bw per day.

For local stomach irritancy in the F0 generation, the $BMDL_{10}$ was estimated to be 4 mg/kg bw per day for males and 11 mg/kg bw per day for females due to histological findings in the non-glandular stomach.

The $BMDL_{10}$ for systemic toxicity in F1 males and females in Cohort 1A and 1B was estimated to be 29 mg/kg bw per day based on opacities/cataracts.

The NOAEL for reproductive toxicity for F1 and F2 offspring was considered to be 30 mg/kg bw per day due to a reduced live birth index, low offspring body weights and a lower fertility index at 100 mg/kg bw per day.

3.2.6 | PFAEO-oleyl[25]

3.2.6.1 | *Gene mutation in bacteria*

N,N-bis(2-hydroxyethyl)oleylamine, i.e. 2,2′-(octadec-9-enylimino)diethanol (CECAJEL 210, CAS No [redacted] batch No [redacted], purity [redacted]%) was tested in a bacterial reverse mutation test (Ames test) with the Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102, in the presence and absence of a metabolic activation system (liver S9 from Aroclor induced rats). The study was conducted in 2005 in compliance with GLP principles and following the OECD TG 471 (OECD, 1997a). Two experiments were performed with triplicate plates, over a dose range determined in a preliminary toxicity test. The first experiment was conducted using the plate incorporation method, with six concentrations from 2.34 to 75 μg/plate in the absence of S9 and five concentrations from 18.75 to 300 μg/plate (TA98, TA1537 and TA102) or 9.38 to 150 μg/plate (tA1535 and TA100) with S9. The repeat experiment was performed using the plate incorporation method without S9, with five concentrations from 1.25 to 20 μg/plate, and with the preincubation method with S9, testing five concentrations from 6.25 to 100 μg/plate. DMSO was used as solvent and vehicle control. Toxicity, as reduction of background lawn and/or decrease in revertant counts, was observed at 10 μg/plate and above in the absence of S9, and at 150 μg/plate and above with S9. In both experiments, no increase in revertant colonies was observed at any dose, either with or without metabolic activation.

The Panel considered the study reliable without restrictions, and the negative results reported highly relevant.

3.2.6.2 | In vitro *mammalian cell gene mutation test*

N,N-bis(2-hydroxyethyl)oleylamine, i.e. 2,2′-(octadec-9-enylimino)diethanol (CAS No 25307–17-9, batch No [redacted], purity [redacted]%) was tested in a gene mutation assay in mouse L5178Y lymphoma TK+/– cells in a fluctuation assay using microtitre plates, with and without metabolic activation with liver S9 obtained from phenobarbital/β-naphtoflavone induced rats. The study was conducted in 2010 in compliance with GLP principles and following the OECD TG 476 (OECD, 1997b). The test item was tested in two assays, with 4 h exposure (in the first experiment with 2% S9 and without S9, and in the second experiment with 1% S9) and 20 h exposure (in the second experiment without S9). Six concentrations in following dose ranges were evaluated: in the first experiment 0.63–7.5 μg/mL without S9 and 5–20 μg/mL with S9; in the second

[25]Additional data/Nouryon/Technical dossier/Section 7 and related annexes.

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experiment 0.31–3.75 μg/mL without S9 and 1.25–15 μg/mL with S9. Duplicate cultures were used for each dose and for solvent (DMSO) and positive controls. Treatments induced a steep increase in toxicity in a narrow concentration interval: relative total growth at the highest analysable concentration was [redacted] and [redacted]% without S9 (in the first and second experiment, respectively) and [redacted] and [redacted]% with S9, with the subsequent concentration, spaced less than a 1.5-fold factor, completely toxic. In both experiments, no increase in mutant frequency which was reproducible, or statistically significant, or above the Global Evaluation Factor, was observed, either in the presence or in the absence of S9-mix.

The Panel noted that the target toxicity level (10%–20% relative total growth) was not reached in the first experiment without S9 due to the steep onset of toxicity, but this was reached, and slightly exceeded, in the repeat experiment. Overall, the study is considered reliable without restrictions, and the results obtained of high relevance.

3.2.6.3 | In vitro *mammalian chromosome aberration test*

N,N-bis(2-hydroxyethyl)oleylamine, i.e. 2,2′-(octadec-9-enylimino)diethanol (CAS No 25307-17-9, batch No [redacted], purity [redacted]%) was tested in a chromosome aberration test in human lymphocytes in vitro, with and without metabolic activation with liver S9 from phenobarbital/β-naphtoflavone induced rats. The study was conducted in 2010 in compliance with GLP principles and following the OECD TG 473 (OECD, 1997c). The test item was tested in two assays, with 4 h exposure (in the first experiment with 2% S9 and without S9, and in the second experiment with 1% S9) and 24 h exposure (in the second experiment without S9). All cultures were harvested 24 h after treatment initiation. Based on the observed reduction in mitotic index, the following doses were tested using duplicate cultures: in the first and second experiment[redacted] μg/mL in the presence of S9, and [redacted] μg/mL in the absence of S9. For each dose and vehicle (DMSO) controls, [redacted]. Treatments induced a dose-related reduction in mitotic activity in both experiments: –[redacted]% with S9 and –[redacted]% without S9 in first experiment, –[redacted]% and –[redacted]% with and without S9, respectively, in the second experiment. No statistically significant increase in the percentage of cells with structural chromosome aberrations or polyploidy was observed in any experiment, either with or without metabolic activation.

The Panel noted that the target toxicity level (55 ± 5% reduction MI) was not reached in the second experiment without S9 due to the steep onset of toxicity, but this was reached, and slightly exceeded in the first experiment. Overall, the study is considered reliable without restrictions, and the results obtained of high relevance.

3.2.6.4 | *Repeated dose 90-day oral toxicity study in Wistar rats*

2,2′-(octadec-9-enylimino)diethanol (PFAEO-oleyl; CAS No 25307–17-9; [redacted] batch No [redacted]; purity > [redacted]%) was tested in a 90-day subchronic toxicity study in Wistar Han™: RccHan™rats. The study was performed in 2014, in compliance with GLP principles and following the OECD TG 408 (OECD, 1998). Three groups of animals (10/sex/group) received the test material by gavage at doses of 5, 30 or 150 mg/kg bw per day based on a 28-day dose range finding study. A similarly constituted control group received the vehicle (arachis oil British Pharmacopoeia).

There were no unscheduled deaths throughout the study.

No clinical signs of toxicological relevance and behavioural effects were observed at any dose level during the study. [redacted] At 150 mg/kg bw per day, body weight and body weight gain of males were lower than those in the controls throughout the treatment period, with an overall body weight gain of [redacted]% compared to that in the control group at the end of treatment. Food consumption of the same animals was generally lower (–[redacted]% at the end of treatment period) than in the control group throughout the study.

Ophthalmic examination did not indicate any effect of treatment. Slight, but statistically significant decreases in clinical haematology parameters were reported, [redacted] However, considering the limited differences observed, the Panel considered them of no toxicological concern.

[redacted] In males of the high-dose group, lower levels of total protein (–[redacted]%) were observed, accompanied by lower blood albumin levels (–[redacted]%) and an increase for albumin/globulin ratio ([redacted]%). All reached statistical significance compared to those of the control groups. In males of the same dose group, higher levels of bile acids ([redacted]%) were measured, the individual values being higher than the 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. Lower levels of creatinine and cholesterol (–[redacted]% and –[redacted]%, respectively) also reached statistical significance compared with to those of the control group.

No macroscopic findings were observed at necropsy that might be considered associated with treatment. At the high dose, changes in liver weights were evidenced both in males (–[redacted]% and +[redacted]% for absolute and relative weights, respectively) and in females (+[redacted] and +[redacted]% for absolute and relative weights, respectively). Increases in kidney weights ([redacted]% and [redacted]% for absolute and relative weights, respectively) were also observed in females. At the high dose, minimal or mild diffuse epithelial hyperplasia and hyperkeratosis of the non-glandular stomach were observed in most of the animals with

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minimal or mild focal erosion, minimal or mild submucosal inflammatory cell infiltration, submucosal oedema and minimal focal dyskeratosis in a few animals. In addition, minimal to marked foamy macrophages were present in the lamina propria of the small intestine (principally the jejunum, but also in the duodenum and ileum) and also in the sinuses of the mesenteric lymph node in both sexes. At 30 mg/kg bw per day, only incidental findings of minimal focal epithelial hyperplasia, minimal focal erosion, submucosal oedema and minimal or mild submucosal inflammatory cell infiltration of the non-glandular stomach were reported.

Significant histopathological findings were observed in the high-dose group, 150 mg/kg bw per day, after 90-day repeated oral administration in Han Wistar rats.

For the histopathological findings, i.e. minimal focal epithelial erosion, minimal submucosal oedema and minimal submucosal inflammatory cell infiltration in the non-glandular stomach, $BMDL_{10}$ estimates in females were 65, 26 and 40 mg 2,2′-(octadec-9-enylimino)diethanol/kg bw per day, respectively.

3.2.6.5 | *Prenatal developmental toxicity study in Sprague Dawley rats by oral administration*

A prenatal developmental toxicity study was performed in female Sprague–Dawley rats (Crl:CD (SD) IGS BR) with 2,2′-(octadec-9-enylimino)diethanol (trade name not indicated, CAS No 25307–17-9; batch No [redacted]; purity > [redacted]%; vehicle: arachis oil). The study was conducted in 2014 in compliance with GLP principles and following the OECD TG 414 (OECD, 2001). The test substance was administered by gavage to three groups of 24 female Sprague–Dawley (Crl:CD® (SD) IGS BR strain) rats between GD 5 and 19 at dose levels of 15, 50 and 150 mg/kg bw per day. Further 24 females were exposed to the vehicle arachis oil to serve as a control group.

There were no unscheduled deaths during the study. Clinical signs did not indicate any effect of treatment (except for noisy respiration in 1 of 24 high-dose females). No effect on body weight and body weight gain was reported, but after adjustment for the contribution of the gravid uterus, a statistically non-significant reduction [redacted] in the weight change (GDs 5–20) was observed in high-dose females. Food and water consumption were unaffected by the treatment. No macroscopic abnormalities were detected for treated parental females.

There were no toxicologically relevant treatment-related findings in the number of implantations, subsequent embryofetal survival and litter size, sex ratio and mean fetal, litter and placental weights on GD 20 of gestation. In the high-dose group, there were some increases in the number of affected fetuses and litters regarding ureter effects and incomplete ossifications. However, considering that the total number of skeletal and visceral findings in fetuses/litters was not changed in the high-dose group compared to controls, the developmental effects were not considered adverse.

Based on these data, the Panel identified for maternal and developmental toxicity of the offspring a NOAEL of 150 mg/kg bw per day, the highest dose tested.

3.2.6.6 | *Extended one-generation reproductive toxicity study in Wistar rats by oral administration*

An EOGRT Study with 2,2′-(octadec-9-enylimino)diethanol (CAS No 25307-17-9; [redacted] batch No [redacted]; purity [redacted]%; vehicle: arachis oil) was performed in the Han Wistar rats (RccHan™;WIST rat). The study was conducted in 2021 in compliance with GLP principles and following the OECD TG 443 (OECD, 2018b).

Methods: The doses for this study were chosen based on results of a dose-range finding study (reductions of body weight gains in F0 and F1, high mortality/litter death and delayed sexual maturation (F1) at 150 mg/kg bw per day). The method reported in the study is given as follows.

In the F0 generation, three groups of 25 male and 25 female rats were treated with the test substance at dose levels of 30, 70 or 150 mg/kg bw per day by gavage. Males were treated for 10 weeks before pairing up to necropsy after litters were weaned. Females were treated for 10 weeks from before pairing, throughout pairing up to necropsy on day 28 of lactation. For the F0 generation, data were recorded on clinical condition, body weight, food consumption, oestrous cycles, mating performance and fertility, gestation length and parturition observations as well as reproductive performance. Clinical pathology (haematology, blood chemistry and thyroid-related hormones), sperm assessment, organ weight, macroscopic pathology and microscopic pathology were investigated.

For F1 offspring, clinical condition, litter size and survival, sex ratio, body weight, anogenital distance, organ weights and macropathology were assessed. Nipple counts were performed on male offspring on day 13 of age. Serum samples that were collected from selected offspring on day 22 of age were analysed for thyroid-related hormones. The F1 generation comprised of two cohorts:

Cohort 1A: 20 male and 20 female progeny were selected from each dose group and continued to receive test substance at doses of 0 (control), 30, 70 or 150 mg/kg bw per day from weaning until scheduled sacrifice at approximately week 13 of age.

Cohort 1B: 20 male and 20 female progeny were selected from each dose group and continued to receive the test substance at doses of 0 (control), 30, 70 or 150 mg/kg/day from weaning until scheduled sacrifice at approximately week 14 of age.

For F1 Cohort 1A, data were recorded on clinical condition, body weight, food consumption, sexual maturation and oestrous cycles. Clinical pathology (haematology, blood chemistry, urinalysis and biomarkers), sperm assessment, ovarian follicle and corpora lutea counts, organ weight, macroscopic pathology, full microscopic pathology and immunophenotyping investigations were performed.

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For F1 Cohort 1B, data were recorded on clinical condition, body weight, food consumption, sexual maturation, organ weight and macroscopic pathology.

Results for the F0 generation: Premature deaths of three high-dose females were considered treatment-related due to macroscopic and histopathological findings related to the stomach and small intestine (e.g. accumulation of the foamy macrophages, hyperplasia of the non-glandular gastric region), atrophy/involution of the thymus and changes in spleen, pancreas and uterus.

Dose-related, statistically significant reductions of body weights were observed in all treated males (e.g. day 120: up to % vs. controls) and of the overall body weight gain in mid- and high-dose males (day 1–120: % and %, respectively). Statistically significant reductions of body weight gain in females were seen temporarily at days 22–27 before pairing (up to % vs. controls) and at days 18–20 during gestation (% in high-dose group vs. controls); increases of body weight gain were observed during the first 2 weeks of lactation. Food consumption was lower in males (dose-dependently) and females before pairing and during gestation (in the high-dose group). Oestrous cycles (except for one high-dose females out of 25), pre-coital interval, mating performance and gestation index (% at the high-dose vs. 100% in controls) were mostly unaffected by treatment. Sperm analysis did not show any treatment-related adverse effects.

While absolute organ weights were unaffected by treatment, increases in relative (to body weight) organ weight were observed in the mid- and high-dose groups of males and females for kidney, In the absence of microscopic findings in these organs, the changes were considered of limited toxicological relevance. Macroscopic findings at terminal sacrifice showed treatment-related and dose-dependent thickening of the stomach for all treated groups and thickened jejunum in the high-dose groups Accumulation of foamy cells was observed in the jejunum of males and females in the mid- and high-dose groups and in the duodenum of high-dose males.

F1 litter responses: The number of implantation sites, post-implantation survival, live birth index and the number of offspring on day 1, litter survival and sex ratio were unaffected by parental treatment. At weaning, reductions of body weights (%) and body weight gain (%) were observed in male and female offspring at the high dose. . No treatment-related macroscopic findings were reported for F1 offspring of day 22.

F1 generation: From eight premature deaths, one high-dose male showed treatment-related effects, e.g. hyperplasia in the non-glandular gastric region, foamy macrophages in the intestine and involution/atrophy of the thymus. The remaining deaths were not treatment-related. At day 64, the reductions of body weight in F1 males (dose-dependent) and F1 females were up to % and %, respectively, compared to controls; the reductions of overall body weight gain (day 1–64) in F1 males (dose-dependent) and for F1 females were up to % and %, respectively. Similarly, food consumption was lower in the F1 males (mid- and high-dose) and F1 females (high-dose).

Cohort 1A: No adverse treatment-related effect was observed on oestrous cycling. Similar to the changes in haematology in F0 animals, there were reductions of red blood cell parameters in F1 animals and increases in white blood cell counts, i.e. leucocytes, neutrophils and monocytes probably reflecting inflammations in gastrointestinal (GI) tract.

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Blood chemistry findings [redacted] as well as other changes of limited toxicological relevance, e.g. an increase in plasma urea level and a decrease in glucose levels in males without any histological correlates.

[redacted]

Statistically significant increases in relative (to body weight) organ weights were observed for kidneys (all treated animals), [redacted]. Statistically significant changes in absolute and relative organ weights were seen for thymus (decreases in males: associated with involution/atrophy), mesenteric lymph nodes (increases in females), associated with histological changes). [redacted].

Thickening of the stomach was seen in males [redacted] and females [redacted] of the mid- and high-dose groups and was associated with microscopic findings of diffuse hyperplasia of the non-glandular mucosa, erosion/vesicles, ulceration, oedema and mixed inflammation. Accumulation of foamy cells was reported for jejunum, duodenum, ileum and mesenteric lymph nodes along with other observations in both sexes. The histopathological findings were considered adverse, except for those in mesenteric lymph nodes. Involution/atrophy of the thymus was observed with higher incidences in males than in females. The data on the endpoint thymus involution/atrophy in males allowed to perform a BMD analysis: the $BMDL_{10}$ estimate was 47 mg/kg bw per day. [redacted]

Cohort 1B: [redacted] Thickening of the stomach was seen in mid- and high-dose males [redacted] and high-dose females [redacted] along with depression in both sexes. The macroscopic findings were correlated with adverse microscopic observations in the stomach, as also seen in Cohort 1A. Diffuse accumulation of foamy cells within the lamina propria were reported in the jejunum.

Conclusion: Considering the premature mortalities at 150 mg/kg bw per day, the reductions in body weight and body weight gain along with adverse macroscopic and microscopic findings at 70 and at 150 mg/kg bw per day, $BMDL_{10}$ values for epithelial hyperplasia, oedema and inflammation were estimated 4, 37 and 46 mg/kg bw per day, respectively, for males and 5, 17 and 11 mg/kg bw per day, respectively, for females. The NOAEL for reproduction was at 150 mg/kg bw per day in the absence of adverse findings on mating performance and fertility. A developmental $BMDL_{10}$ was estimated for the accumulation of foamy cells in the jejunum in F1 males and females at 26 mg/kg bw per day.

3.2.7 | PFAEO-hydrogenated tallow[26]

3.2.7.1 | *Combined repeated dose toxicity study with reproduction/developmental toxicity screening test in Wistar rats by oral administration*

A combined repeated dose toxicity study and reproductive/developmental toxicity screening study with 2,2′-(C16/18 alkylimino)diethanol (Ethomeen HT/12; CAS No 1218787-30-4; batch No [redacted]; purity [redacted]%; vehicle: arachis oil) was performed in Han Wistar rats. The study was conducted in 2023 in compliance with GLP principles and following the OECD TG 422 (OECD, 2016c). The dose levels for this study were selected based on the results of a preliminary study [redacted] at 150, 175 or 200 mg/kg per day, with an initial toxicity phase followed by an embryo-fetal phase with females dosed at 125 or 175 mg/kg bw per day.

In males, doses of 150, 175 or 200 mg/kg bw per day were not tolerated, resulting in body weight loss and reduced food intake with no dose–response as well as premature death of two males receiving 175 mg/kg bw per day and two males receiving 150 mg/kg bw per day. Based on these results, for this OECD TG 422 study, three groups of 10 male and 10 female rats were treated with Ethomeen HT/12 at doses of 10, 30 or 100 mg/kg bw per day by gavage. One male receiving a dose of 100 mg/kg bw per day was prematurely sacrificed on day 11 due to the extent of the body weight loss. Therefore, from day 12 of dosing, the high-dose level was reduced to 75 mg/kg per day for males. Males were treated daily for 2 weeks before pairing, up to necropsy after a minimum of four consecutive weeks. Females were treated daily for 2 weeks before pairing until day 12 of lactation. Females were killed on day 13 of lactation. The F1 generation was exposed in utero and via the milk. A similarly constituted control group received the vehicle (arachis oil) at the same volume dose as the treated groups.

There were five unscheduled deaths in F0 animals. In addition to one high-dose male euthanised because of acute weight loss on day 11, one female was found dead because of misdosing and three females were euthanised due to failure to litter/total litter loss. After reduction of the high-dose for males, the overall weight gain in this group remained markedly lower [redacted] compared to controls. In high-dose females, the overall body weight gain was [redacted] lower

[26] Additional data/Nouryon/Technical dossier/Section 7 and related annexes.

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before pairing (day 1 to day 15; however, not statistically significant) and [redacted] lower during gestation (GDs 0–20; however, not statistically significant). Reduced food intakes from day 1 to 15 [redacted] may have contributed to the lower body weight gains in parental animals.

Sensory reactivity, grip strength and motor activity were unaffected by treatment, except for some changes (i.e. reductions in total high/low beam breaks in mid- and high-dose males), which did not reach statistical significance.

Oestrous cycles, pre-coital interval, mating performance, fertility and gestation length were unaffected by the treatment. Lower sperm count estimates were within the historical control range.

Treatment-related statistically significant decreases in red blood cell parameters (mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration) were observed in all treated males, along with higher red cell distribution width and higher reticulocyte counts that attained statistical significance in the high-dose group. In high-dose females, only red cell distribution width was statistically significantly increased. Changes in blood chemistry in the high-dose group of males were mainly related to high bile acid and lower creatinine, sodium, chloride and protein concentrations. Lower cholesterol levels in males and lower levels of ALT in females were not considered to be of toxicological relevance. T4 levels in mid- and high-dose F0 males were increased compared to controls. However, these findings were not considered adverse in the absence of changes in thyroid weights and histological findings. No effect on T4 levels was found in male and female offspring.

Statistically significant changes in organ weights of F0 animals were restricted to a decrease in the absolute prostate weight in high-dose males and higher relative liver weight in mid- and high-dose males without histological correlates – except for two out of five high-dose males with minimal acinar atrophy in the prostate. Other microscopic findings were observed in testes (tubular atrophy) in high-dose males, mesenteric lymph nodes (increased cellularity of intrasinusoidal macrophages), ileum, jejunum (foamy macrophage infiltrates) and non-glandular stomach (epithelial hyperplasia) in the high-dose groups and in mesenteric lymph nodes and the jejunum also in the mid-dose groups of males and females.

F1 Litter responses: litter size, implantation count, sex ratio, offspring survival and anogenital distances were unaffected by treatment. There were no treatment-related clinical signs.

The male and female offspring in the high-dose groups gained slightly less weight than the offspring of control animals from day 1 to 4 of age (only significant for female F1). However, there was no effect on overall weight gain from day 1 to day 13 of age. No treatment-related macroscopic findings were reported.

In conclusion, the Panel considered that, due to the adverse effect on body weight gain in males receiving a dose of 100/75 mg/kg bw per day, the NOAEL of Ethomeen HT/12 for systemic toxicity was 30 mg/kg bw per day for males and 100 mg/kg bw per day for females. Based on the absence of adverse effects on reproductive performance, fertility, litter size, offspring survival and growth in this study, the NOAEL for reproductive/developmental toxicity was considered to be 100 mg/kg bw per day for F0 females and offspring, the highest dose tested. Regarding local irritant effects, $BMDL_{10}$ estimates for inflammatory cell infiltrate in the jejunum were 7 and 19 mg/kg bw per day for F0 males and females, respectively.

3.2.7.2 | *Prenatal developmental toxicity study in Wistar rats by oral administration*

A prenatal developmental toxicity study on 2,2′-(C16/18alkylimino)diethanol (Ethomeen HT/12; CAS No 1218787-30-4; batch No [redacted]; purity [redacted]%; vehicle: arachis oil) was performed in Han Wistar rats (RccHan®:WIST). The study was conducted in 2023 in compliance with GLP principles and following the OECD TG 414 (OECD, 2018c). Two groups of 22 females and one group of 24 females received Ethomeen HT/12 at doses of 15, 50 or 150 mg/kg bw per day, respectively, by gavage administration, from day 6 to day 20 after mating. A similarly constituted control group received the vehicle, arachis oil, at the same volume dose as the treated groups. Animals were killed on day 21 after mating for reproductive assessment and fetal examination. The doses used in this study were based considering results of a preliminary toxicity and embryo-fetal toxicity study in rats [redacted] and modelling data from studies with similar substances.

In F0 females (GD 21), there was a dose-dependent decrease in the mean serum T4 and triiodothyronine (T3) concentrations, correlated with a dose-dependent increase in serum TSH concentration. However, due to high inter-group variability, these values did not attain statistical significance and were within historical control range. No significant weight change and no microscopic findings were observed in the thyroid glands.

Treatment with the highest dose, i.e. 150 mg/kg bw per day, was not well tolerated: seven females with multiple common signs, including hunched posture, decreased activity and piloerection, were euthanised for welfare reasons in late gestation. Two further females of this dose group were euthanised: one with signs of general poor condition and one with a dosing trauma.

Clinical signs were reported for all high-dose females. In the high-dose group, substantially lower body weight [redacted], body weight gain [redacted]), lower gravid uterine weight [redacted] and an adjusted mean weight loss of 28 g compared to a mean weight gain of 26 g in controls were observed. The mean food consumption was also lower in this group [redacted].

Statistically significant reductions of placental, litter and fetal weights were found in the high-dose group. There were no significant changes in the number of resorptions, pre- and post-implantation losses (%), the number of live offspring and the sex ratio in treated animals. However, only 13 litters were available at the high dose. The anogenital distance of fetuses was not affected by maternal treatment.

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[redacted]

In the absence of treatment-related adverse effects in the low- and mid-dose groups of this study, the Panel considered that the NOAEL for maternal toxicity was 50 mg/kg bw per day due to effects on mortality, clinical signs, body weight and body weight gain at 150 mg/kg bw per day. The Panel noted that only 13 litters were available for evaluation at 150 mg/kg bw per day, which is not in compliance with the requirements of the Guidelines and, therefore, considered that the results on developmental toxicity from this study can only be used as supportive evidence.

3.2.8 | PFAEO-tallow[27]

3.2.8.1 | *Gene mutation in bacteria*

A short report with the results of a mutagenicity test in bacteria with PFAEO-tallow ([redacted], Lot No [redacted], purity and CAS No not indicated) was also available for evaluation. The study was conducted in 1978. The compound was tested with Salmonella Typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 at five concentrations in the range of 0.2–500 μg/plate, in the presence and absence of metabolic activation. No increase in revertant colonies was reported.

The Panel noted that this study was inadequately reported, with no information on experimental procedures, no criteria for dose selection, lack of concurrent positive control and an inadequate set of strains compared to the recommendation of OECD TG 471 (OECD, 1997a; OECD, 2020) and considered the study of insufficient reliability and the results of low relevance.

Intermediate conclusion on the genotoxic potential of the assessed Nouryon substances: PFAOE-coco and PFAEO-oleyl were tested with negative results in reliable in vitro assays for the induction of gene mutations in bacteria and in mammalian cells and for the induction of structural chromosome aberrations in mammalian cells. In the latter test, no increase of polyploidy was observed, suggesting the lack of aneugenic potential. Negative results were also obtained in a bacterial mutation test with PFAEO-tallow, but the study was evaluated of insufficient reliability and low relevance as inadequately reported. Overall, the data indicate that PFAOE-coco and PFAEO-oleyl do not raise concern for genotoxicity.

3.2.8.2 | *Repeated dose 90-day oral toxicity study in Wistar rats*

Ethomeen T/12 (PFAEO-tallow; batch No, purity and CAS No not stated) was administered via the diet daily for 90 days to Wistar rats. The study was performed in 1965. Five groups of animals (25/sex/group, except for the high-dose group with 10 animals/sex) received the test material at levels of 0, 50, 170, 500, 1500 or 4500 ppm. A further group of 14 rats, 7 males and 7 females was fed a diet containing 4500 ppm Ethomeen T/12 and sacrificed at intervals up to 6 weeks after the start of the experiment. Tissues from these animals were examined for sudanophilic material.

There were no unscheduled deaths throughout the study.

At the highest dose level, hair loss was observed and the animals were generally lethargic throughout the experiment. A decreased body weight gain [redacted] was reported at 4500 and 1500 ppm, respectively. The mean daily food consumption of the same animals was generally lower [redacted] than the controls.

No effects were noted in any of the measured haematological parameters [redacted].

Treatment-related changes, seen in the highest dose group, included yellow stomach and bowel content of all rats, thickened and yellow mucosa of the small intestine. The mean organ body weight ratios, measured for liver, kidney, adrenals, heart, lungs and spleen only in few animals (2–5 in total), were not different from controls. Microscopic findings at 4500 ppm and 1500 ppm, as described in the original report, included abnormalities in the small intestine and regional mesenteric nodes. All animals in the 4500 ppm dose group showed inflammation of the villi and lamina propria of the small intestine with swollen foamy macrophages. Similar macrophages were occasionally seen to a lesser degree in Peyer's patches and in the regional lymph nodes. Changes were most pronounced in the jejunum and upper ileum but could be detected throughout the small intestine. The macrophages were sudanophilic and were presumed to contain deposits of the test item. Similar changes were observed, though in a lesser degree, in rats fed at a dietary level of 1500 ppm.

Based on effects on body weight gain, food consumption, microscopic changes in small intestines and its regional lymph nodes, the Panel identified a NOAEL of 500 ppm, corresponding to approximately 35 mg Ethomeen T/12/kg bw per day (based on mean food intake and the mean body weight throughout the study) after 90-day repeated oral administration in Wistar rats.

In the study report, the OECD TG,[28] the batch No, purity and CAS No as well as the CoA of the test item were not reported. The raw data, the histopathology report, the data on frequency as well as the set-up of the observations and clinical signs were not available (NA). The evaluation of parameters was limited (only in 5 animals), as for haematology or absent, as for

[27]Additional data/Nouryon/Technical dossier/Section 7 and related annexes.

[28]The study was performed before the publication of OECD TGs.

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biochemistry and ophthalmology and functional observations. Based on these limitations, the Panel considered the study reliable with restrictions and, therefore, as a supporting study.

3.2.8.3 | *Repeated dose 90-day oral toxicity study in dogs*

Ethomeen T/12 (PFAEO-tallow; batch No, purity and CAS No not stated) was administered via the diet daily for 90 days to Beagle dogs. The study was performed in 1965. Four groups of animals (four/sex/group) received the test material at 0, 13, 40 or 120 mg/kg bw per day (low-, medium-, high-dose).

The high-dose group was terminated after 5–6 weeks due to the poor conditions of the animals: sickness and loss of approximately 20% body weight.

Body weight gain was decreased (–[redacted]%) in the males at mid-dose. Clinical signs observed at the mid-dose included sporadic vomiting and anorexia, subdued physical activity associated with the occasional vomiting. Haematological examinations showed a degree of simple hypochromic anaemia in females treated with 120 mg/kg bw per day. Increases in both measured biochemical parameters, i.e. blood urea [redacted] and serum alkaline phosphatase [redacted] levels were noticed at the high-dose. An increase [redacted] was also noted in the retention of bromsulphalein, used at that time to assess liver functionality, in the high-dose group. No treatment-related changes were seen in urine analysis, relative organ weights and at macroscopic examination. Animals treated with 40 or 120 mg/kg bw per day showed increased numbers of foamy macrophages in the intestinal villi and in the regional lymph nodes.

Based on effects on body weight gain, microscopic changes in small intestines and its regional lymph nodes, the Panel identified a NOAEL of 13 mg Ethomeen T/12/kg bw per day after 90-day repeated oral administration in Beagle dogs.

In the study report, the OECD TG,[29] the batch No, purity, the CAS No, as well as the CoA of the test item were not reported. The raw data, histopathology report and data on frequency, set up of the observations and clinical signs were not available. The evaluation of haematological and biochemical parameters was limited. Ophthalmological examinations and functional observations were not performed. Based on these limitations, the Panel considered the study reliable with restrictions and, therefore, as a supporting study.

3.2.8.4 | *Combined repeated dose toxicity study and reproduction/developmental toxicity screening study in Wistar rats by oral administration*

A combined repeated dose toxicity study and reproductive/developmental toxicity screening study with 2,2′-(C16/18, C18 unsaturated) alkylimino)diethanol (Ethomeen T/12; CAS No 1218787-32-6; batch No [redacted]; purity [redacted]%; vehicle: arachis oil) was performed in Han Wistar rats. The study was conducted in 2022 in compliance with GLP principles and following the OECD TG 422 (OECD, 2016c). Three groups with 10 male and 10 female Wistar rats (RccHan™;WIST) received the test substance at doses of 30, 75 or 175 mg/kg bw per day by gavage. A similarly constituted control group received the vehicle, arachis oil. Males were treated from 2 weeks pre-pairing up to necropsy after a minimum of 5 weeks; females were treated from 2 weeks before pairing (except 3 weeks before pairing for Group 4) throughout pairing and gestation until day 13 of lactation.

In high-dose (175 mg/kg bw per day) female F1 offspring on day 13, T4 levels were lower [redacted] than in concurrent controls and were slightly lower than the historical control data minimum. Changes in T4 levels of F1 and F0 males were not statistically significant.

In the high-dose groups, premature deaths were reported for four males – the remaining males in this group were euthanised for welfare reasons – and three females with intestinal lesions as a major factor contributing to death. Treatment-related adverse findings in the small intestine included foamy macrophages expanding the lamina propria. The accumulation of foamy macrophages in the mesenteric lymph nodes may have been related to an impairment of the clearance of the test substance.

Body weight losses in males at 75 and 175 mg/kg bw per day [redacted] and reductions of body weight gain in high-dose females before mating [redacted] and during gestation [redacted] were reported along with reduced food intake.

Changes in red blood cell parameters were observed in males at 75 mg/kg bw per day, i.e. low haemoglobin concentration (–[redacted]%, outside historical control range), mean cell haemoglobin concentration (–[redacted]%) and increased red cell distribution width (+[redacted]%) along with a dose-related increase in reticulocyte counts (+[redacted]%). Similar changes were observed in high-dose females with statistically significant effects only for haemoglobin and red cell distribution width. In addition, a dose-related increase in platelet counts was observed in males.

Changes in blood chemistry in females included dose-related decreases in liver enzymes (ALP, aspartate aminotransferase (AST)), which were not considered toxicologically relevant.

Higher mean relative (to body weight) organ weights were observed for kidneys (+[redacted]%) and livers (+[redacted]%) of high-dose females and for livers of males dosed at 75 mg/kg bw per day (+[redacted]%). These treatment-related increases in relative organ weights were not correlated with macroscopic or microscopic findings.

[29]The study was performed before the publication of OECD TGs.

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Changes in motor activity parameters of high-dose females were observed at days 7–9 of lactation, e.g. low beam scores. However, the extent of these changes was not considered adverse by the authors.

High-dose females were mated with males dosed at 75 mg/kg bw per day. Three high-dose females showed an irregular oestrous cycle, which was above the historical control data maximum. However, fertility and conception were unaffected by the treatment and no microscopic pathology changes were reported for the female reproductive organs.

Histopathology revealed epithelial hyperplasia in the non-glandular stomach of males dosed at 75 mg/kg bw per day and mid- and high-dose females. In the jejunum and ileum, foamy macrophages expanding the lamina propria of villi were observed in males at 75 mg/kg bw per day and as well as in mid- (jejunum) and high-dose (jejunum, ileum) females. Similarly, in the mesenteric lymph node, foamy macrophages expanding the sinuses were reported for males dosed at 75 mg/kg bw per day as well as mid- and high-dose females.

Treatment with 175 mg/kg bw per day resulted in a low post-implantation survival index, a lower total litter size, lower indices for viability on day 4 of lactation and lactation on day 13. No treatment-related effects were observed for the sex ratio, the anogenital distance in the male and female offspring and nipple retention in the male offspring. Treatment at 175 mg/kg per day was associated with some adverse clinical signs (e.g. offspring in three litters being recorded as cold to touch, with offspring in one of these litters having little/no milk in stomach and offspring in another litter being scattered in the cage). Male and female offspring from high- and mid-dose mothers had low body weights on day 1 of age and lower overall (day 1–13 of age) mean body weight gain for high-dose offspring.

In conclusion, the Panel considered that, due to the treatment-related mortality that occurred in four males and in three females administered with 175 mg/kg bw per day, caused by intestinal lesions, this dose level exceeded the maximum tolerated dose. The Panel noted that reductions in red blood cell parameters in males and microscopic findings in the GI-tract (foamy macrophages) in males and females were also present at 75 mg/kg bw per day. $BMDL_{10}$ estimates for epithelial hyperplasia in the stomach of F0 males and F0 females were 7 and 18 mg/kg bw per day, respectively.

Due to mortalities, clinical signs in offspring and reduced offspring survival (day 4 and day 13 lactation) at 175 mg/kg bw per day, the NOAEL for developmental toxicity was considered to be 75 mg/kg bw per day.

3.2.8.5 | *Prenatal developmental toxicity study in Wistar rats by oral administration*

A prenatal developmental toxicity study with 2,2′-((C16/18, C18 unsaturated)alkylimino)diethanol (Ethomeen T/12; CAS No 1218787-32-6; batch No [redacted]; purity [redacted]%; vehicle: arachis oil) was performed in Han Wistar rats (RccHan®:WIST). The study was performed in 2023 in compliance with GLP principles and following the OECD TG 414 (OECD, 2018c). Three groups of 22 females received the test substance at doses of 15, 50 or 150 mg/kg bw per day by gavage administration, from GDs 6 to 20. A similarly constituted control group received the vehicle, arachis oil. Animals were killed on day 21 after mating for reproductive assessment and fetal examination. The highest dose, i.e. 150 mg/kg bw per day, was chosen, based on results from a preliminary study showing a reduction of body weight gain of 40% in females treated with this dose compared to controls. When adjusted for gravid uterus weights, the treatment resulted in an overall loss of body weight (GD 6–21).

Thyroid hormone analysis in dams revealed a decrease of T4 and T3 concentrations. The latter attained statistical significance in the high-dose group (–[redacted]%). The TSH concentration was not statistically significantly increased in low- and high-dose dams.

The maternal responses were restricted to the high-dose group. Overall body weight gain during gestation (GDs 6–21) was reduced by 50%. On GD 21, there was a mean loss of body weight [redacted] when adjusted for the contribution of the gravid uterus. Overall food intake was [redacted] lower compared to controls. Absolute weights of thyroids and parathyroids were statistically significantly reduced in all treatment groups without dose–response. In one high-dose female, a macroscopically small thyroid gland was found. However, no microscopic findings in the thyroids were reported.

The numbers of non-pregnant females were two in controls, three in the low-dose group, one in the mid-dose group and four in the high-dose group. One female of each treatment group had a total litter resorption. In the high-dose group, the lower number of live offspring and higher pre- and post-implantation loss were outside of the historical control range. However, these effects did not attain statistical significance. A statistically significant reduction of total litter weight [redacted] was only observed in the high-dose group. There were no treatment-related effects on male, female or overall fetal weights, on the anogenital distance or on the incidence of major and minor abnormalities and skeletal variants.

In conclusion, the Panel considered that the NOAEL for maternal toxicity was 50 mg/kg bw per day, based on the effects on the body weight gain and body weight loss when corrected for gravid uterus at 150 mg/kg bw per day. Due to higher pre- and post-implantation losses which were outside of the historical control data and the reduction of total litter weight at 150 mg/kg bw per day, the developmental NOAEL was 50 mg/kg bw per day.

3.2.8.6 | *Potential for accumulation in humans*

No clear information was provided on the potential for accumulation of PFAEOs in humans. The foamy macrophage formation observed in toxicity studies with 2,2′-((C16/18, C18 unsaturated)alkylamino)diethanol (PFAEO-tallow) can be ascribed to phospholipid accumulation within cells. This effect may be considered as a common non-adverse response to this type of cationic surfactants. However, this assumption does not clearly describe the possible depot effect resulting in slow release of the substance.

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For all the representative PFAEOs, a prediction performed by the OECD QSAR Toolbox supports the same first metabolic reactions: oxidative N-dealkylation and hydroxylation of the penultimate or ultimate position of the alkyl chain.

3.2.9 | Impurities and degradation/reaction products

A comprehensive assessment of the safety of impurities was performed for the test material Atmer 163 (N,N-bis(2-hydroxyethyl)alkyl(C13-C15)amine). Under the SCF default exposure scenario, [redacted] impurities[30] migrating above 0.15 μg/kg food (equivalent to the threshold of toxicological concern (TTC) of 0.0025 μg/kg bw), were identified (see Section 3.1.7).

[redacted] of them identified by LC–MS were grouped in [redacted] **groups** of structurally related impurities; [redacted]

In addition, [redacted] **compounds** were identified by GC–MS. These compounds had functional groups that were not shared by other impurities and were considered separately: [redacted] The migration was below the SML available for two of them [redacted]. For the other [redacted] impurities, [redacted] a SML is not available.

For the [redacted] **groups of interest** [redacted] **and structurally related impurities and the additional** [redacted] **unrelated impurities without a SML**, an in silico assessment of genotoxicity was performed using the OECD QSAR Toolbox (profilers 'DNA alerts for AMES, CA and MNT by OASIS' and 'In vitro mutagenicity (Ames test) alerts by ISS').

- No alert was detected for the [redacted] **groups** [redacted] **of structurally related impurities** and for [redacted].
- No alert was identified by the QSAR Toolbox for [redacted].
- For [redacted], the presence of a simple aldehyde group was identified as a potential structural alert by the ISS and OASIS profilers. However, this prediction was overruled by the negative experimental data for Ames and in vitro micronucleus test available on [redacted] in the Toolbox database.
- [redacted] is closely related to [redacted] that has an SML hence does not raise concern for genotoxicity.
- [redacted] **and** [redacted] are closely related to [redacted] that have no SML hence do not raise concern for genotoxicity. They are grouped in Table 5.

The results obtained are summarised in Table 5.

TABLE 5 In silico assessment of the genotoxicity potential of the five groups of structurally related impurities and the additional four unrelated impurities.

Compound/group	Alerts for genotoxicity/profiler
[redacted]	No alert
[redacted]	No alert
[redacted]	No alert
[redacted]	No alert
[redacted]	SML
[redacted]	No alert
[redacted]	No alert
[redacted]	Negative results
[redacted]	SML
[redacted]	Covered by [redacted]
[redacted]	Covered by [redacted]
[redacted]	SML

[redacted]

In addition to the [redacted] impurities described above, which are related to (N,N-Bis(2-hydroxyethyl)alkyl(C13-C15)amine), the three other business operators reported the other [redacted] **impurities** below, for which the genotoxic hazard was evaluated based on available literature data [redacted] or using the OECD QSAR Toolbox [redacted]:

[30] [redacted]

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- [redacted]
- [redacted] **(#2)** and [redacted] **(#3)** are not classified for genotoxicity under the Regulation (EC) 1271/2008 on classification, labelling and packaging of substances and mixtures.[32] Hence, they do not raise concern for genotoxicity. [redacted]
- For the three remaining impurities [redacted] the assessment of genotoxicity was based on in silico data. Both general mechanistic (DNA binding capacity) and end-point specific (reverse mutations in bacteria (Ames test), chromosomal aberrations and micronuclei) profilers of the OECD QSAR Toolbox were interrogated. No structural alert was identified in any substance by end-point specific profilers. The indication of potential non-covalent DNA binding provided by the alert Hacceptor-path3-Hacceptor was not considered as shown to lack positive predictivity (Pradeep et al., 2021).

3.3 | Discussion

3.3.1 | Identity and migration potential

Data were submitted from four business operators on various substances falling strictly or not within the definition of FCM substance No 19 N,N-bis(2-hydroxyethyl)alkyl(C8-18)amine and reported to be used or not in FCM. No data or use were reported on the FCM substance No 20.

Two substances were clearly within the definition of the FCM substance No 19 and intended to be used in FCM. They were characterised and data on their migration potential were available. These are N,N-bis(2-hydroxyethyl)alkyl(C13/15) amine from [redacted] source (Croda/Cargill and LyondellBasel) and N,N-bis(2-hydroxyethyl)alkyl(C16/18)amine from [redacted] (Clariant).

[redacted]

These two substances are used as polymer processing aids and aid to polymerisation at up to 0.1% to manufacture PO intended for contact with all food types with a range of time/temperature conditions including frozen and ambient storage (Clariant) and up to no restrictions in time and temperature (Croda/Cargill).

The migration potential was tested for a range of thicknesses (from 80 µm films up to 2.5 mm plaques), amount used (from 0.058% up to 0.1% w/w, the maximum amount used), food simulant types (3% acetic acid, 20% ethanol, olive oil, 95% ethanol, isooctane, Tenax®), as well as contact time and temperature (up to 10 days at 60°C). Data, especially those complying with the testing conditions required for the contact conditions for all types of food and with no restriction of time, and temperature including hotfill and heating up to 100°C, showed that the migration (a) exceeds the current SML(T) of 1.2 mg/kg food in most of the tested conditions and (b) has the potential to largely exceed 5 mg/kg food depending on the use level and the type of application(e.g. at the maximum intended use level, at higher FCM thicknesses and higher contact temperatures). Migration from film of 80 µm thickness made with up to ca. 0.1% was shown to be below 5 mg/kg food. Migration into dry foods was compliant with the SML(T) of 1.2 mg/kg food. Migration into 95% ethanol, iso-octane, 20% ethanol and 3% acetic acid for 10 days at 60°C was above 1.2 and below 5 mg/kg food.

Insufficient data were provided from Nouryon on five PFAEO from natural origin, in particular on their manufacture, their characterisation and their migration potential. Moreover, no indication was provided with regard to their use to manufacture FCM.

The impurities were tentatively identified and summed up to [redacted] individual compounds for N,N-bis(2-hydroxyethyl) alkyl(C13/15)amine plus an additional [redacted] compounds identified in N,N-bis(2-hydroxyethyl)alkyl(C16/18)amine and/or the PFAEO substances from Nouryon (see 3.1.7). Considering the impurities individually, as a group of structurally related impurities or as a sum of all impurities (specifications up to 5%), their potential migration pro-rata to a potential migration of the substance up to 5 mg/kg food is expected to be below 0.05 mg/kg food and in the range of 0.05 mg/kg food for [redacted].

[31] [redacted]

[32] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

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3.3.2 | Genotoxicity

Several adequate in vitro genotoxicity studies with N,N-bis(2-hydroxyethyl)alkylamines were available.

Tests for the induction of gene mutations in bacteria and/or mammalian cells with N,N-bis(2-hydroxyethyl)alkyl(C8), alkyl(C13/C15) and alkyl(C16/C18)amines provided consistent negative results.

An in vitro micronucleus test in human lymphocytes provided negative results with bis(2-hydroxyethyl)alkyl(C13/C15) amine, considered as a suitable representative substance for the FCM substance No 19, since it includes components with linear and branched alkyl groups and is in the mid-range of the alkyl chain length. An in vitro test for induction of structural chromosome aberrations in human lymphocytes also produced negative results in the presence of metabolic activation, while an equivocal response was obtained in the absence of metabolic activation.

In vivo micronucleus tests in mouse bone marrow with C8 and tallow C18 compounds, although considered of limited relevance, provided overall negative results.

The Panel noted that negative results were also obtained in the supportive studies provided by Nouryon with oleyl(C18:9) and coco-alkyl(C12-18)compounds in gene mutation tests in bacteria and mammalian cells, and for in vitro chromosomal aberrations, including polyploidy, in human lymphocytes.

The Panel considered that the studies provided adequate information on all genotoxicity endpoints and, based on the negative results obtained in the highly relevant in vitro studies, concluded that the FCM substance No 19 does not raise concern for genotoxicity.

Based on in silico assessment, read-across with authorised substances or available studies, the Panel considered that the identified impurities do not raise concern for genotoxicity.

3.3.3 | Subchronic toxicity

Seven 90-day studies were submitted with different N,N-bis(2-hydroxyethyl)alkylamines. Three studies, two on (C13/C15)- and one on (C16/C18)alkyl derivatives, were considered reliable for risk assessment. The four other studies provided by Nouryon on the related PFAEO substances were considered as supportive.

- The subchronic oral toxicity of N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine in rats and dogs was evaluated in two reliable studies.
    - In rats, the observed findings included ophthalmological effects ($BMDL_{10}$: 27 mg/kg bw per day in males), gross findings (thickened mucosa; $BMDL_{10}$ values: 24 and 22 mg/kg bw per day in males and females, respectively) and histopathological findings (acanthosis) in non-glandular stomach ($BMDL_{10}$ values: 20 and 23 mg/kg bw per day in males and females, respectively), indicative of an inflammatory process likely due to the irritative properties of the test substance.
    - In dogs, clinical signs (i.e. salivation and emesis) in both sexes and liver histopathological changes, correlated with circulating alanine aminotransferase (ALT) and pigment accumulation in Kupffer cells, specified the liver as one of the target organs of the substance. The Panel identified a NOAEL of 30 mg/kg bw per day.

- The subchronic oral toxicity of N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine in rats was evaluated in a reliable study. Based on treatment-related macroscopic (thickening) and histopathological findings (epithelial hyperplasia and sub-mucosal inflammation) at the first site of contact (non-glandular stomach), considered as the main critical effects, $BMDL_{10}$ values of 44 and 20 mg/kg bw per day were estimated.
- In supporting subchronic toxicity studies with different primary ethoxylated fatty amines from natural sources (Nouryon), the toxicological profile is coherent, despite the different saturated or unsaturated chain length and origins of each test substance. Treatment-related macroscopic (thickening) and histopathological findings (epithelial hyperplasia and hyperkeratosis) at the first site of contact (non-glandular stomach) were observed.[33]

The relevance for humans of the changes observed in the non-glandular stomach can be questioned. However, it is considered that the mucosa of other parts of the GI tract (in species with no forestomach) can react to the test substance in a similar way as the non-glandular stomach epithelium in rodents, if equivalent exposure levels are reached. Moreover, the Panel noted that gavage administration to experimental animals generally results in higher substance concentration in the GI tract compared with those after dietary exposure. Therefore, it can be expected that local irritating effects in the GI tract seen in gavage studies lead to an overestimation of the risk in humans who are exposed to the substances via the diet. In addition, effects in the small intestine were also reported in two 90-day dietary studies with PFAEO-tallow, one in rats and one in dogs (with limitations in these studies and used only as supporting evidence). Overall, these findings from

[33]In the supporting studies with the Nouryon PFAEO substances, likely related to the irritant properties of the PFAEO-coco, a lower $BMDL_{10}$ would equal to 5 (thickening and acanthosis).

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the PFAEO studies strengthen the relevance of the local effects in the non-glandular stomach after gavage administration for the risk assessment.

Based on the histopathological findings observed at the level of the first site of contact (non-glandular stomach) in the subchronic toxicity studies with N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine and N,N-bis(2-hydroxyethyl)alkyl(C16/C18) amine, the Panel identified the lowest $BMDL_{10}$ equal to 20 mg/kg bw per day.

3.3.4 | Reproductive/developmental toxicity

The studies on reproductive/developmental toxicity comprise two prenatal developmental studies with N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine and one reproduction/developmental toxicity screening test with N,N-bis(2-hydroxy) alkyl(C8)amine. In addition, 10 supporting reproductive/developmental studies (two in combination with repeated dose toxicity) were performed with PFAEOs (Nouryon).

Among the two prenatal developmental studies in rats with N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine, the substance was administered by gavage in one study and via the diet in the other. While maternal toxicity was observed in both studies (reduced body weight gain), litter effects (post-implantation loss) were only observed in the gavage study at the highest dose (90 mg/kg bw per day). There were developmental effects observed in both studies, but the developmental toxicity in the dietary study (increase in fetal external malformations) was reported at a threefold higher dose compared to that in the gavage study. Based on increased incidences of skeletal abnormalities (cervical vertebrae) observed in the latter study, the Panel considered that the lowest BMDL for systemic effects was a $BMDL_5$ of 12 mg/kg bw per day.

In these two prenatal developmental studies with N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine, local/histological effects were not investigated. With N,N-bis(2-hydroxyethyl)alkyl(C8)amine, there were no local and no clearly adverse systemic effects up to the highest dose tested (320 mg/kg bw per day).

In the supporting reproductive/developmental studies performed with PFAEOs, no adverse effects on reproductive parameters (mating performance, fertility and gestation length) were reported. Litter effects in the high-dose groups were considered to be related to toxic effects in dams.[34] No adverse endocrine effects were reported in males and females. The Panel noted that the general toxicity pattern in the studies on reproductive/developmental toxicity with the PFAEOs was in line with the findings in the related subchronic studies, including macroscopic and histopathological changes in the GI tract, reductions in body weight gain and food intake as well as changes in haematological parameters and organ weights.

3.3.5 | Potential for accumulation in humans

No experimental data have been provided on the accumulation potential of the entire category of N,N-ethoxylated alkylamines in humans.

However, in a repeated 90-day oral toxicity study with linear N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine, the urinary excretion of the metabolite DEA, produced by the cleavage of the alkyl chain, was evaluated. Its formation and urinary elimination were followed during the 4-week recovery period of the 90-day study. During the recovery period, no new DEA was formed from potentially bioaccumulated parental substance since DEA was almost completely eliminated in 4 weeks.

In addition, two in silico prediction models ([redacted]) were used to evaluate the potential for accumulation in humans of linear N,N-bis(2-hydroxyethyl)alkylamines. [redacted]

Based on the experimental data and in silico predictions provided, the Panel concluded that the metabolism of the linear N,N-bis(2-hydroxyethyl)alkylamines results in molecules that enter well-known metabolic pathways, which ultimately result in the excretion of degradation products through air (carbon dioxide) or urine. Therefore, the Panel considered that the potential accumulation in humans of linear N,N-bis(2-hydroxyethyl)alkylamines is not of concern.

The FCM substance No 19 includes branched alkylamines, such as Atmer 163 ((C13/C15)alkyl derivatives) that may undergo different metabolic pathways. However, the available general toxicity studies on linear and branched derivatives showed consistent toxicological patterns.

Therefore, the Panel concluded that the potential accumulation in humans of linear and branched N,N-bis(2-hydroxyethyl)alkylamines is not of concern.

[34] In the supporting studies with the Nouryon PFAEO substances, the lowest BMDL for systemic effect was a $BMDL_1$ of 18 mg/kg bw per day (PFAEO-coco) based on rare visceral abnormalities (microphthalmia and anophthalmia).

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3.3.6 | Point of departure and margin of safety

3.3.6.1 | *Systemic effects*

For systemic effects, the Panel noted that the lowest BMDL was derived from developmental effects in rats treated with N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine, i.e. skeletal abnormalities with a $BMDL_5$ of 12 mg/kg bw per day. As supporting evidence, another developmental effect (visceral abnormality) was observed after treatment with PFAEO-coco ($BMDL_1$ of 18 mg/kg bw per day). The Panel noted that the $BMDL_5$ of 12 mg/kg bw per day is the most suitable point of departure for systemic effects, because it would also cover other findings of N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine, such as ophthalmological effects ($BMDL_{10}$ of 27 mg/kg bw per day). Taking into account the data set available, the Panel considered that a margin of exposure (MoE) approach could be applied and that according to the EFSA Note for Guidance the migration should not exceed 5 mg/kg food.

Given an exposure of 0.08 mg/kg bw per day resulting from a maximum migration of 5 mg/kg food when using standard assumptions for FCM (1 kg food/person; 60 kg bw) and the $BMDL_5$ of 12 mg/kg bw per day for developmental effects, a margin of safety (MoS) of 150 was calculated. Considering the toxicological data provided and an overall default uncertainty factor (UF) of 100 for interspecies (extrapolation from rat studies to the human situation) and intra-individual differences (in the human population), the Panel considered the MoS of 150 as sufficient to conclude on no safety concern for systemic toxicity.

3.3.6.2 | *Local effects*

In a subchronic toxicity studies in rats treated by gavage with N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine, local effects in the non-glandular stomach were observed with estimated $BMDL_{10}$ values of 20 and 23 mg/kg bw per day in males and females, respectively. Similar effects in the non-glandular stomach were observed in another subchronic rat study with N,N-bis(2-hydroxyethyl)alkyl(C16/C18)amine with an estimated $BMDL_{10}$ of 20 mg/kg bw per day.

As supportive evidence, further rat studies with gavage administration of different N,N-ethoxylated alkylamines from natural sources (PFAEOs substances from Nouryon) showed a coherent toxicological profile with local irritant effects in different parts of the GI tract (including in the jejunum).[35] These findings from the PFAEO studies strengthen the relevance of the local effects in non-glandular stomach after gavage administration for the risk assessment.

Applying a conservative approach for the risk assessment, the Panel considered the lowest $BMDL_{10}$ for local effects of 20 mg/kg bw per day as a possible point of departure for local effects.

Given a maximum exposure of 0.08 mg/kg bw per day resulting from a maximum migration of 5 mg/kg food and the $BMDL_{10}$ of 20 mg/kg bw per day (same for N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine and N,N-bis(2-hydroxyethyl) alkyl(C16/C18)amine), an MoS of 250 was calculated for local irritant effects. The Panel considered this MoS of 250 sufficient to conclude on no safety concern for local toxicity.

3.3.7 | Overall discussion

Based on the available data and the EFSA Note for Guidance, the Panel considers a migration of up to 5 mg/kg food to be of no safety concern.

Except for dry food, the migration tests showed that the current SML(T) of 1.2 mg/kg food is exceeded in many cases, even from thin films.

The migration does not exceed 5 mg/kg food under the test conditions of 10 days at 60°C for 80 µm PO films with up to 0.1% of the substance. However, for other uses, the migration of the substance has the potential to clearly exceed 5 mg/kg, depending on the combination of the three main factors: (i) the use level, (ii) the FCM thickness and (iii) the condition of food contact. In that situation, the EFSA Note for Guidance requires either a full toxicological data set – that was not provided – or to reduce the intended uses so that the migration is in accordance with the toxicological data provided.

The Panel noted that, when derived from fatty acids, the alkyl group is almost exclusively linear. However, in N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine (Atmer 163), [redacted]% the alkyl group was reported to be [redacted]. This fraction was assessed by the toxicological data, but the data do not cover other branching that could occur when the alkyl amine is from other sources. Therefore, the Panel recommends a restriction to alkyl groups derived from hydrogenated vegetable oils or ethylene oligomers[36] with a high degree of linear structure.

If the migration of the substance does not exceed 5 mg/kg food, the potential migration of the impurities is not expected to exceed 0.05 mg/kg food or in the range of 0.05 mg/kg food for [redacted] that is covered by the absence of genotoxicity of the detected impurities as long as the impurities do not exceed 5% and the substance is manufactured from the two sources reported above.

[35] In the supporting studies with the Nouryon PFAEO substances, the lowest $BMDL_{10}$ of around 4 mg/kg bw per day was estimated ((C12-18)alkyl derivatives (PFAEO-coco) and (C18:9)alkenyl derivatives (PFAEO-oleyl).

[36] The C13 and C15 alkyl groups are obtained from C14 and C16 α-olefins by oxidation. The α-olefins are made of ethylene oligomers.

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None of the business operators indicated uses in contact with infant foods, such as human milk and infant formulae or water that could be used to reconstitute such formulae. The available toxicological data do not cover food for infants below 16 weeks of age. Thus, in principle, contact with water[37] is not covered by the assessment. While some of the indicated uses (film, trays) do not lead to expect such contact, N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine (Atmer 163) was indicated to be used to manufacture caps, e.g. for PET bottles, hence in articles in contact with water. The thickness of caps, said to be around 1 mm, is expected by the Panel to be up to 1.5 mm. Considering the limited solubility of the FCM substance No 19 in water and the low surface to volume ratio when used in caps for bottles in contact with drinking water, the migration arising from the use in caps to close bottles in contact with water is expected to be negligible. Therefore, the Panel considered that the use of the substance in caps of bottles does not raise a safety concern, including bottles in contact with water used to reconstitute infant formulae.

The three business operators use the substance as a 'polymer production aid' and/or 'processing aid', and the data provided correspond to these uses. None of them is claiming its use as an 'additive' as defined by the Regulation (EU) No 10/2011 on plastics. The Panel noted that such a use would not affect the safety assessment and its conclusion.

3.3.8 | N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides

No business operator provided information on the substance N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides (FCM No 20) in response to the call for data. Following the EFSA request for additional data, LyondellBasell, Clariant, Nouryon and Croda declared not using the FCM substance No 20.

No information was submitted through the EFSA call of data[38] and no uses were claimed. Since no data nor argumentation were provided to support its safety assessment, the FCM Panel did not assess the safety of the use of the substance N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides in plastic FCM.

4 | CONCLUSION

Based on the provided data, the FCM Panel concluded that the authorisation of the substance N,N-bis(2-hydroxyethyl) alkyl(C8-C18)amine (FCM substance No 19) is not in accordance with the Regulation (EC) No 1935/2004, since the migration is likely to exceed the current SML(T) of 1.2 mg/kg food under certain conditions of use.

Based on the provided data, the FCM Panel concluded that the FCM substance No 19, N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine is not of safety concern for the consumer if:

1. the substance is used at up to 0.1% w/w as polymer production aid and as processing aid to manufacture polyolefin materials and articles of thickness up to 1 mm that are intended for contact with all types of food except infant foods. This exception for infant foods and the restriction for maximum thickness do not apply to caps of bottles;
2. the migration does not exceed 5 mg/kg food;
3. the source of the alkyl group is either from hydrogenated vegetable oil or synthetic from ethylene oligomers with a high degree of linear structure;
4. the impurities do not exceed 5% w/w.

The Panel noted that the migration of the substance is expected to exceed 5 mg/kg food under the combination of high amount and/or high thickness and/or long-term storage and/or elevated temperature.

The information available from REACH and submitted on the five substances, PFAEO-coco, PFAEO-oleyl, PFAEO-HT, PFAEO-T and PFAEO-C18 were considered supportive. They bear unsaturation, thus do not fall within the scope of the FCM substance No 19, and their use to manufacture FCM was not confirmed in the submissions. If they were intended to be used to manufacture plastic FCM, a proper application following the EFSA Guidance documents should be submitted.

No uses of the FCM substance No 20, N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine hydrochlorides, were claimed and no information was provided to support that its current authorisation is in accordance with the Regulation (EC) No 1935/2004.

5 | DOCUMENTATION AS PROVIDED TO EFSA

1. Cargill Corporate France, April 2024. Additional information provided during the assessment process in response to the requests from EFSA sent on 10 November 2022 to Croda International PLC.
2. Croda International PLC, June 2022. Submission of data in response to the call for technical and toxicological data on the substances on N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine (FCM No 19) and N,N-bis(2-hydroxyethyl)alkyl (C8-C18)

[37]Contact with water is understood as foreseen like in the case of water bottle and unforeseen in the case of other bottles (e.g. soft drinks).

[38]https://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-substances-nn-bis2.

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amine hydrochlorides (FCM No 20) for their use in plastic materials and articles intended to come into contact with food, including in food for infants.
3. Croda Europa Limited, October 2019. Dossier for the re-evaluation of N,N-Bis(2-hydroxyethyl) alkyl (C8-C18) amine (FCM PM Ref No 39090), gathering the submission from the Consortium in 2006 and the submission from Croda in 2014.
4. Croda International PLC, November 2014. Dossier for the re-evaluation of N,N-Bis(2-hydroxyethyl) alkyl (C8-C18) amine (PM Ref No 39090).
5. Consortium made of the Uniqema division of ICI, CECA, Akzo-Nobel, Clariant and Kao, March 2006. Dossier on N,N-Bis(2-hydroxyethyl) alkyl (C8-C18) amine providing additional data to the initial application in April 1994.
6. Clariant Produkte GmbH, July 2022. Submission of data in response to the call for technical and toxicological data on the substances N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine (FCM No 19) and N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine hydrochlorides (FCM No 20) for their use in plastic materials and articles intended to come into contact with food, including in food for infants.
7. Clariant Produkte GmbH, September 2023. Additional information provided during the assessment process in response to the requests from EFSA sent on 10 November 2022.
8. European Commission, April 2019. Summary datasheet of the EC SCF Working Group on Food Contact Materials and exchange of information on the substances N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine (PM Ref No 39090 (FCM No 19)) and N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine hydrochlorides (PM Ref No 39120 (FCM No 20)).
9. Nouryon Surface Chemistry AB on behalf of the Primary Fatty Amine Ethoxylates (PFAEO) Consortium, June 2022. Submission of data in response to the call for technical and toxicological data on the substances N,N-bis(2-hydroxyethyl) alkyl (C8-C18) amine (FCM No 19) and N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine hydrochlorides (FCM No 20) for their use in plastic materials and articles intended to come into contact with food, including in food for infants.
10. Nouryon Surface Chemistry AB on behalf of the Primary Fatty Amine Ethoxylates (PFAEO) Consortium, November 2023. Additional information provided during the assessment process in response to the requests from EFSA sent on 10 November 2022.
11. LyondellBasell Industries N.V., July 2022. Submission of data in response to the call for technical and toxicological data on the substances N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine (FCM No 19) and N,N-bis(2-hydroxyethyl)alkyl (C8-C18) amine hydrochlorides (FCM No 20) for their use in plastic materials and articles intended to come into contact with food, including in food for infants.
12. LyondellBasell Industries N.V., May 2023. Additional information provided during the assessment process in response to the requests from EFSA sent on 10 November 2022.
13. RIVM, January 2020. Summary datasheet of the SCF Working Group on Food Contact Materials supporting application to the EC on N,N-bis(2-hydroxyethyl)lauramide and coconut oil fatty acids diethanolamide.

ABBREVIATIONS

ABS	acrylonitrile/butadiene/styrene
ADI	acceptable daily intake
AFC Panel	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
ALP	alanine aminotransferase
ALT	alanine transaminase
AST	aspartate aminotransferase
BMD	benchmark dose
BMDL	lower confidence limit of the benchmark dose
BMR	benchmark response
BOPP	biaxially oriented polypropylene
bw	body weight
CAS	Chemical Abstracts Service
CBPI	Cytokinesis-Block Proliferation Index
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoA	certificate of analysis
DEA	diethanolamine
DMSO	dimethyl sulfoxide
DSC	differential scanning calorimetry
ECHA	European Chemicals Agency
EOGRT	Extended One Generation Reproductive Toxicity
FCM	food contact materials
FDA	US Foods and Drugs Administration
FTIR	Fourier-transform infrared spectroscopy
GC-FID	gas chromatography-flame ionisation detection
GC-MS	gas chromatography-mass spectrometry
GD	gestational day
GGT	gamma-glutamyl transferase
GI	gastrointestinal

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GLP	good laboratory practices
HDL	high-density lipoprotein
HDPE	high-density polyethylene
IARC	International Agency for Research on Cancer
IR	infrared spectroscopy
LC-MS	liquid chromatography-mass spectrometry
LD_{10}	lethal dose 10%
LDL	low-density lipoprotein
LDPE	low-density polyethylene
LOAEL	lowest observed adverse effect level
LOD	limit of detection
MEA	monoethaloamine
MoE	margin of exposure
MoS	Margin of safety
MTD	maximum tolerated dose
NA	not available
NCE	normochromatic erythrocytes
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD TG	Organisation for Economic Co-operation and Development Testing Guidelines
p.c.	post coitum
PCE	polychromatic erythrocytes
PE	polyethylene
PEG	polyethylene glycol
PET	polyethylene terephthalate
PFAEO	primary fatty amine ethoxylates
PO	polyolefins
Po/w	octanol/water partition coefficient
PS	polystyrene
PP	polypropylene
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation
RIVM	Dutch National Institute for Public Health and the Environment
SCF	Scientific Committee on Food
SML	specific migration limit
T3	triiodothyronine
T4	thyroxine
TDI	tolerable daily intake
TMS	trimethylsilyl
ToR	terms of reference
TSH	thyroid-stimulating hormone
TTC	threshold of toxicological concern
t-TDI	temporary tolerable daily intake
UF	uncertainty factor
UVCB	unknown or variable composition, complex reaction products and biological materials
UV/VIS	ultraviolet/visible spectroscopy
WG	working group

REQUESTOR

European Commission

QUESTION NUMBERS

EFSA-Q-2019-00483, EFSA-Q-2019-00484

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REFERENCES

EFSA (European Food Safety Authority). (2008). Note for Guidance for the preparation of an application for the safety assessment of a substance to be used in plastic food contact materials. *EFSA Journal*, *6*(7), 21r. https://doi.org/10.2903/j.efsa.2008.21r

EFSA (European Food Safety Authority). (2009). Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. *EFSA Journal*, *7*(5), 1051. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Silano, V., Solecki, R., Turck, D., Younes, M., Aquilina, G., Crebelli, R., Gürtler, R., … Schlatter, J. (2017). Clarification of some aspects related to genotoxicity assessment. *EFSA Journal*, *15*(12), 5113. https://doi.org/10.2903/j.efsa.2017.5113

EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T. I., Hernández-Jerez, A. F., Bennekou, S. H., Koutsoumanis, K., Lambré, C., Machera, K., Mennes, W., Mullins, E., Nielsen, S. S., Schrenk, D., Turck, D., Younes, M., Aerts, M., Edler, L., … Schlatter, J. (2022). Guidance on the use of the benchmark dose approach in risk assessment. *EFSA Journal*, *20*(10), 7584. https://doi.org/10.2903/j.efsa.2022.7584

European Commission. (1997). Reports of the scientific committee for food (thirty-third series); first report of the scientific committee for food on certain additives used in the manufacture of plastic materials intended to come into contact with foodstuffs (opinions expressed until 3 May 1992). https://aei.pitt.edu/40842/1/33rd_food.pdf

European Commission. (2001a). Guidelines of the scientific committee on food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out82_en.pdf

European Commission. (2001b). Opinion of the scientific committee on food on the 13th additional list of monomers and additives for food contact materials. https://ec.europa.eu/food/system/files/2020-12/sci-com_scf_out86_en.pdf

Kaling, M. (2017). Determination of monoethanolamine and diethanolamine in rat urine and serum. Clariant Produkte (Deutschland) GmbH CLAS, R&D Analytics ClC. Study no. 16-005211. Unpublished report.

Lee, M. S., Kaling, M., Dierdof, I., & Kreiling, R. (2017). Assessment of systemic exposure to metabolically generated mono- and diethanolamine in rats treated with 2,2′-(C12-18 alkylimino)-diethanol. Unpublished report.

Mathews, J. M., Garner, C. E., Black, S. L., & Matthews, H. B. (1997). Diethanolamine absorption, metabolism and disposition in rat and mouse following oral, intravenous and dermal administration. *Xenobiotica*, *27*(7), 733–746. https://doi.org/10.1080/004982597240316

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1983). *Test no. 474: Mammalian erythrocyte micronucleus test*. OECD Publishing. https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/testing-of-chemicals/previous-test-guidelines/section-4/replaced-test-guidelines/replaced-test-guideline-474-1983.pdf

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1995a). *Test no. 102: Melting point/melting range, OECD guidelines for the testing of chemicals, section 1*. OECD Publishing. https://doi.org/10.1787/9789264069527-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1995b). *Test no. 103: Boiling point, OECD guidelines for the testing of chemicals, section 1*. OECD Publishing. https://doi.org/10.1787/9789264069541-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1995c). *Test no. 105: Water solubility, OECD guidelines for the testing of chemicals, section 1*. OECD Publishing. https://doi.org/10.1787/9789264069589-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1995d). *Test no. 421: Reproduction/developmental toxicity screening test*. OECD Publishing. https://doi.org/10.1787/9789264070967-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1996). *Test no. 422: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test*. OECD Publishing. https://doi.org/10.1787/9789264070981-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1997a). *Test no. 471: Bacterial reverse mutation test, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/testing-of-chemicals/previous-test-guidelines/section-4/replaced-test-guidelines/replaced-test-guideline-471-1997.pdf

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1997b). *Test no. 476: in vitro mammalian cell gene mutation test*. OECD Publishing. https://doi.org/10.1787/9789264071322-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1997c). *Test no. 473: In vitro mammalian chromosome aberration test*. OECD Publishing. https://doi.org/10.1787/9789264071261-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1997d). *Test no. 474: Mammalian erythrocyte micronucleus test*. OECD Publishing. https://doi.org/10.1787/9789264071285-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (1998). *Test no. 408: Repeated dose 90-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/testing-of-chemicals/previous-test-guidelines/section-4/replaced-test-guidelines/e408_1998.pdf

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2001). *Test no. 414: Prenatal developmental toxicity study, OECD guidelines for the testing of chemicals, section 4*. OECD publishing. https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/testing-of-chemicals/previous-test-guidelines/section-4/replaced-test-guidelines/replaced-test-guideline-414-2001.pdf

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2008). *Test no. 407: Repeated dose 28-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264070684-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2016a). *Test no. 490: in vitro mammalian cell gene mutation tests using the thymidine kinase gene, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264264908-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2016b). *Test no. 474: Mammalian erythrocyte micronucleus test, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264264762-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2016c). *Test no. 422: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264264403-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2018a). *Test no. 408: Repeated dose 90-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264070707-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2018b). *Test no. 443: Extended one-generation reproductive toxicity study, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/97892641853 71-en

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2018c). *Test no. 414: Prenatal developmental toxicity study, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264070820-en

18314732, 2025, 2, Downloaded from https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2025.9104 by Turkey Cochrane Evidence Aid, Wiley Online Library on [11/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

OECD (Organisation for Economic Co-operation and Development Testing Guidelines). (2023). *Test no. 487: In vitro mammalian cell micronucleus test, OECD guidelines for the testing of chemicals, section 4*. OECD publishing. https://doi.org/10.1787/9789264264861-en

Pradeep, P., Judson, R., DeMarini, D. M., Keshava, N., Martin, T. M., Dean, J., Gibbons, C. F., Simha, A., Warren, S. H., Gwinn, M. R., & Patlewicz, G. (2021). Evaluation of existing QSAR models and structural alerts and development of new ensemble models for Genotoxicity using a newly compiled experimental dataset. *Computational Toxicology*, *18*, 100167. https://doi.org/10.1016/j.comtox.2021.100167

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APPENDIX A

Data submitted on toxicological studies

	Chemical name	Trade name	Cas no	Lot/batch no	Purity	GLP (Y/N; date)	OECD TG (Y/N; date)
CRODA/CARGIL							
N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine							
Gene mutation in bacteria	NA	Atmer 163	NA	310F0262	99%	Yes, 1997	OECD TG 471 (1997)
In vitro mammalian cell gene mutation test	NA	Atmer 163	NA	310F0262	99%	Yes, 1997	OECD TG 476 (1997)
In vitro mammalian chromosomal aberration test	NA	Atmer 163	NA	310F0262	99%	Yes, 1997	OECD TG 473 (1997)
In vitro micronucleus test	NA	Atmer 163	NA	1212BD7545	> 99%	Yes, date NA	OECD TG 487 (2016) (corr. 2023)
Subchronic tox study in dogs	NA	Atmer 163	NA	NA	NA*	Yes, 21CFR, part 58 (FDA)	NA
Subchronic tox study in rats	NA	Atmer 163	NA	NA	NA	Yes, 21CFR, part 58 (FDA)	NA
Prenatal Developmental Toxicity Study in the Han Wistar Rat	Ethanol,2,2′-iminobis-, N-(C13/15-branched and linear alkyl) derivatives	Atmer 163	NA	2206ND5315	99% (as supplied) 100% (for formulation)	Yes, 2005	OECD TG 414 (2001)
LYONDELLBASELL							
Prenatal developmental toxicity study in Sprague Dawley rats	NA	Atmer 163-LQ-(AP)	NA	0000870154	99%	EPA GLP Standards 40 CFR Part 160 and 40 CFR Part 792	OECD TG 414 (2001)
CLARIANT							
N,N-bis(2-hydroxyethyl)alkyl(C8)amine							
Gene mutation in bacteria	2,2′-(Octylimino)diethanol	Genamin 3920	15520-05-5	[redacted]	[redacted]	Yes, 1998	OECD TG 471 (1997)
In vitro mammalian cell gene mutation test	2,2′-(Octylimino)diethanol	Genamin 3920	15520-05-5	[redacted]	[redacted]	Yes, 1998	OECD TG 476 (1997)
In vivo micronucleus test	2,2′-(Octylimino)diethanol	Genamin 3920	15520-05-5	[redacted]	[redacted]	Yes, 1998	OECD TG 474 (1997)
28-day repeated dose oral toxicity study in Wistar rats, including 14-day recovery period	2,2′-(Octylimino)diethanol	Genamin 3920	15520-05-5	[redacted]	[redacted]	Yes, 1998	OECD TG 407 (2008)
Reproduction/developmental Tox screening test in Wistar rats	2,2′-(Octylimino)diethanol	Genamin 3920	15520-05-5	[redacted]	[redacted]	Yes, 1998	OECD TG 421 (1995)

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(Continued)

	Chemical name	Trade name	Cas no	Lot/batch no	Purity	GLP (Y/N; date)	OECD TG (Y/N; date)
N,N-bis(2-hydroxyethyl)alkyl (C16/C18)amine							
Gene mutation in bacteria	N,N-bis(2-hydroxyethyl) alkyl(C16/C18)amine	Hostastat FA 68 V	1218787-30-4	CNP1015848	[redacted]%	Yes, 1998	OECD TG 471, 1997 corr. (2020)
Repeated dose 90-day oral toxicity study by oral gavage in Wistar rats with 28-day recovery period	N,N-bis(2-hydroxyethyl) alkyl(C16/C 18)amine	Hostastat FA 68 V	1218787-30-4	CNP1028238	[redacted]	Yes, 1997	OECD TG 408 (2018)
Urinary excretion of the metabolite diethanolamine after repeated 90-day treatment by oral gavage in Wistar rats and bioaccumulation potential	N,N-bis(2-hydroxyethyl) alkyl(C16/C 18)amine	Hostastat FA 68 V	1218787-30-4	CNP1028238	[redacted]	Yes, 1997	OECD TG 408 (2018)
N,N-bis(2-hydroxyethyl)alkyl (tallow C18)amine							
In vivo micronucleus test	Tallow Amine Ethoxylate	[redacted]	NA	NA	[redacted]%	Yes, date NA	OECD TG 474 (1983)
NOURYON							
PFAEO-coco							
Gene mutation in bacteria	N,N-bis(2-hydroxyethyl) alkyl(coco)amine	[redacted]	61791-31-9	[redacted]	[redacted]%	Yes, 1999	OECD TG 471 (1997)
In vitro mammalian cell gene mutation test	N,N-bis(2-hydroxyethyl) alkyl(coco)amine	[redacted]	61791-31-9	[redacted]	[redacted]%	Yes, 1999	OECD TG 476 (1997)
In vitro mammalian chromosome aberration test	N,N-bis(2-hydroxyethyl) alkyl(coco) alkylamine	[redacted]	61791-31-9	[redacted]	[redacted]%	Yes, 1999	OECD TG 473 (1997)
90-day toxicity study by oral gavage administration to Han Wistar Rats	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives 2,2′-(C12-18 even-numbered alkyl imino) diethanol N,N-bis(2-hydroxyethyl) alkyl(coco)amine	[redacted]	71786-60-2	[redacted]	[redacted]%	Yes, 1999	OECD TG 408 (1998)
Oral combined repeat dose toxicity study with reproduction/developmental toxicity screening test in rats	N,N-bis(2-hydroxyethyl) alkyl(coco)amine	[redacted]	61791-31-9	[redacted]	NA	Yes, 1999	OECD TG 422 (1996)

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	Chemical name	Trade name	Cas no	Lot/batch no	Purity	GLP (Y/N; date)	OECD TG (Y/N; date)
Study for Effects on Embryo-Fetal Development in Han Wistar Rats	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives 2,2′-(C12-18 even numbered alkylimino) diethanol N,N-bis(2-hydroxyethyl) alkyl(coco)amine.	[redacted]	71786-60-2 and 61791-31-9	[redacted]	[redacted]%	Yes, 1999	OECD TG 414, date NA
Study for Effects on Embryo-Fetal Development in New Zealand White Rabbits by Oral Gavage Administration	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives. 2,2′-(C12-18 even numbered alkylimino) diethanol. N,N-bis(2-hydroxyethyl) alkyl(coco)amine	[redacted]	71786-60-2	[redacted]	[redacted]%	Yes, 1999	OECD TG 414, date NA
Extended One Generation Reproductive Toxicity Study in Han Wistar Rats by Oral Gavage Administration	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives. 2,2′-(C12-18 even numbered alkylimino) diethanol. N,N-bis(2-hydroxyethyl) alkyl(coco)amine	[redacted]	71786-60-2	[redacted]	99.6%	Yes, date NA	OECD TG 443 (2018)
PFAEO-oleyl							
Gene mutation in bacteria	NA	CECAJEL 210	[redacted]-[redacted]	[redacted]	[redacted]%	Yes, date NA	OECD TG 471 (1997)
In vitro mammalian cell gene mutation test	2,2′-(octadec-9-enylimino) diethanol	NA	25307-17-9	[redacted]	[redacted]%	Yes, 1997	OECD TG 476 (1997)
In vitro mammalian chromosome aberration test	2,2′-(octadec-9-enylimino) diethanol	NA	25307-17-9	[redacted]	[redacted]%	Yes, 1997	OECD TG 473 (1997)
90-day oral toxicity study in rats	2,2′-(octadec-9-enylimino) diethanol	[redacted]	25307-17-9	[redacted]	[redacted]%	Yes, 1997	OECD TG 408 (1998)
Oral (Gavage) Pre-Natal Development Toxicity Study in Rats	2,2′-(octadec-9-enylimino) diethanol	NA	25307-17-9	[redacted]	[redacted]%	Yes, 1997	OECD TG 414 (2001)
Extended One Generation Reproductive Toxicity Study in Han Wistar Rats by Oral Gavage Administration	2,2′-(Octadec-9-enylimino) diethanol	[redacted]	25307-17-9	[redacted]	[redacted]%	Yes, 1997	OECD TG 443 (2018)

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	Chemical name	Trade name	Cas no	Lot/batch no	Purity	GLP (Y/N; date)	OECD TG (Y/N; date)
PFAEO-hydrogenated tallow							
Combined Repeated Dose Toxicity Study and Reproductive/Developmental Toxicity Screening Study in the Han Wistar Rat by Oral Gavage Administration	2,2′-(C16/18 alkylimino) diethanol	Ethomeen HT/12	1218787-30-4	[redacted]	[redacted]% UVCB	Yes, date NA	OECD TG 422 (2016)
Study for Effects on Embryo-Fetal Development in the Han Wistar Rat by Oral Gavage Administration	2,2′-(C16-18 alkylimino) diethanol	Ethomeen HT/12	1218787-30-4	[redacted]	[redacted]%	Yes, 1999	OECD TG 414 (2018)
PFAEO-tallow							
Gene mutation in bacteria	PFAEO-tallow	[redacted]	NA	[redacted]	NA	NA	NA
90-day oral toxicity study in Wistar rats	PFAEO-tallow	Ethomeen T/12	NA	NA	NA	NA	NA
90-day oral toxicity study in Beagle dogs	PFAEO-tallow	Ethomeen T/12	NA	NA	NA	NA	NA
Combined Repeated Dose Toxicity Study and Reproductive/Developmental Toxicity Screening Study in Han Wistar Rats by Oral Gavage Administration	2,2′-(C16-18, C18 unsaturated) alkylimino)diethanol	Ethomeen T/12	1218787-32-6	[redacted]	[redacted]%	Yes, 1999	OECD TG 422 (2016)
Study for Effects on Embryo-Fetal Development in Han Wistar Rats by Oral Gavage Administration	2,2′-(C16-18, C18 unsaturated) alkylimino)diethanol	Ethomeen T/12	1218787-32-6	[redacted]	[redacted]%	Yes, 1999	OECD TG 414 (2018)

* In the original report, the study director reported '100% assumed' because no quantification of the substance's purity was made at that time.

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APPENDIX B

Clarification of chemical names for FCM 19, impurities and analogues used in this opinion

FCM No 19 is listed in Regulation (EU) 10/2011 with the chemical name 'N,N-bis(2-hydroxyethyl)alkyl(C8-C18)amine'. The following synonyms are also used for commercial products and have been presented by the different data providers of the BOs and are reported in the opinion: (C8-C18)alkyl diethanolamine, 2,2′-((C8-C18)alkylimino)diethanol; or expressed as PFAEO (primary fatty alkyl ethoxylate): PFAEO-(C8-C18).

Concerning impurities, the FCM substance No 19 is manufactured via ethoxylation of fatty amines. In addition to the FCM substance No 19 *N,N-bis(2-hydroxyethyl)alkylamine* (a tertiary amine), di-ethoxylation yields reaction products that are under/mono-ethoxylated yielding *N-alkyl monoethanolamine* (a secondary amine) and overethoxylated (a tertiary amine) yielding at least tri-ethoxylated species, i.e. for instance with one N-(hydroxyethyl) group, and one N-(2-hydroxyethoxy) ethyl group (3-ethoxylated species) or N-(2-hydroxyethoxyethoxy)ethyl group (4-ethoxylated species) and other higher ethoxylated species.

For analogous compounds with other alkyl chains (saturated or unsaturated) the same analogous variability in the nomenclature is used and can be found in the opinion.

B.1. | Constituents of the PFAEO-coco, PFAEO-oleyl, PFAEO-HT, PFAEO-T, PFAO-C18

Preamble: Appendix B.1 should be visited before reading this Appendix.

PFAEO-coco is identified as (C12-C18)alkyl diethanolamine with C12 [redacted] and C14 [redacted] diethanolamine as the main constituents. Considering the boundary composition given by Nouryon, it includes [redacted]

According to the information reported on the ECHA website and in ScienceFinder, only the substance CAS No 71786-60-2 is a diethanolamine (with an alkyl C12 – C18 chain), while the CAS No 61791-31-9 covers a mixture of substances based on coco nut oil and includes alkyl diethanolamines and fatty acid diethanolamides (and possibly others). Moreover, no chemical structure could be found under this CAS No from the information submitted by Nouryon.

PFAEO-HT is identified as C18 [redacted] and C16 [redacted] diethanolamine that are the main constituents, and with two CAS numbers (1218787-30-4 and 90367-28-5). According to the given boundary composition, it includes [redacted]

PFAEO-T is identified as C18:1 [redacted], C16 [redacted] and C18 [redacted] diethanolamine that are the main constituents, and with two CAS numbers (1218787-32-6 and 61791–44-4). According to the given boundary composition, it includes [redacted]

PFAEO-C18 is identified as C18 [redacted] diethanolamine that is the main constituent, and with one CAS No (10213–78-2). According to the given boundary composition, it includes [redacted]

PFAEO-oleyl is identified as C18:1 [redacted] diethanolamine that is in fact the main constituent, and with two CAS numbers (25307-17-9 and [redacted]). According to the given boundary composition, it includes [redacted]

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APPENDIX C

Points of departure for repeated toxicity studies from which an MoS could be derived

TABLE C.1 Repeated dose 90-day oral toxicity studies from which lowest observed adverse effect level (LOAELs)/BMDLs were identified.

Study/TG/GLP/treatment/species/dose range/LOAEL	Substance	Endpoint	Covariate (sex or litter)	$BMDL_{10}$ (mg/kg bw per day)
90-day Repeated Dose Toxicity Study/FDA/Yes/Capsule/Beagle Dog/15, 30, 100 mg/kg bw per day LOAEL: 100 mg/kg bw per day	Atmer 163; N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine	Increased clinical pathology parameters (ALT); liver histopathological changes (pigment accumulation in Kupffer cells)	Not applicable	Not determined
90-day Repeated Dose Toxicity study/FDA/Yes/Gavage/SD rats/0, 15, 30, 150 mg/kg bw per day LOAEL:30 mg/kg bw per day	Atmer 163; N,N-bis(2-hydroxyethyl)alkyl(C13/C15)amine	Ophthalmological findings (Chromodacryorrhoea, Retinal atrophy, Cataract) Gross findings in the non-glandular stomach (thickened mucosa) Histopathological findings in the non-glandular stomach (acanthosis)	Males only Males Females Males Females	27 24 22 20 23
90-day Repeated Dose Toxicity study/OECD 408/Yes/Gavage/Wistar rats/40, 160 or 320/240 mg/kg bw per day LOAEL: 40 mg/kg bw per day	Hostastat FA 68 V; N,N-bis(2-hydroxyethyl) alkyl (C16/C18)amine	Macroscopic findings in the non-glandular stomach (focal, multifocal thickened mucosa) Histopathological findings in non-glandular stomach (epithelial hyperplasia)	Females only Females only	44 20
Supporting studies				
90-day Repeated Dose toxicity study OECD 408/Yes/Gavage/RccHan™ Wistar rats/0, 10, 30, 125 mg/kg bw per day LOAEL: 30 mg/kg bw per day	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives (PFAEO-coco/[redacted])	Macroscopic findings in the non-glandular stomach (thickening) Histopathological findings in the non-glandular stomach: Epithelial hyperplasia Hyperkeratosis	Females only Males Females Males Females	5 8 5 8 6
90-day Repeated Dose toxicity study/OECD 408/Yes/Gavage/RccHan™ Wistar rats/0, 5, 30 or 150 mg/kg bw per day LOAEL: 150 mg/kg bw per day	2,2′-(octadec-9- enylimino) diethanol (PFAEO-oleyl) C18′; octadecenyl, oleyl)	Minimal focal epithelial, erosion of the non-glandular stomach Minimal submucosal oedema of the non-glandular stomach Minimal submucosal inflammatory cell infiltration in the non-glandular stomach	Females only Females only Females only	65 26 40

TABLE C.2 Reproduction and developmental toxicity endpoints from which L/NOAELs were identified.

Study/TG/GLP/treatment/species	Test substance/batch/purity	Main observations	LOAEL (mg/kg bw day)	NOAEL (mg/kg bw day)
Prenatal developmental toxicity (OECD, 414)/GLP/gavage/Han Wistar rat	Atmer 163; Ethanol, 2,2′-iminobis-, N-(C13/15-branched and linear alkyl) derivatives	Decreases in both the food consumption and body weight gain; increase in post-implantation loss and decrease in the number of fetuses per litter. *Skeletal abnormalities of the fetuses (cervical vertebrae)**	Maternal: 90; developmental: 30	Maternal: 30
Prenatal developmental toxicity (OECD 414)/GLP/dietary/SD rat	Atmer 163-LQ-(AP)	Lower mean body weight gains. Increase in fetal external malformations	Maternal/developmental: 90	Maternal/developmental: 30
Reproduction/developmental toxicity screening test (OECD 421, 1995)/GLP/gavage/Wistar rat	2,2′-(Octylimino) diethanol (N,N-bis(2-hydroxyethyl) octylamine)	No clearly adverse effects	> 320	320

(Continues)

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TABLE C .2 (Continued)

Study/TG/GLP/ treatment/species	Test substance/batch/ purity	Main observations	LOAEL (mg/kg bw day)	NOAEL (mg/kg bw day)
Supporting studies				
Combined repeat dose toxicity study with reproduction/ developmental toxicity screening test (OECD 422, 1996)/GLP/ gavage/Wistar rat	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives PFAEO-coco	Reductions in maternal bw gain (lactation), in litter sizes and weights; increases in post-implantation loss and deaths of offspring.	Maternal: 125; developmental: 30	Maternal: 30; developmental: 10
Prenatal developmental toxicity (OECD 414)/ GLP/gavage/Wistar rats	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives PFAEO-coco	Increases in post-implantation loss and total resorptions; major abnormalities in fetuses (head, eye and vertebral column). *Microphthalmia, anophthalmia**.	Maternal: 125; Fetal dev.: 30	Maternal: 30; Fetal dev.: 30
Prenatal developmental toxicity (OECD 414)/ gavage/New Zealand White Rabbit	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives PFAEO-coco	Maternal body weight loss, low food intake, clinical signs; reductions in fetal weights	175	60
Extended One Generation Reproductive Toxicity Study (EOGRTS; OECD 443)/gavage/Han Wistar rats	Ethanol, 2,2′-iminobis-, N-C12-18-alkyl derivatives PFAEO-coco	Increased liver weights, higher cholesterol level (F0, females). *Macroscopic and histological changes in GI tract (F0)**. *Opacity/cataracts (F1)**.	100	30 for systemic parental toxicity
Prenatal developmental reproductive/ developmental toxicity (OECD 414)/gavage/ Sprague–Dawley rats	2,2′-(Octadec-9-enylimino)diethanol PFAEO-oleyl	No effects observed	> 150	150
Extended One Generation Reproductive Toxicity Study (EOGRTS; OECD 443)/gavage Han Wistar rats	2,2′-(Octadec-9-enylimino)diethanol PFAEO-oleyl	Four premature deaths; body weight gain, food consumption; organ weight changes. *Histopathological changes (stomach, GI-tract)**.	70	30 for systemic parental toxicity
Combined repeated dose toxicity study and reproductive/ developmental toxicity screening study OECD 422/gavage/Han Wistar rats	2,2′-(C16/18) alkylimino) diethanol PFAEO-HT	Mortality (F0/F1); Body weight gain (F0/F1), food intake Red blood cell parameters, Rel. organ weights (liver). *Histological changes (GI tract)**.	75	30(m) and 100 (f) for systemic parental toxicity
Developmental toxicity (OECD 414)/gavage/ Han Wistar rats	2,2′-(C16/18) alkylimino) diethanol PFAEO-HT	Body weight gain, food intake; Total litter weight, number of litters insufficient.	150	50 for maternal toxicity
Combined repeated dose toxicity study and reproductive/ developmental toxicity screening study OECD 422/gavage/Han Wistar rats	2,2′-(C16/18, C18 unsaturated) alkylimino)diethanol PFAEO-T	Mortality (F0/F1); Body weight gain (F0/F1), food intake; rel. organ weights (liver, kidney). *Histological changes (GI tract)**. Clinical signs (F1); reduced offspring survival.	175	75 for systemic parental and developmental toxicity
Developmental toxicity (OECD 414)/gavage/ Han Wistar rats	2,2′-(C16/18, C18 unsaturated) alkylimino)diethanol PFAEO-T	Body weight gain, food intake; Total litter weight Number of offspring.	150	50 for maternal and developmental toxicity

*See Table C.3 hereafter with BMDL values for these effects.

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TABLE C.3 Reproduction and developmental toxicity endpoints from which a BMDL was estimated.

Study/species/dose range/NOAEL	Substance	Endpoint	Subgroup	BMDL (mg/kg bw per day)	BMD_{10} (mg/kg bw per day)	$BMDU_{10}$ (mg/kg bw per day)
Prenatal dev. toxicity study in rats (414) 0, 10, 30, 90 mg/kg bw per day	N,N-bis(2-hydroxyethyl) alkyl(C13/C15)amine	Skeletal abnormalities of the fetuses (cervical vertebrae/skull cranial bone)	Effect on litters	$BMDL_5$ 12**	42	80
Supporting studies						
Prenatal dev. study (OECD 414) 10, 30, 125 mg/kg bw per day	2,2′-(C12-18 even numbered alkyl imino) diethanol. PFAEO-coco	Micro–/anophthalmia	Effect on litters	$BMDL_1$ 18***	75	119
EOGRT study (OECD 443) 10, 30, 100 mg/kg bw per day	2,2′-(C12-18 even numbered alkyl imino) diethanol PFAEO-coco	Stomach, thickened Hyperkeratosis Epithelial hyperplasia	F0 males F0 females F0 males F0 females F0 males F0 females	$BMDL_{10}$ 4 22 4 11 4 11	8 32 9 19 8 21	12 43 13 28 14 31
EOGRT study (OECD 443) 10, 30, 100 mg/kg bw per day	2,2′-(C12-18 even numbered alkyl imino) diethanol. PFAEO-coco	Opacity/cataract in F1	Same BMD values for males and females	$BMDL_{10}$ 29	34	42
EOGRT study (OECD 443) 30, 70, 150 mg/kg bw per day	2,2′-(octadec-9-enylimino) diethanol PFAEO-oleyl	Epithelial hyperplasia Oedema Inflammation	F0 males F0 females F0 males F0 females F0 males F0 females	$BMDL_{10}$ 4 5 37 17 46 11	7 7 100 31 82 29	15 22 274 55 146 56
EOGRT study (OECD 443) 30, 70, 150 mg/kg bw per day	2,2′-(octadec-9-enylimino) diethanol PFAEO-oleyl	Accumulation of foamy cells in the jejunum Thymus involution/ atrophy	F1 males F1 females Cohort 1A, males	$BMDL_{10}$ 26 26 46	36 38	49 54
Toxicity and Repro/Dev Screening study (OECD422) 10, 30, 100 (f) or 100/75 (m) mg/kg bw per day	2,2′-(C16/18)alkylimino) diethanol PFAEO-HT	Inflammatory cell infiltrate in the jejunum	F0 males (75/100)* F0 females (100)	$BMDL_{10}$ 7 19	16 27	42 37
Toxicity and Reprod/ Dev Screening study (OECD 422) 30, 75, 175 mg/kg bw per day	2,2′-(C16-18, C18 unsaturated) alkylimino)diethanol PFAEO-T	Epithelial hyperplasia in the stomach Foamy cell infiltrate in the jejunum	F0 males (75) F0 females (175) F0 males (75) F0 females (175)	$BMDL_{10}$ 7 18 22 28	32 47 39 53	66 118 84 89

*One male at 100 mg/kg bw per day was sacrificed on Day 11 due to the extend of body weight loss. From Day 12 of dosing, the high dose level was reduced to 75 mg/kg bw per day for males.

**BMR5 was applied in the BMD analysis due to the rarity of effect in the historical control data.

***BMR1 was applied in the BMD analysis due to the extreme rarity of the effect in historical control data.

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APPENDIX D

Benchmark Dose Modelling Report

D.1 | Data Description

The endpoint to be analysed: number of litters, where fetuses were affected by skeletal abnormalities.

Data used for analysis:

Dose (mg/kg bw per day)	No of litters affected	No of litters
0	1	21
10	2	22
30	3	20
90	7	20

D.2 | Selection of the BMR

BMR of 5% was selected based on the historical control data, where skeletal abnormalities in fetuses (i.e. cervical vertebrae) are very rare.

D.3 | Software Used

Results are obtained using the EFSA web-tool for Bayesian BMD analysis, which uses the R-package [BMABMDR] version 0.0.0.9083 for the underlying calculations.

D.4 | Results

Information pertaining to this endpoint.

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor in favour of saturated model is 4.19e-01).

Model Averaged BMD

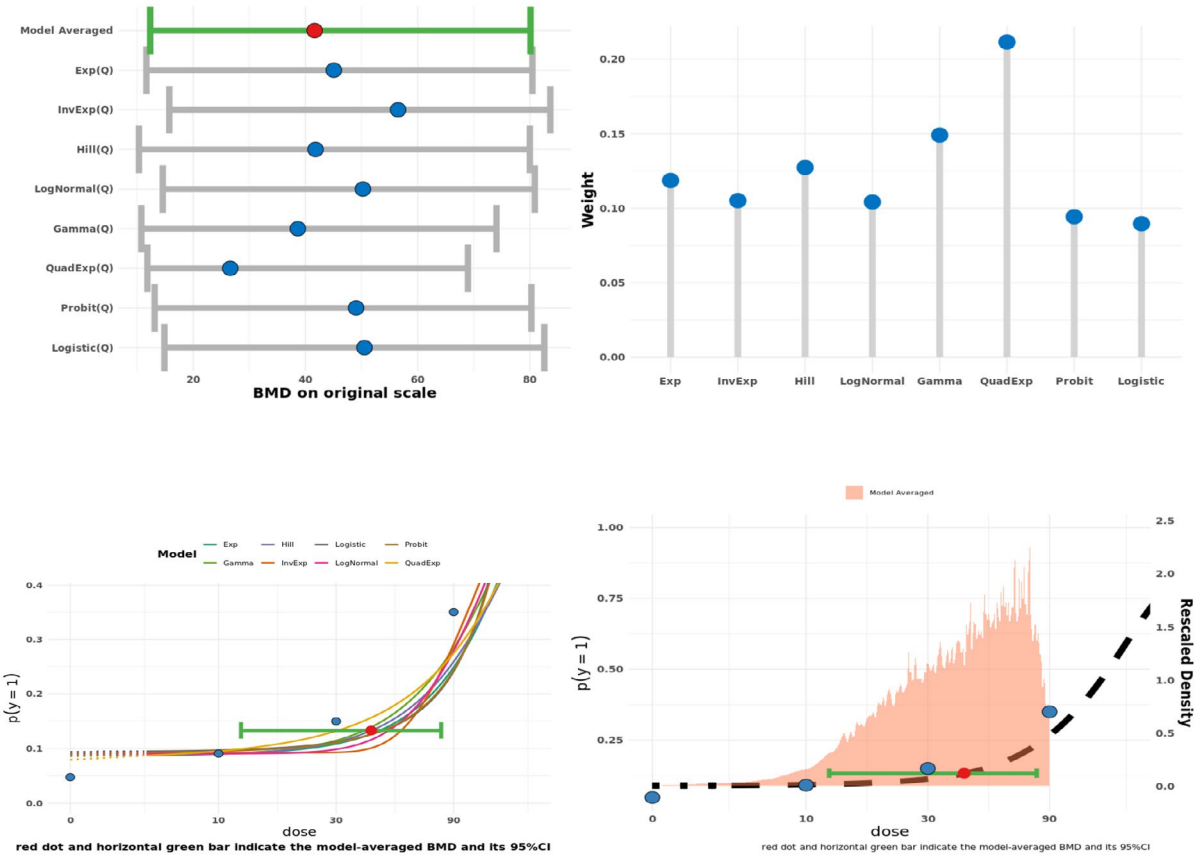
Model	Type	BMDL	BMD	BMDU
Model averaged	BS	12.334	41.638	80.113

Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model weights	Converged
E4_Q	11.672	45.061	80.441	0.119	1
IE4_Q	15.750	56.508	83.633	0.105	1
H4_Q	10.341	41.800	79.992	0.127	1
LN4_Q	14.583	50.245	80.868	0.104	1
G4_Q	10.766	38.644	74.043	0.149	1
QE4_Q	11.843	26.604	68.959	0.212	1
P4_Q	13.140	49.031	80.261	0.094	1
L4_Q	14.876	50.520	82.563	0.090	1

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Plots of Fitted Models



D.5 | Conclusions

The BMD analysis was performed for the incidence of skeletal abnormalities in fetuses (cervical vertebra, cranial bone) from a prenatal developmental toxicity study in rats treated with Atmer 163 by gavage (Section 3.2.1.7). Using the litter data (number of affected litters), the BMD calculated for 5% response is 42 mg/kg bw per day, with a lower bound of 90% confidence interval (BMDL) of 12 mg/kg bw per day and upper bound of 90% confidence interval (BMDU) of 80 mg/kg bw per day. The uncertainty of the BMD estimation characterised by the BMDU/BMDL ratio (6.5) is acceptable since it is below 50.

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