Wave 2 FA Data Supplement

Fatty Amphocarboxylates

EXPERT PANEL MEETING
JUNE 12-13, 2023

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR
Date: June 2, 2023
Subject: Safety Assessment of Fatty Amphocarboxylates as Used in Cosmetics – Wave 2

In response to the SLR issued on fatty amphocarboxylates (all of which are mixtures of several fatty acid chain lengths), multiple submissions were received, including comments, links to REACH dossiers, and limited unpublished data. One of these submissions includes an analogue approach applied for the REACH registration of alkylamphoacetates containing data on fatty acid chain mixtures (amphoacetates C8-C18, amphoacetates C12-14, and amphoacetates C12) that comprise ingredients reviewed in the fatty amphocarboxylates report. Upon review of these test substances, it appears to CIR staff that these test substances could directly correlate to several of the ingredients reviewed in the report (and therefore, for our purposes, these data appear to be data on ingredients in this report themselves, and not on read-across sources). The correlation of the test substances to the related INCI ingredients included in the report can be found below. ***Does the Panel agree that these data are directly applicable to ingredients under review in the CIR report?***

Test Substance Name	Related INCI Ingredient
amphoacetates C8-18	Disodium Cocoamphodiacetate; Sodium Cocoamphoacetate
amphoacetates C12-C14	Disodium Lauroamphodiacetate; Sodium Lauroamphoacetate
amphoacetates C12	Disodium Lauroamphodiacetate; Sodium Lauroamphoacetate

It should be noted that while the suggested additional ECHA dossiers contain information on a variety of endpoints, data on many of these endpoints have already been provided in the Draft Report (and may actually be the same data cited in the proposed ECHA dossier). An overview of what was received in each submission, as well as the information provided in the additional ECHA dossiers, is described below, as is a comparison to what data are currently provided in the report.

Data Submission	Summary of Data Received	Similar data currently in Draft Report

data1_FattyAmphocarboxylates_Wave2_062023	• Corrected technical data sheet information on Disodium Lauroamphodiacetate (***Does the Panel feel that the data in the Draft Report should be altered to reflect the data presented in this submission?)***
	
	• Responses to CIR request for data including the following:
	  • Dermal absorption data on dodecylamidopropylbetaine (potential read-across ingredient)
	  • EpiOcular assay on Sodium Lauroamphoacetate (4% solids, water)
	  • HET-CAM assay on Disodium Cocoamphodiacetate (4% solids, water)
		Dermal absorption data are not currently provided for any of the ingredients reviewed in the report.

Data Submission	Summary of Data Received	Similar data currently in Draft Report
data2_FattyAmphocarboxylates_Wave2_062023	• Comments on the SLR by the Amphoacetate Consortium (comments presented as marked-up copy of the SLR) • Submission of a REACH dossier on amphoacetates C12; dossier includes data on the following endpoints: • Physical and chemical properties • Acute toxicity (oral and dermal) • Repeated dose toxicity (oral) • DART • Genotoxicity (in vitro) • Dermal irritation • Dermal sensitization • Ocular irritation • Submission of REACH dossier on amphoacetates C12-C14; dossier includes data on the following endpoints: • Physical and chemical properties • Acute toxicity (oral and dermal) • Repeated dose toxicity (oral) • DART • Genotoxicity (in vitro and in vivo) • Dermal irritation • Dermal sensitization • Ocular irritation • Submission of REACH dossier on amphoacetates C8-C18; dossier includes data on the following endpoints: • Physical and chemical properties • Acute toxicity (oral and dermal) • Repeated dose toxicity (oral) • DART • Genotoxicity (in vitro and in vivo) • Dermal irritation • Dermal sensitization • Ocular irritation • Case report of dermatitis following exposure to test substance	For the endpoints listed under the REACH dossiers (e.g., acute toxicity, genotoxicity), data are provided in the current Draft Report (including data from the previous report published in 1990) for Disodium Cocoamphodiacetate, Sodium Cocoamphoacetate, Sodium Lauroamphoacetate (with the exception of repeated dose toxicity data (this endpoint is only available for Disodium Cocoamphodiacetate). No data on these endpoints were available in the Draft Report for Disodium Lauroamphodiacetate.
data3_FattyAmphocarboxylates_Wave2_062023	• Analogue approach applied for the REACH registration of alkylamphoacetates; document contains the following: • Read-across hypothesis • General chemistry/synthesis of alkylamphoacetates • Functional groups of alkylamphodiacetates • General composition of alkylamphoacetates • Variability of fatty acid chain lengths • General physicochemical properties of alkylamphoacetates	For the toxicological endpoints (toxicity studies only (e.g., acute toxicity, genotoxicity) listed under the REACH registration of alkylamphoacetates, data are provided in the current Draft Report (including data from the previous report published in 1990) for Disodium Cocoamphodiacetate, Sodium Cocoamphoacetate, Sodium Lauroamphoacetate

Data Submission	Summary of Data Received	Similar data currently in Draft Report
	• Physicochemical properties of amphoacetates C8-18, C12-14, and C12 (including water solubility, log P_{ow}, and vapor pressure) • Composition of amphoacetates C8-18, C12-14, and C12 as manufactured • Toxicokinetic studies on amphoacetates C8-18, C12-14, and C12 • Acute toxicity data on amphoacetates C8-18, C12-14, and C12 • Repeated dose toxicity on amphoacetates C8-18 and C12-14 • DART data on amphoacetates C8-18 • Genotoxicity data on amphoacetates C8-18, C12-14, and C12 • Dermal irritation data on amphoacetates C8-18 and C12 • QSAR predictions for dermal sensitization on amphoacetates C8-18, C12-14, and C12 • Dermal sensitization data amphoacetates C8-18, C12-14, and C12 • Ocular irritation data on amphoacetates C8-18 and C12	(with the exception of repeated dose toxicity data (this endpoint is only available for Disodium Cocoamphodiacetate). No data on these endpoints were available in the Draft Report for Disodium Lauroamphodiacetate. Toxicokinetic studies are not currently provided in the Draft Report, on any ingredient.
	• Expert review of available repeated-dose and DART studies for amphoacetates	• Several of these studies are already in the report)
data4_FattyAmphocarboxylates_Wave2_062023	• Submission of the REACH dossiers on the following potential read-across test substances, particularly for the propionate ingredients reviewed in this report: • Reaction products of 1H-imidazole-1-ethanol, 4-5-dihydro-, 2-(C11-17 and C17 unsatd. alkyl) derivs. and sodium hydroxide and 2-propenoic acid; dossier contains data on the following endpoints: • Physical and chemical properties • Acute toxicity (oral and dermal) • Repeated dose toxicity (oral) • DART • Genotoxicity (in vitro) • Dermal irritation • Dermal sensitization • Ocular irritation	Data available in the report on Disodium Cocoamphodipropionate and Sodium Cocoamphopropionate include acute toxicity, dermal irritation, dermal sensitization, and ocular irritation data. Genotoxicity data are also available for Disodium Cocoamphodipropionate.

Data Submission	Summary of Data Received	Similar data currently in Draft Report
	• *N*-(2-hydroxyethl)-*N*-[2-[(1-oxooctyl)amino]ethyl]-β-alanine; dossier contains data on the following endpoints: • Physical and chemical properties • Acute toxicity (oral and dermal) • Repeated dose toxicity (oral) • DART • Genotoxicity (in vitro) • Dermal irritation • Dermal sensitization • Ocular irritation	
data5_FattyAmphocarboxylates_Wave2_062023	• Composition data on trade name mixtures containing Disodium Cocoamphodiacetate and Disodium Lauroamphodiacetate • Manufacturing data on Disodium Cocoamphodiacetate	Not applicable_

The Panel should consider these data and answer the following questions:

1. ***Does the Panel agree that the data on amphoacetates C8-18, amphoacetates C12-C14, and amphoacetates C12 directly correlate to the ingredients listed above?***

2. ***Should the data on the following potential read-across sources be included in the report?***
• dodecylamidopropylbetaine
• reaction products of 1*H*-imidazole-1-ethanol, 4-5-dihydro-, 2-(C11-17 and C17 unsatd. alkyl) derivs. and sodium hydroxide and 2-propenoic acid
• *N*-(2-hydroxyethl)-*N*-[2-[(1-oxooctyl)amino]ethyl]-β-alanine

3. ***Are the data in the Draft Report, along with the information provided in these data supplements, sufficient for the Panel to determine the safety of this ingredient group? If not, what additional information is needed?***

If the Panel determines the data provided in the Draft Report, as well as the data suggested for addition from the ECHA dossiers and the unpublished data that were submitted, are sufficient to conclude on the safety of these ingredients, CIR Staff requests that the Panel consider tabling this report so that these data can summarized and included in a new iteration of the report. Accordingly, a Revised Draft Report would be prepared for a future meeting.

However, if the Panel decides that additional information (in addition to the data included in these submissions presented herein) is required to conclude on the safety of this ingredient group, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein. The next iteration of the report would then include all the data received from the IDA, as well as the data suggested herein.

In addition, it should be noted that comments on the Draft Report have been provided from Council and are attached herein (*PCPCComments_FattyAmphocarboxylates_Wave2_062023*).

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: June 1, 2023

SUBJECT: Draft Report: Safety Assessment of Fatty Amphocarboxylates as Used in Cosmetics (draft prepared for the June 2023 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft report, Safety Assessment of Fatty Amphocarboxylates as Used in Cosmetics.

Introduction – Is the date of the literature search (April 2022) correct? Please correct: "was las conducted" (add "t")

Developmental and Reproductive Toxicity – Regarding the developmental toxicity study of Sodium Cocoamphodiacetate, the text should also note: "A test-item related effect could not be excluded as the right-sided aortic arch incidence was above historical control range. Other visceral malformations observed were within the historical control data range." (as stated in Table 8).

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 24, 2023

SUBJECT: Disodium Lauroamphodiacetate and Sodium Lauroamphoacetate

Colonial Chemical. 2023. Comments and information on Disodium Lauroamphodiacetate and Sodium Lauroamphoacetate.

May 2023

Comment on Disodium Lauroamphodiacetate composition, table 4 reference 45

Reference 45 relates to the Colonial Chemical Safety Data Sheet for Cola®Teric J49. Please note that the link relates to a joint venture in the Middle East and should instead point to https://colonialchem.com/products/colateric-j49/ which is the US-based company having the up-to-date safety data sheets.

Table 4 currently gives the composition of Disodium Lauroamphodiacetate as "30-60% Sodium Lauroamphoacetate and < 0.1% dichloracetic acid (remaining components not stated)" but should instead state "31% Sodium Lauroamphoacetate and 7% sodium chloride (remaining components not stated)" based on the TDS that can be found in the link above. Alternatively, "30-60% Sodium Lauroamphoacetate (remaining components not stated)" based on the safety data sheet which can be retrieved following the link above. The active substance is present in an aqueous solution. The water content is in the 55-65% range. As the technical data sheet and safety data sheet state, the pH is 9. At this pH, 100% of dichloroacetic acid will be in the form of sodium dichloroacetic acid. For this reason, we have corrected the safety data sheet. Moreover, since sodium dichloroacetic acid is present well below 0.1%, does not contribute to the product classification nor does it have an occupational exposure limit, it is therefore not required to be present in section 3 of the safety data sheet.

CIR staff request for information and response:

Request 1: "Composition and impurities data on all ingredients; specifically, the constituents and percent solids of these ingredients as finished solutions"

Response 1: Safety data sheets of all products listed are publicly available on https://colonialchem.com/

Request 2: Method of manufacturing data

Response 2: No additional data to what is provided in the draft.

Request 3: Dermal absorption data; if absorbed, additional toxicity studies may be needed

Response 3: Experimental data on a structurally related amphoteric surfactant, dodecylamidopropylbetaine (CAS# 4292-10-8) shows dermal absorption of less than 3.5% in Wistar rats (Reference: HERA (Human and Environmental Risk Assessment on ingredients of household cleaning products), 2005, http://www.heraproject.com/RiskAssessment.cfm)

Request 4: Irritation and sensitization data, at maximum concentrations of use

Response 4: In vitro ocular irritation at concentration of use for ColaTeric SLAA and ColaTeric 2C are summarized in the following table.

Test Article	Vehicle	Test Population	Procedure	Summary/Results	Reference
Sodium Lauroamphoacetate (4% solids, water)	Water	Not applicable / number of replicates not stated	EpiOcular MTT ET-50 (Dilution Method)	Under the test conditions, Sodium Lauroamphoacetate, 4% solids, at 20%, elicited in vitro results which indicate that its ET-50 is 87.6 minutes. Therefore, at 100% (i.e. 4% solids), the test article's estimated Draize ocular irritation score is approximately 6.1 with a "minimally irritating" irritancy classification.	Own data
Disodium Cocoamphodiacetate (4% solids, water)	Water	4 eggs	HET-CAM assay	Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than the rabbit eye. Therefore, a 50% dilution of the liquid test article, in distilled water, was used. Under the conditions of this test, the results indicate that Disodium Cocoamphodiacetate, 4% solids, at 100% (i.e. 4% solids) would have a moderate ocular irritation potential in vivo.	Own data

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 26, 2023

SUBJECT: Amphoacetates

Alkylamphoacetate Consortium. 2023. Comments on the CIR Scientific Literature Review on Amphoacetates.

Further information on the requested endpoints (specifically skin irritation/sensitisation) can be found in the submitted read-across justification document as well as the disseminated information of the three jointly submitted REACH dossiers:

Amphoacetates C12 (EC No. 271-794-6) Registration Dossier - ECHA (europa.eu)
Amphoacetates C12-C14 (938-645-3) Registration Dossier - ECHA (europa.eu)
Amphoacetates C8-C18 (931-291-0) Registration Dossier - ECHA (europa.eu)

In addition, we would also like to inform you that there are still two toxicological studies on-going that may be of relevance for your assessment:

- A Pre-natal Developmental Toxicity Study (OECD 414) in rabbits with Amphoacetates C8-C18 (final report expected in April 2024)
- An Extended One-Generation Reproduction Toxicity Study (OECD 443) with Amphoacetates C8-C18 (final report expected in 2025)

Safety Assessment of Amphoacetates as Used in Cosmetics

Status: Scientific Literature Review for Public Comment
Release Date: March 30, 2023
Panel Meeting Date: June 12-13, 2023

All interested persons are provided 60 days from the above release date (i.e., May 29, 2023) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651
cirinfo@cir-safety.org

ABBREVIATIONS

AEEA	aminoethylethanolamine
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CIR	Cosmetic Ingredient Review
CLP	Classification, Labeling, and Packaging
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DI	denaturation index
ECHA	European Chemicals Agency
European Chemicals Agency	ECHA
ET_{50}	effective time of exposure to reduce tissue viability to 50%
EU	European Union
FDA	Food and Drug Administration
H_{50}	half-maximal effective concentration for hemolysis
HET-CAM	hen's egg test-chorioallantoic membrane
K_{ow}	n-octanol/water partition coefficient
HRIPT	human repeated-insult patch test
LD_{50}	median lethal dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NR	not reported
NOAEL	no-observed-adverse-effect-level
OECD	Organisation for Economic Cooperation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
SIDS	screening information dataset
SLS	sodium lauryl sulfate
TG	test guideline
TUNEL	TdT-dUTP terminal nick-end labeling
US	United States
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

INTRODUCTION

This assessment reviews the safety of the following 11 amphoacetates as used in cosmetic formulations:

Disodium Cocoamphodiacetate*
Disodium Cocoamphodipropionate*
Disodium Lauroamphodiacetate
Disodium Wheatgermamphodiacetate
Sodium Arganamphoacetate
Sodium Cocoamphoacetate*
Sodium Cocoamphopropionate*
Sodium Cottonseedamphoacetate
Sodium Lauroamphoacetate
Sodium Olivamphoacetate
Sodium Sweetalmondamphoacetate

** previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel)*

Sodium Lauroamphoacetate was included on the Cosmetic Ingredient Review (CIR) 2021 Priority List due to high reported frequencies of use in the US Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP). Four structurally-similar ingredients (i.e., Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate) have previously been reviewed by the Panel in a safety assessment that was published in 1990,[1] and a re-review published in 2008.[2] Accordingly, in that these ingredients would soon be considered for another re-review, it was deemed appropriate to include the 4 previously-reviewed ingredients in this safety assessment. Additionally, 6 other amphoacetate ingredients are included in this grouping. Hence, all ingredients reviewed in this report are structurally similar as they are alkylamido alkylamines.

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), these ingredients are reported to function in cosmetics as various types of surfactants (cleansing agents, foam boosters, hydrotropes).[3] The majority of these ingredients are also reported to function as hair-conditioning agents (Table 1).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment was found on the European Chemicals Agency (ECHA) website.[4] Please note that the ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited.

In its original 1990 review of Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate, the Panel concluded that these ingredients are safe in the present practices of use and concentration, as described in that assessment.[1] This conclusion was re-affirmed in a re-review published in 2008.[2] Excerpts of summarized data from the original 1990 safety assessment are included throughout the text of this document, as appropriate, and are identified as italicized text. (This information is not included in the tables or Summary section.) For complete and detailed information, the original report can be accessed on the CIR website (https://www.cir-safety.org/ingredients). Accordingly, for these 4 ingredients, an extensive search of the world's literature was performed for studies dated 1985 forward, and relevant new data were included.

Based on the research that was performed on this ingredient group, these ingredients are typically provided as solutions (usually 40-50% of the ingredient itself (represented as percent solids)) instead of standalone ingredients, and commonly include other salts (e.g., sodium chloride and sodium glycolate). When this information is provided, the percent solids and the specific constituents of these solutions are provided herein (e.g., Sodium Lauroamphoacetate (50% solids; water and sodium chloride)); however, it should be noted that these constituents are not provided for all studies included in this report. Clarification is needed regarding the compositions of these ingredients/percentages of these ingredients in finished solutions as used in cosmetics. It should be noted that sodium glycolate has previously been reviewed by the Panel (published in 1998), and it was concluded that this ingredient is safe for use in cosmetic products at concentrations ≤ 10%, at final formulation pH ≥ 3.5, when formulated to avoid increasing sun sensitivity, or when directions for use include the daily use of sun protection.[5] This conclusion was re-affirmed in a 2017 published re-review summary.[6]

In addition, it should be noted that these ingredients may contain amidopropyl dimethylamine (a.k.a. amidoamine) impurities, which is a known sensitizer.[7,8] Cocamidopropyl betaine, a surfactant that has been previously reviewed by the Panel (published in 2012) has similar issues of impurities (e.g., amidoamine) and mechanisms of toxicity to the ingredients reviewed in this report.[8] The Panel concluded that the ingredients in the cocamidopropyl betaine report were safe for use as cosmetic ingredients in the practices of use and concentration as stated in that safety assessment, when formulated to be non-sensitizing (which may be based on a quantitative risk assessment).

CHEMISTRY

Definition and Structure

The ingredients reviewed in this report (e.g., Sodium Lauroamphoacetate; CAS No. 68608-66-2; 156028-14-7; 66161-62-4; formula weight = 349.5 g/mol; log K_{ow} = -1) are compounds with both anionic and cationic structures.[9,10] According to the *Dictionary*, Sodium Lauroamphoacetate is an amphoteric organic compound that generally conforms to the structure:

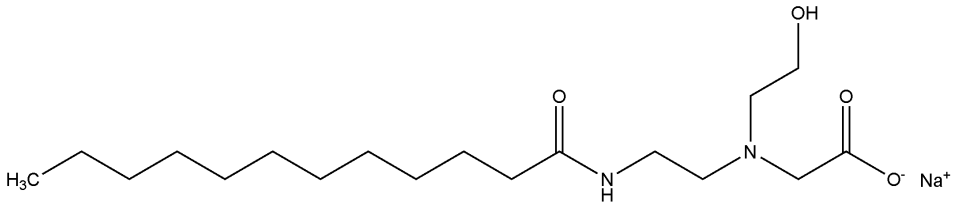


Figure 1. Sodium Lauroamphoacetate

The definitions and structures of all the amphoacetates included in this review are provided in Table 1.

Chemical Properties

Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate are supplied as amber liquids, usually containing 40-50% solids.[1] These ingredients are soluble in water and insoluble in nonpolar organic solvents.

Sodium Lauroamphoacetate is a highly water-soluble, light yellow powder that is typically available as an aqueous solution.[4] Chemical properties of the ingredients in this grouping (some of which may be properties of the ingredient as a solution) are provided in Table 2.

Method of Manufacture

According to the *Dictionary* and published literature, these ingredients are prepared by reacting fatty acid derivatives (e.g., coco fatty acid for Sodium Cocoamphoacetate) with hydroxyethyl ethylenediamine or aminoethylethanolamine (AEEA).[3,11] This reaction produces a substituted imidazoline which is subsequently split via a reaction with an acid (e.g., chloroacetic acid) to yield an amphoteric compound. Compositions of relevant fatty acids (e.g., coconut fatty acid, cottonseed fatty acid) used in the synthesis of these amphoacetates are provided in Table 3.

Composition and Impurities

The compositions of these ingredients as used in cosmetics were not found in the published literature, or provided via unpublished data; however, chemical safety data sheets on trade name products corresponding to several of the ingredients reviewed in this report have been found. These compositions can be found in Table 4. The majority of these ingredients consist of mixtures containing 30 - 60% of the active ingredient, water, dichloroacetic acid, and salts.

AEEA, a potential allergen, may be present in coco-and lauroamphoacetates, amphopropionates, amphodiacetates, and amphodipropionates as an impurity, as it is used as a reagent in the synthesis of these ingredients.[11] The concentration of AEEA in several amphoteric trade name ingredients (corresponding to Disodium Cocoamphodiacetate, Sodium Cocoamphoacetate, and Sodium Lauroamphoacetate) ranged from 4.9 ± 0.2 to 1130 ± 50 ppm. In addition, it should be noted that amidoamine (fatty acid esters of amidopropyl dimethylamine) may be present as an impurity in these ingredients (e.g., a trade name corresponding to Sodium Lauroamphoacetate was reported to contain up to 5% amidoamine).[7,8]

According to a report published by the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) Disodium Wheatgermamphodiacetate contains 15% saturated fatty acids (e.g., stearic acid), 30% oleic acid, 44% linoleic acid, and 11% linolenic acid.[12] This report states that Disodium Wheatgermamphodiacetate has a purity level of > 99.9%, and may contain chloroacetic acid as an impurity in amounts of < 100 ppm.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the FDA and the cosmetics industry on the expected use of these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's VCRP (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council; maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer

habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 FDA VCRP data, Sodium Lauroamphoacetate is reported to be used in 202 total formulations (183 rinse-off formulations; 17 rinse-off formulations; and 2 formulations diluted for bath use; Table 5).[13] Disodium Cocoamphodiacetate has the highest frequency of use (220 total formulations; 40 leave-on formulations, 179 rinse-off formulations, and 1 formulation diluted for bath use; Table 6). The number of uses for this ingredient has increased since it was last reviewed; it was previously reported to be used in 194 formulations in 2005.[2] Sodium Cocoamphoacetate is reported to be used in 121 formulations, and all other ingredients are reported to be used in 73 formulations or less. The results of the 2021 concentration of use survey conducted by Council indicate that Disodium Cocoamphodiacetate has the highest concentration of use in rinse-off products; it is used at up to 20% in cleansing products.[14] Disodium Lauroamphodiacetate has the highest concentration of use reported in leave-on products; it is used at up to 5.4% in other hair preparations. In 2006, the ingredient with the highest reported concentration of use was Sodium Cocoamphoacetate (used at up to 18% in bath soaps and detergents).

Several of these ingredients are reported to be used in products that are applied near the eye; for example, Sodium Lauroamphoacetate is used at 1.3% in eye makeup removers. In addition, these ingredients are reported to be used in products that may result in mucous membrane exposure (e.g., Disodium Cocoamphodiacetate is reported to be used in other personal cleanliness products at up to 3.3%) and in baby products (Disodium Cocoamphodiacetate is used in baby shampoos at up to 5.4%).

Disodium Lauroamphodiacetate is used in a perfume (concentration not reported) and could possibly be inhaled. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The amphoacetates reviewed in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[15]

Non-Cosmetic

Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate are used in cleaning products (all-purpose, oven, floor, dishwashing, metal, and hard-surface) and in the caustic lye peeling of fruit and potatoes.[1] Disodium Cocoamphodiacetate is used at 0.2% in pharmaceutical glaucoma treatment, and in bandage materials. Disodium Cocoamphodipropionate is used at 0.35% in hemorrhoid treatment formulations and up to 0.04% in contact lens disinfecting solutions.

Sodium Lauroamphoacetate is used as a surfactant in various industrial and household cleaning products, including dishwashing and laundry detergents.[4,16] This ingredient is used as an FDA-approved sanitizing agent for food-processing equipment and utensils (21CFR178.1010). Disodium Cocoamphodiacetate is reported to be used as an inactive ingredient in a pharmaceutical shampoo formulation at 5%.[17]

TOXICOKINETIC STUDIES

Toxicokinetics studies were not found in the published literature, and unpublished data were not submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal acute toxicity assays were performed in rabbits using shampoo creams containing 4% Disodium Cocoamphodiacetate (24-h application; occlusive conditions; undiluted).[1] Signs of clinical toxicity (depression, labored respiration, phonation, tremors) and dermal toxicity (reversible gross dermal lesions, atonia, desquamation, fissures, sloughing) were observed during the 14-d observation period. Several acute oral toxicity assays were performed using Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate (as commercially supplied) in mice and rats. All test substances were considered to be nontoxic (median lethal dose (LD_{50}s) ranged from >5 to 28 ml/kg).

Oral

The acute oral toxicity studies on Sodium Lauroamphoacetate summarized here are described in Table 7. An LD_{50} of 6116 mg/kg for Sodium Lauroamphoacetate (% solids not stated; water and sodium chloride) was determined in mice.[4] The lowest LD_{50} in rats was reported to be > 2000 mg/kg bw Sodium Lauroamphoacetate (50% solids; water and sodium

chloride; tested as provided). The same LD_{50} was reported for a 20% aqueous dilution of Sodium Lauroamphoacetate (35% solids; water, sodium chloride, sodium glycolate).

Subchronic Toxicity Studies

Oral

Disodium Cocoamphodiacetate

Wistar Han rats (10/sex/group in main study; 5/sex/group in recovery group) were given Disodium Cocoamphodiacetate (47.2 - 48% solids) in water, via gavage, in doses of either 0, 100, 300, or 1000 mg/kg bw/d for 90 d.[4] Recovery groups received either the vehicle only or 1000 mg/kg bw/d of the test substance, for 90 d, followed by a 28-d treatment-free period. Body weight changes, food consumption, mortality, behavior, ophthalmological, hematological, gross pathological, reproductive, and histopathological parameters were evaluated. No deaths occurred throughout the study. Mild respiratory difficulty, fur loss, and hunched posture were observed in several animals of treated groups. Lowered body weight compared to controls was observed in males treated with 1000 mg/kg bw/d. Slightly lower food consumption was observed in treated males (at all test concentrations). Histopathological changes included non-adverse squamous cell hyperplasia accompanied with hyperkeratosis in the stomach of female rats (dosed with 300 mg/kg bw/d and higher) and goblet cell hyperplasia of the rectum of a few male rats (dosed with 1000 mg/kg bw/d). In addition, higher kidney and liver weights were noted in females dosed with 1000 mg/kg bw/d. Histopathological and organ weight changes were fully reversed at the end of the recovery period. No toxicologically-relevant adverse effects were noted in any of the remaining parameters evaluated. The no-observed-adverse-effect-level (NOAEL) was determined to be 1000 mg/kg bw/d. The reproductive effects evaluated in this assay are found in the Developmental and Reproductive Toxicity section of this report.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

The oral developmental and reproductive toxicity studies summarized here can be found in Table 8. A reproductive toxicity assay was performed on Disodium Cocoamphodiacetate (0, 100, 300, or 1000 mg/kg bw/d; in water; gavage administration; treated days 6 - 20 post-coitum) using female Wistar Han rats (22/group).[4] No maternal toxicity was observed in this assay (maternal NOAEL = 1000 mg/kg bw/d). Severe cardiac abnormalities were observed in fetuses in all test groups (not including control), in a non-dose-dependent manner; accordingly, the developmental NOAEL could not be determined. Disodium Cocoamphodiacetate (0, 100, 300, or 1000 mg/kg bw/d; in water; gavage administration) was given to Wistar Han rats (10/sex/group) to evaluate parental toxicity. In this assay, males were treated for 29 d (before, during, and after mating), and females were treated for 50 - 54 d (before and during mating, throughout pregnancy, and during lactation). Females without offspring were treated for 41 d. No reproductive toxicity was observed in either the parent or F1 generation. The reproductive NOAEL was determined to be 1000 mg/kg bw/d. Wistar Han rats (10/sex/dose) were treated with Disodium Cocoamphodiacetate (47 - 48% solids; in water; 0, 100, 30, or 1000 mg/kg bw/d; 90-d gavage administration). Animals were evaluated for changes in reproductive parameters such as estrous cycle length, spermatogenesis, and histopathology of reproductive organs; no adverse effects were observed. [Results for the non-reproductive parameters evaluated in this study can be found in the Subchronic Toxicity section of this report.] A reproductive NOAEL of 1000 mg/kg bw/d was established in a reproductive toxicity assay performed in Wistar Han rats (10/sex/group) using Sodium Cocoamphoacetate (0, 100, 300, or 1000 mg/kg bw/d; in water; gavage administration). A developmental and maternal NOAEL of 1000 mg/kg bw was established in a developmental toxicity assay performed in female Wistar Han rats (22/group) given Sodium Lauroamphoacetate (0, 100, 300, or 1000 mg/kg bw/d; in water; gavage administration).

GENOTOXICITY STUDIES

Ames assays were performed with Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, and Sodium Cocoamphoacetate (up to 1 µl/plate; with and without metabolic activation) using Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100.[1] The test substances were not considered to be mutagenic.

Details on the in vitro genotoxicity assays summarized here can be found in Table 9. The genotoxic potential of Sodium Lauroamphoacetate was evaluated in three in vitro assays.[4] Sodium Lauroamphoacetate (35% solids; water, sodium chloride, and sodium glycolate; up to 4375 µg/plate) was considered to be non-genotoxic in an Ames assay performed on *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100. Similarly, no genotoxicity was observed in an Ames assay performed on Sodium Lauroamphoacetate (water and sodium chloride; up to 5000 µg/plate) using *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 uvr A. Sodium Lauroamphoacetate (water, sodium chloride, and sodium glycolate; up to 250 µg/ml) was considered non-clastogenic in a mammalian chromosome aberration assay performed using human peripheral blood lymphocytes. All assays were performed with and without metabolic activation.

CARCINOGENICITY STUDIES

Carcinogenicity studies were not found in the literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Corneal Epithelium Impairment

Disodium Cocoamphodiacetate

The following study is included as it may be helpful in addressing cosmetic safety concerns following ocular exposure to Disodium Cocoamphodiacetate. The right eye of C5BL/6 mice (n = 8) was anesthetized with isoflurane, and either the control (10 µl phosphate-buffered saline (PBS)), 0.1% Disodium Cocoamphodiacetate in PBS, or 1% Disodium Cocoamphodiacetate in PBS was administered.[18] Treatment was performed once per day, for 7 or 14 consecutive days. Morphological and pathological changes in the murine ocular surface were evaluated. After one day of treatment, slit lamp images revealed that no obvious alterations were observed in corneas treated with 0.1% Disodium Cocoamphodiacetate; however, corneas treated with 1% Disodium Cocoamphodiacetate manifested diffuse sodium fluorescein staining in the central area. After 7 d of treatment punctuate staining of fluorescein was observed in 0.1% Disodium Cocoamphodiacetate-treated animals, and haze appeared in the central cornea of 1% Disodium Cocoamphodiacetate-treated animals. Hematoxylin and eosin staining performed on eyes treated with 0.1% Disodium Cocoamphodiacetate and control eyes for 14 d revealed a statistically significant decrease of epithelial thickness in the Disodium Cocoamphodiacetate-treated group compared to the control ($P < 0.05$). To determine if the test substances promoted corneal epithelial apoptosis, a TdT-dUTP terminal nick-end labeling (TUNEL) assay was performed after 14 d of treatment. Very few TUNEL-positive cells were observed in the control group, while an increased number of TUNEL-positive cells were found in the Disodium Cocoamphodiacetate-treated groups, in a dose-dependent manner.

Co-Reactivity of Surfactant Allergens

Disodium Lauroamphodiacetate

The following study is included as it may be helpful in addressing irritation/hypersensitivity concerns following exposure to Disodium Lauroamphodiacetate. Previously patch-tested, surfactant-positive subjects (n = 47) were patch-tested with 1 and 2% aqueous Disodium Lauroamphodiacetate, screening surfactants (cocamidopropyl betaine, amidoamine, dimethylaminopropylamine, cocamide diethanolamine, oleamidopropyl dimethylamine, and decyl glucoside), the novel surfactants sodium lauroyl sarcosinate and isostearamidopropyl morpholine lactate, and a hypoallergenic liquid cleanser.[19] Patch testing occurred for 5-8 d under occlusive conditions for all test substances except for the hypoallergenic liquid cleanser, which was tested in a semi-open fashion. Doubtful, mild, and moderate reactions to Disodium Lauroamphodiacetate (concentration at which reactions were noted was not specified) were observed in 7, 2, and 1 subjects, respectively. Of the three participants who displayed a mild or moderate reaction to Disodium Lauroamphodiacetate, 2 reacted to isostearamidopropyl morpholine lactate and 1 reacted to dimethylaminopropylamine, oleamidopropyl dimethylamine, amidoamine, cocamidopropyl betaine, or sodium lauroyl sarcosinate.

Reactivity to Irritants in Atopic and Non-Atopic Patients

Sodium Cocoamphoacetate

The following study is included as it may be helpful in addressing irritation concerns following exposure to Sodium Cocoamphoacetate. Patch testing was performed in 40 healthy volunteers and 480 atopic subjects (affected by atopic dermatitis, psoriasis, or eczema) using several irritants, including 15 µl aqueous solutions of Sodium Cocoamphoacetate (3 and 5%).[20] Patch tests were applied to the back for 2 d (level of occlusion not stated). Readings were performed 1 h after patch removal. No reactions were observed in healthy subjects treated with 3% Sodium Cocoamphoacetate; however, 2 healthy subjects displayed positive reactions to 5% Sodium Cocoamphoacetate. Three and 11 atopic subjects displayed positive reactions to 3% Sodium Cocoamphoacetate and 5% Sodium Cocoamphoacetate, respectively.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Single patch tests were performed using Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate (ingredients were as commercially supplied) in rabbits (occlusive conditions; abraded and unabraded skin; 24-h applications).[1] Disodium Cocoamphodiacetate and Sodium Cocoamphoacetate ranged from non-irritating to severely irritating. Disodium Cocoamphopropionate was observed to be non-irritating in rabbits, and slight irritation was observed in assays performed using Sodium Cocoamphopropionate. Dermal irritation was also evaluated in rabbits via a single intradermal injection of Disodium Cocoamphodiacetate (tested at 1%), Disodium Cocoamphodipropionate (tested at 1%), and Sodium Cocoamphopropionate (tested at 0.1%). All test substances resulted in less irritation compared to control shampoos (olive oil castile shampoo). Cleansing creams containing 5% Disodium Cocoamphodipropionate very mildly irritating in 12 subjects in a 21-d cumulative irritation assay (occlusive), and were non-irritating when products were applied daily for 2 wk (n = 24) or 1 mo (n = 53). A facial cleanser containing 25% Disodium Cocoamphodiacetate (45.6% solids) that was routinely used by subjects (n = 54) for 1 mo produced no adverse effects.

A human repeated insult patch test (HRIPT) evaluating the sensitization potential of 10% Sodium Cocoamphoacetate and 10% Sodium Cocoamphopropionate in human subjects yielded negative results (n = 141; non-occlusive conditions). No sensitization was observed in a maximization assay performed in 25 subjects using a diluted hair product containing 0.1%

Disodium Cocoamphodipropionate. A cleansing cream containing 5% Disodium Cocoamphodipropionate was non-irritating and non-sensitizing in an HRIPT. In addition, no sensitization was observed in an HRIPT using Disodium Cocoamphodiacetate (32% solids), under semi-occlusive conditions; however, some irritation was noted under occlusive conditions.

Details regarding the animal and human dermal irritation and sensitization studies summarized here can be found in Table 10. Test substances were considered to be non-irritating in two irritation assays performed in rabbits using Sodium Lauroamphoacetate (35-50% solids).[4] Severe dermal irritation was noted in two assays performed in the intact and abraded skin of New Zealand albino rabbits using a trade name mixture containing Sodium Lauroamphoacetate (36 - < 67.9%).[21,22] Test substances (Disodium Cocoamphodiacetate (up to 5%), Sodium Cocoamphoacetate (up to 5%), and Sodium Lauroamphoacetate (35% solids; tested undiluted)) produced none to slight irritation in irritation assays performed in humans.[4,16,23,24] Erythema and scaling was observed in in a 48-h occlusive patch test performed in 12 subjects using Sodium Cocoamphoacetate (10%) in citrate buffer.[25] Irritation was observed in a soap chamber and epicutaneous dermal irritation assay using 1% Sodium Lauroamphoacetate and 2% Sodium Lauroamphoacetate, respectively.[16]

No sensitization was observed in a guinea pig maximization test using Sodium Cocoamphoacetate (water, sodium chloride, and sodium glycolate).[4] The test substance was evaluated as a 1% (0.394% solids), 5%, and 75% dilution in water for the intradermal, epicutaneous, and challenge exposures, respectively. A two-part local lymph node assay was performed in female CBA/J mice (4/group). Animals were exposed to the test article (Sodium Lauroamphoacetate (water and sodium chloride)), in propylene glycol, at up to 30% in experiment 1 and up to 50% in experiment 2. No signs of hypersensitivity was observed in experiment 1; however, delayed contact hypersensitivity was noted at concentrations of 50%. A guinea pig maximization test was performed using Sodium Lauroamphoacetate (0.18 - 17.5% solids). The test substance, tested at 0.5% for the intradermal induction, 50% for the epicutaneous induction, and at 20% for the challenge exposure, was considered to be non-sensitizing. The sensitization potential of a 0.5% aqueous solution of Sodium Lauroamphoacetate (0.15% solids) was evaluated in an HRIPT in 99 subjects.[4] Subjects were exposed to the test substance, under occlusive conditions for 9, 24-h induction periods, followed by a 24-h challenge exposure. The test substance was considered to be non-irritating and non-sensitizing.

Photosensitization/Phototoxicity

Sodium Cocoamphoacetate, Sodium Cocoamphopropionate, and Disodium Cocoamphoacetate (tested at 10% in distilled water) did not cause photo-allergic reactions or delayed contact hypersensitivity in an assay performed in 30 subjects.[1]

OCULAR IRRITATION STUDIES

Several ocular irritation assays were performed using Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate (ingredients were as commercially supplied; 0.1 ml), predominantly via the Draize method, using rabbits.[1] For some assays, rinse-out procedures were performed prior to scoring irritation. Disodium Cocoamphodiacetate was considered to be moderately to severely irritating when the test substance was not rinsed from the eyes, and minimally to mildly irritating when the test substance was rinsed from the eyes. Disodium Cocoamphopropionate was non-irritating under unrinsed conditions. Sodium Cocoamphoacetate was considered to be minimally to severely irritating under unrinsed conditions. Sodium Cocoamphopropionate was non-irritating to minimally irritating under unrinsed conditions. In some assays, Disodium Cocoamphodiacetate was observed to have an anti-irritation effect on rabbit corneas. In a human ocular irritation assay, a shampoo containing 28.1% Disodium Cocoamphodiacetate (diluted up to 10% in distilled water) was evaluated in 30 subjects. Irritation was similar among the test substance and control-treated groups (treated with distilled water).

Details regarding the ocular irritation studies summarized here are provided in Table 11. The majority of in vitro ocular irritation assays performed using Disodium Cocoamphodiacetate (up to 3%), Sodium Cocoamphodiacetate (up to 3%), and Sodium Lauroamphoacetate (up to 3%) reported no to slight irritation; however, a red blood cell test using 1% Disodium Cocoamphodiacetate resulted in moderate irritation.[16] However, severe irritation potential was observed with higher concentrations. Severe irritation was noted in an EpiOcular™ assay evaluating the ocular irritation potential of 50% Disodium Cocoamphodiacetate.[26] Severe ocular irritation was also observed in a hen's egg test-chorioallantoic membrane (HET-CAM) assay using 40% Sodium Lauroamphoacetate.[27] In several studies, Sodium Lauroamphoacetate (tested as 10 - 50% solids; water and sodium chloride; tested undiluted) was not considered to be an ocular irritant based on Classification, Labelling, and Packaging (CLP) criteria in three assays performed in New Zealand White rabbits (n = 3 - 6). However, in one study Sodium Lauroamphoacetate (50% solids; water and sodium chloride; tested undiluted) was considered to be a category 2 ocular irritant (based on CLP criteria) when evaluated in 3 New Zealand White rabbits. All signs of irritation were fully reversible within 7 d post-administration. No symptoms of eye irritation were observed in assays performed in humans (n = 10), in which subjects were reported to use a micellar water cleanser containing Disodium Cocoamphodiacetate (0.4 and 1.2%) once per day for 21 d.[28]

CLINICAL STUDIES

Case Reports

Disodium Cocoamphodipropionate

A 28-yr-old woman with a history of eczema reported worsened dermatitis following dermal exposure to contact lens solution (containing 38-40% Disodium Cocoamphodipropionate).[29] Patch tests were performed using the undiluted contact lens fluid, as well as the contact lens fluid ingredients, including Disodium Cocoamphodipropionate (0.1 - 1%; aqueous solution). Positive reactions were observed following testing with Disodium Cocoamphodipropionate at all tested concentrations, as well as the undiluted contact lens fluid. Twenty-one non-atopic control individuals were patch tested with a 1% aqueous solution of Disodium Cocoamphodipropionate. No positive reactions were observed.

Disodium Lauroamphodiacetate

A 46-yr-old massage therapist with a history of contact allergies presented with hand dermatitis following use of a hypoallergenic liquid cleanser.[30] In addition, a 57-yr-old woman with a history of hand dermatitis displayed atopic symptoms following the use of the same cleanser. Semi-open patch tests were performed on both individuals using the liquid cleanser itself (1, 10, and 100%; aqueous solution), and the cleanser ingredients, including Disodium Lauroamphodiacetate (1 and 2%; aqueous solution). Patch tests were also performed in 10 healthy control subjects. Positive responses were observed in both atopic patients following testing with Disodium Lauroamphoacetate (at both test concentrations), and the liquid cleanser (tested at 100%). No positive responses were observed in control subjects.

Sodium Cocoamphoacetate

A 45-yr-old woman with a history of eczema and rhinoconjunctivitis reported facial dermatitis following the use of a makeup remover containing Sodium Cocoamphoacetate (concentration not specified).[31] Patch tests were performed using the eye makeup remover and Sodium Cocoamphoacetate (1 and 2%; aqueous solution). Thirty-three non-atopic control subjects underwent the same patch testing. Positive reactions were observed in the atopic individual for both concentrations of Sodium Cocoamphoacetate, and the eye makeup remover. Some weak irritant reactions were noted in control subjects treated with 2% Sodium Cocoamphoacetate. No reactions were observed in control subjects following testing with 1% Sodium Cocoamphoacetate. It was not stated whether control subjects elicited a response to the eye makeup remover formulation.

Sodium Cocoamphopropionate

Four individuals reported hand and forearm dermatitis following use of a skin protection cream containing Sodium Cocoamphopropionate.[32] One of the four individuals had a history of atopic disease (allergic rhinoconjunctivitis). Occlusive patch tests (24-h) were performed on the individuals using the cream itself, as well as the cream ingredients, including Sodium Cocoamphopropionate (1%; aqueous solution). Positive reactions were observed in all individuals following testing with the cream and 1% Sodium Cocoamphopropionate. Eczema improved in all patients following elimination of exposure to Sodium Cocoamphopropionate.

Sodium Lauroamphoacetate

Four cases of atopic dermatitis were reported in individuals following exposure to detergents containing amphoacetates.[11] Patch tests of aqueous solutions of Sodium Lauroamphoacetate (1, 5, and 10%), as well as undiluted Sodium Lauroamphoacetate, were administered to patients under occlusive conditions, for 2 d. Other substances tested include ethylenediamine (concentration not reported) and AEEA (1%). Twenty non-allergic control subjects were patch tested with Sodium Lauroamphoacetate (1, 5, 10, and 100%) and AEEA (1%). All four atopic individuals displayed positive reactions to Sodium Lauroamphoacetate and AEEA at all tested concentrations. Six of the 20 non-atopic control subjects responded with an irritation reaction to Sodium Lauroamphoacetate tested at 100%. No other reactions were reported in control subjects.

Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, Sodium Cocoamphopropionate, and Sodium Lauroamphoacetate

A 34-yr-old nurse working in a surgical department reported hand and forearm dermatitis following use of a disinfectant hand cleanser containing 2% Sodium Cocoamphopropionate.[33] Patch tests of the diluted hand soap (3.2 – 20%), as well as patch tests of the individual hand soap ingredients, including Sodium Cocoamphopropionate (1 – 10%), were performed. Related surfactants that were not ingredients of the hand soap were also patch tested (Sodium Cocoamphoacetate (1 – 10%), Sodium Lauroamphoacetate (1 – 10%), Disodium Cocoamphodipropionate (10%), and AEEA (0.1 – 1%)). Positive patch test results were observed for the hand cleanser (at all concentrations), Sodium Cocoamphopropionate (at 3.2% and higher), Sodium Cocoamphoacetate (at 3.2% and higher), Sodium Lauroamphoacetate (at 3.2% and higher), and AEEA (at 0.32% and higher). Four fast-food restaurant workers also reported atopic dermatitis following exposure to the same hand cleanser containing 2% Sodium Cocoamphopropionate. Patch tests were performed in these individuals according to similar procedures as mentioned above. Positive reactions were observed for all tested substances (hand cleanser (at all concentrations), Sodium Cocoamphopropionate (at all concentrations), Sodium Cocoamphoacetate (at 3.2% and higher),

Sodium Lauroamphoacetate (at 3.2% and higher), Disodium Cocoamphodipropionate (at all concentrations), and AEEA (at all concentrations). Other reports of hand irritation following use of this hand cleanser were reported in 24-yr-old and 27-yr old fast-food workers with recurrent eczema.[34] These patients were patch tested with several materials including ethylenediamine (1%), the hand soap (100%), and Sodium Cocoamphopropionate (1%; aqueous solution). Both patients showed positive reactions to all test substances. Sodium Cocoamphopropionate (1%; aqueous solution) was also tested in 20 non-atopic control individuals. No irritation or allergic reactions were observed.

SUMMARY

The safety of 11 amphoacetate ingredients is reviewed in this safety assessment. These ingredients are reported to function as various types of surfactants (cleansing agents, foam boosters, hydrotropes) and hair-conditioning agents in cosmetics. Disodium Cocoamphodiacetate, Disodium Cocoamphodipropionate, Sodium Cocoamphoacetate, and Sodium Cocoamphopropionate have been previously reviewed by the Panel and were considered safe in the present practices of use and concentration as described in the safety assessment published in 1990. This conclusion was re-affirmed in 2008.

According to 2023 VCRP survey data, Disodium Cocoamphodiacetate has the highest frequency of use (220 total formulations; 40 leave-on formulations, 179 rinse-off formulations, and 1 formulation diluted for bath use. Sodium Lauroamphoacetate is reported to be used in 202 total formulations (183 rinse-off formulations; 17 rinse-off formulations; and 2 formulations diluted for bath use). All other ingredients are reported to be used in 121 formulations or less. The results of the 2021 concentration of use survey conducted by Council indicate that Disodium Lauroamphodiacetate has the highest concentration of use in leave-on products; it is used at up to 5.4% in other hair preparations.

Acute oral toxicity studies were performed using Sodium Lauroamphoacetate in mice and rats. An LD_{50} of 6116 mg/kg for Sodium Lauroamphoacetate (% solids not stated; water and sodium chloride) was determined in mice. The lowest LD_{50} in rats was reported to be > 2000 mg/kg bw (using Sodium Lauroamphoacetate (50% solids; water and sodium chloride; tested as provided) and Sodium Lauroamphoacetate (35% solids; water, sodium chloride, sodium glycolate; tested as a 20% aqueous dilution). An NOAEL of 1000 mg/kg bw/d was established in a 90-d oral subchronic toxicity assay in which Wistar Han rats (10/sex/group in main study; 5/sex/group in recovery group) were given Disodium Cocoamphodiacetate (47.2 – 48% solids), in water, via gavage, in doses of up to 1000 mg/kg bw/d.

A maternal NOAEL of 1000 mg/kg bw/d was established in a reproductive toxicity assay in which Disodium Cocoamphodiacetate (up to 1000 mg/kg bw/d; in water; gavage administration; treated days 6 - 20 post-coitum) was given to female Wistar Han rats (22/group). Severe cardiac abnormalities were observed in fetuses in all treated test groups (not including control group). A parental NOAEL of 300 mg/kg bw/d was determined in an assay in which Disodium Cocoamphodiacetate (up to 1000 mg/kg bw/d; in water; gavage administration) was given to Wistar Han rats (10/sex/dose). Males were treated before, during, and after mating, and females were treated before and during mating, throughout pregnancy, and during lactation. No reproductive toxicity was observed in either the parent or F1 generation. No adverse effects regarding estrous cycle length, spermatogenesis, and histopathology of reproductive organs were observed in an assay in which Wistar Han rats (10/sex/dose) were treated with Disodium Cocoamphodiacetate (47 - 48% solids; in water; up to 1000 mg/kg bw/d; 90-d gavage administration). A parental NOAEL of 1000 mg/kg bw/d was established in a reproductive toxicity assay performed in Wistar Han rats (10/sex/group) using Sodium Cocoamphoacetate (up to 1000 mg/kg bw/d; in water; gavage administration). Similarly, a developmental and maternal NOAEL of 1000 mg/kg bw was established in a developmental toxicity assay performed in female Wistar Han rats (22/group) given Sodium Lauroamphoacetate (up to 1000 mg/kg bw/d; in water; gavage administration).

No genotoxicity was observed in Ames assays performed using Sodium Lauroamphoacetate (35% solids; water, sodium chloride, and sodium glycolate; up to 4375 µg/plate) and Sodium Lauroamphoacetate (water and sodium chloride; up to 5000 µg/plate). Similarly, Sodium Lauroamphoacetate (water, sodium chloride, and sodium glycolate; up to 250 µg/ml) was considered to be non-clastogenic in a mammalian chromosome aberration assay. All assays were performed with and without metabolic activation.

In an assay performed to evaluate the potential corneal epithelium impairment effects of Disodium Cocoamphodiacetate, C5BL/6 mice (n = 8) were administered either the control (10 µl phosphate-buffered saline (PBS)), 1% Disodium Cocoamphodiacetate in PBS, or 0.1% Disodium Cocoamphodiacetate in PBS, in the right eye, once a day, for 7 or 14 d. Treatment with both 0.1 and 1% Disodium Cocoamphodiacetate resulted in corneal impairment (e.g., decreased thickness, increased apoptosis of corneal cells).

Previously patch-tested, surfactant-positive subjects (n = 47) were patch-tested (5 - 8 d testing duration) with several types of surfactants, including Disodium Lauroamphodiacetate (aqueous solution; 1 and 2%). Doubtful, mild, and moderate reactions to Disodium Lauroamphodiacetate (concentration at which reactions were noted was not specified) were observed in 7, 2, and 1 subjects.

Patch testing was performed in 40 healthy volunteers and 480 atopic subjects (affected by atopic dermatitis, psoriasis, or eczema) using several irritants, including Sodium Cocoamphoacetate (aqueous solution; 3 and 5%). No reactions were observed in healthy subjects treated with 3% Sodium Cocoamphoacetate; however, 2 healthy subjects displayed positive

reactions to 5% Sodium Cocoamphoacetate. Three and 11 atopic subjects displayed positive reactions to 3% Sodium Cocoamphoacetate and 5% Sodium Cocoamphoacetate, respectively.

Test substances were considered to be non-irritating in two irritation assays performed in rabbits using Sodium Lauroamphoacetate (35-50% solids). Severe dermal irritation was noted in two assays performed in the intact and abraded skin of New Zealand albino rabbits using a trade name mixture containing Sodium Lauroamphoacetate (36 - < 67.9%). Test substances (Disodium Cocoamphodiacetate (up to 5%), Disodium Cocoamphodiacetate (up to 2%), Sodium Cocoamphoacetate (up to 5%), and Sodium Lauroamphoacetate (35% solids)) produced none to slight irritation in irritation assays performed in humans. Erythema and scaling were observed in in a 48-h occlusive patch test performed in 12 subjects using Sodium Cocoamphoacetate (10%) in citrate buffer. Irritation was observed in a soap chamber and epicutaneous dermal irritation assay using 1% Sodium Lauroamphoacetate and 2% Sodium Lauroamphoacetate, respectively.

No sensitization was observed in a guinea pig maximization test using Sodium Cocoamphoacetate (water, sodium chloride, and sodium glycolate; tested as a 1% (0.394% solids), 5%, and 75% dilution in water for the intradermal, epicutaneous, and challenge exposures, respectively). Delayed contact hypersensitivity was observed in a local lymph node assay performed in mice using Sodium Lauroamphoacetate (water and sodium chloride; vehicle of propylene glycol) when tested at 50%. No hypersensitivity was observed when this test substance was used at 30%. No sensitization was observed in a guinea pig maximization test performed using Sodium Lauroamphoacetate (0.18 – 17.5% solids; water, sodium chloride and sodium glycolate (tested at 0.5% for the intradermal induction, 50% for the epicutaneous induction, and at 20% for the challenge exposure)). A 0.5% aqueous solution of Sodium Lauroamphoacetate (0.15% solids) was considered to be non-irritating and non-sensitizing in an HRIPT performed in 99 subjects.

The majority of in vitro ocular irritation assays performed using Disodium Cocoamphodiacetate (up to 3%), Sodium Cocoamphodiacetate, (up to 3%) and Sodium Lauroamphoacetate (up to 3%) reported none to slight irritation; however, a red blood cell test using 1% Disodium Cocoamphodiacetate resulted in moderate irritation. However, severe irritation potential was observed with higher concentrations. Severe irritation was noted in an EpiOcular^{TM} assay evaluating the ocular irritation potential of 50% Disodium Cocoamphodiacetate. Severe ocular irritation was also observed in a HET-CAM assay using 40% Sodium Lauroamphoacetate. Sodium Lauroamphoacetate (tested as 10 - 50% solids; water and sodium chloride; tested undiluted) was not considered to be an ocular irritant when tested in rabbits. However, Sodium Lauroamphoacetate (50% solids; water and sodium chloride; tested undiluted) was considered to be a category 2 ocular irritant when evaluated in rabbits. No eye irritation was observed in assays performed in humans (n = 10), in which subjects were reported to use a micellar water cleanser containing Disodium Cocoamphodiacetate (0.4% and 1.2%) once per day for 21 d.

Several case reports were found in the literature regarding dermatitis following the use of products containing amphoacetates. A positive patch test reaction to Disodium Cocoamphodipropionate (0.1 – 1%; aqueous solution) was observed in a 28-yr-old woman experiencing dermatitis following exposure to a contact lens solution containing Disodium Cocoamphodipropionate. Two women presented with hand dermatitis following exposure to a cleanser containing Disodium Lauroamphodiacetate. Positive patch tests were observed in both patients for both the cleanser and Disodium Lauroamphodiacetate (1 and 2%; aqueous solution). A 45-yr-old woman reported facial dermatitis following the use of a makeup remover containing Sodium Cocoamphoacetate. Patch tests for the eye makeup remover and for Sodium Cocoamphoacetate (1 and 2%; aqueous solution) were positive. Four individuals with a history of allergies reported dermatitis following the use of a cream containing Sodium Cocoamphopropionate. All subjects had positive patch test reactions to the cream and 1% Sodium Cocoamphopropionate (aqueous solution). Four cases of atopic dermatitis were reported in individuals following exposure to detergents containing amphoacetates. All four individuals displayed positive patch test reactions to Sodium Lauroamphoacetate (1, 5, 10, and 100%; aqueous solutions) and AEEA (1%). Several cases of dermatitis have been reported following exposures to hand cleansers containing amphoacetates. Patch testing using several amphoacetates (Disodium Cocoamphodipropionate (1 - 10%) Sodium Cocoamphoacetate (1 - 10%), Sodium Cocoamphopropionate (1 - 10%), Sodium Lauroamphoacetate (1 - 10%)), performed in these individuals, yielded positive results.

INFORMATION SOUGHT

The following information on the amphoacetates reviewed in this report is being sought for use in the resulting safety assessment:

- Composition and impurities data on all ingredients; specifically, the constituents and percent solids of these ingredients as finished solutions
- Method of manufacturing data
- Dermal absorption data; if absorbed, additional toxicity studies may be needed
- Irritation and sensitization data, at maximum concentrations of use

TABLES

Table 1. INCI names, definitions, structures, and functions of the amphoacetate ingredients reviewed in this safety assessment[3]

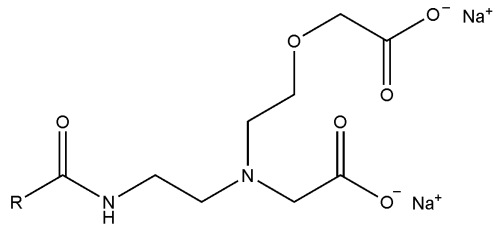
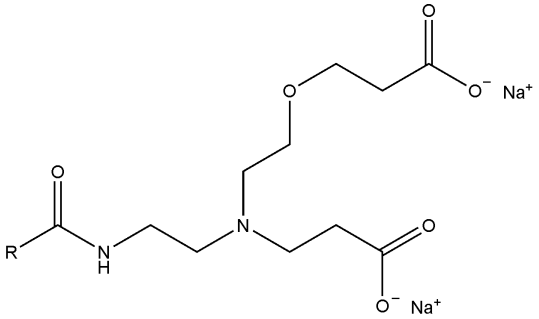
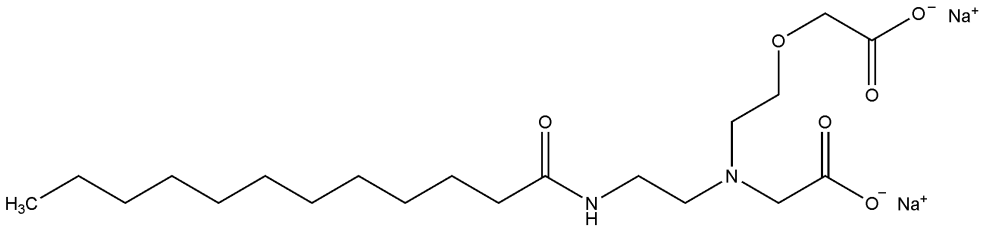
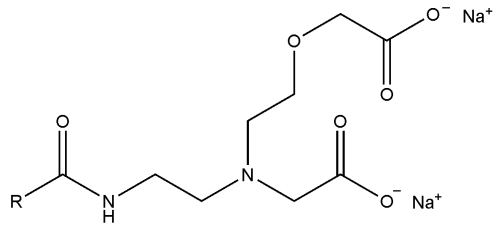
Ingredient	Definition	Function
Disodium Cocoamphodiacetate [CAS: 68650-39-5]	Disodium Cocoamphodiacetate is the amphoteric organic compound that conforms generally to the structure:  where RC(O)- represents the acyl groups derived from coconut oil.	Hair Conditioning Agents; Surfactants – Cleansing Agents; Surfactants – Foam Boosters; Surfactants – Hydrotropes
Disodium Cocoamphodipropionate [CAS: 68411-57-4; 86438-79-1]	Disodium Cocoamphodipropionate is the amphoteric organic compound that conforms generally to the structure:  where RC(O)- represents the acyl groups derived from coconut oil.	Hair Conditioning Agents; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Surfactants - Hydrotropes
Disodium Lauroamphodiacetate [CAS: 14350-97-1]	Disodium Lauroamphodiacetate is the amphoteric organic compound that conforms generally to the formula: 	Hair Conditioning Agents; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Surfactants - Hydrotropes
Disodium Wheatgermamphodiacetate	Disodium Wheatgermamphodiacetate is the organic compound that conforms to the formula:  where RC(O)- represents the acyl groups derived from wheat germ oil.	Hair Conditioning Agents Surfactants - Cleansing Agents Surfactants - Foam Boosters Surfactants - Hydrotropes

Table 1. INCI names, definitions, structures, and functions of the amphoacetate ingredients reviewed in this safety assessment[3]

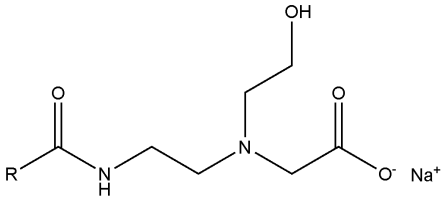
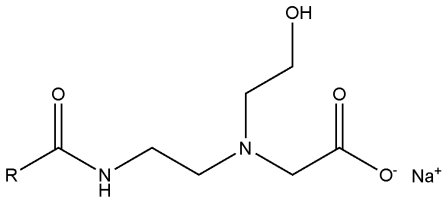
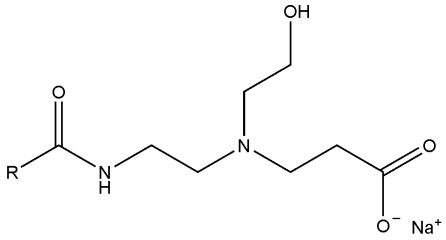
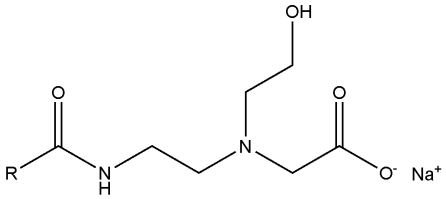
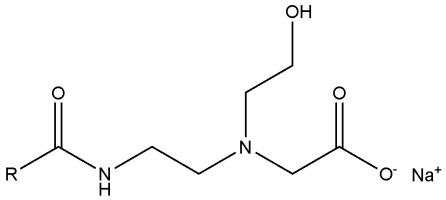
Ingredient	Definition	Function
Sodium Arganamphoacetate	Sodium Arganamphoacetate is the amphoteric organic compound that conforms generally to the formula:  where RC(O)- represents the acyl groups derived from Argania Spinosa Kernel Oil.	Surfactants - Cleansing Agents
Sodium Cocoamphoacetate [CAS: 90387-76-1; 68334-21-4; 68608-65-1]	Sodium Cocoamphoacetate is the amphoteric organic compound that conforms generally to the formula:  where RC(O)- represents the acyl groups derived from coconut oil.	Hair Conditioning Agents; Surfactants - Cleansing Agents; Surfactants - Foam Boosters
Sodium Cocoamphopropionate	Sodium Cocoamphopropionate is the amphoteric organic compound that conforms generally to the formula:  where RC(O)- represents the acyl groups derived from coconut oil.	Hair Conditioning Agents; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Surfactants - Hydrotropes
Sodium Cottonseedamphoacetate	Sodium Cottonseedamphoacetate is the amphoteric organic compound that conforms generally to the formula:  where RC(O)- represents the acyl groups derived from cottonseed oil.	Surfactants - Cleansing Agents
Sodium Lauroamphoacetate [CAS: 68608-66-2; 156028-14-7; 66161-62-4]	Sodium Lauroamphoacetate is the amphoteric organic compound that conforms generally to the structure in *Figure 1*.	Hair Conditioning Agents; Surfactants - Cleansing Agents; Surfactants - Foam Boosters
Sodium Olivamphoacetate	Sodium Olivamphoacetate is the amphoteric organic compound that conforms generally to the formula:  where RC(O)- represents the acyl groups derived from olive oil.	Hair Conditioning Agents Surfactants - Cleansing Agents Surfactants - Foam Boosters

Table 1. INCI names, definitions, structures, and functions of the amphoacetate ingredients reviewed in this safety assessment[3]

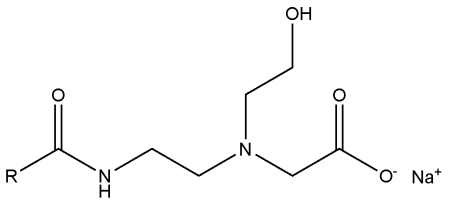
Ingredient	Definition	Function
Sodium Sweetalmondamphoacetate	Sodium Sweetalmondamphoacetate is the amphoteric organic compound that conforms generally to the formula:  where RC(O)- represents the acyl groups derived from sweet almond oil.	Hair Conditioning Agents; Surfactants - Cleansing Agents; Surfactants - Foam Boosters

Table 2. Chemical properties

Property	Value	Reference
Disodium Cocoamphodiacetate		
Physical Form	liquid	1
Color	light tan	1
Odor	faintly fruity	1
Specific Gravity (@ 25ºC)	1.17	35
Water Solubility	soluble	1
Alcohol Solubility	insoluble	1
Nonpolar Organic Solvent Solubility	insoluble	1
Disodium Cocoamphodipropionate		
Physical Form	liquid	1
Color	light amber	1
Odor	faintly fruity	1
Molecular Weight (g/mol)	292.24	36
Specific Gravity (@ 25ºC)	1.05	37
Vapor Pressure (mmHg @ 25ºC)	0.0000225	38
Boiling Point (ºC)	≥ 100; ≤ 101	38
log K_{ow}	-7.57	38
Water Solubility	soluble	1
Alcohol Solubility	soluble	1
Nonpolar Organic Solvent Solubility	insoluble	1
Disodium Lauroamphodiacetate		
Physical Form	liquid	39
Formula Weight (g/mol)	446.5	39
Disodium Wheatgermamphodiacetate		
Physical Form	liquid	1
Color	clear-amber	1
Odor	mild organic	1
Formula Weight (g/mol)	525 – 531	1
Specific Gravity	1.02	1
Boiling Point (ºC)	105	1
log K_{ow}	0.5	1
Sodium Cocoamphoacetate		
Physical Form	liquid	40
Color	clear – light amber	1
Odor	faintly fruity	1
Formula Weight (g/mol)	270.62	40
Water Solubility	soluble	1
Alcohol Solubility	insoluble	1
Nonpolar Organic Solvent Solubility	insoluble	1
Sodium Cocoamphopropionate		
Physical Form	liquid	1
Color	light amber	1
Odor	faintly fruity	1
Water Solubility	soluble	1
Alcohol Solubility	soluble	1
Nonpolar Organic Solvent Solubility	insoluble	1
Sodium Lauroamphoacetate		
Physical Form	powder	4

Table 2. Chemical properties

Property	Value	Reference
Disodium Cocoamphodiacetate		
Physical Form	liquid	[1]
Color	light tan	[1]
Odor	faintly fruity	[1]
Specific Gravity (@ 25ºC)	1.17	[35]
Water Solubility	soluble	[1]
Alcohol Solubility	insoluble	[1]
Nonpolar Organic Solvent Solubility	insoluble	[1]
Disodium Cocoamphodipropionate		
Physical Form	liquid	[1]
Color	light amber	[1]
Odor	faintly fruity	[1]
Molecular Weight (g/mol)	292.24	[36]
Specific Gravity (@ 25ºC)	1.05	[37]
Vapor Pressure (mmHg @ 25ºC)	0.0000225	[38]
Boiling Point (ºC)	≥ 100; ≤ 101	[38]
log K_{ow}	-7.57	[38]
Water Solubility	soluble	[1]
Alcohol Solubility	soluble	[1]
Nonpolar Organic Solvent Solubility	insoluble	[1]
Disodium Lauroamphodiacetate		
Physical Form	liquid	[39]
Formula Weight (g/mol)	446.5	[39]
Disodium Wheatgermamphodiacetate		
Physical Form	liquid	[1]
Color	clear-amber	[1]
Odor	mild organic	[1]
Formula Weight (g/mol)	525 – 531	[1]
Specific Gravity	1.02	[1]
Boiling Point (ºC)	105	[1]
log K_{ow}	0.5	[1]
Sodium Cocoamphoacetate		
Physical Form	liquid	[40]
Color	clear – light amber	[1]
Odor	faintly fruity	[1]
Formula Weight (g/mol)	270.62	[40]
Water Solubility	soluble	[1]
Alcohol Solubility	insoluble	[1]
Nonpolar Organic Solvent Solubility	insoluble	[1]
Sodium Cocoamphopropionate		
Physical Form	liquid	[1]
Color	light amber	[1]
Odor	faintly fruity	[1]
Water Solubility	soluble	[1]
Alcohol Solubility	soluble	[1]
Nonpolar Organic Solvent Solubility	insoluble	[1]
Sodium Lauroamphoacetate		
Color	light yellow	[4]
Formula Weight (g/mol)	349.5	[41]
Specific Gravity (@ 20ºC)	1.09	[4]
Vapor Pressure (mmHg @ 20ºC)	< 0.000011	[4]
Melting Point (ºC)	40	[4]
Water Solubility (g/l @ 20ºC)	1000	[4]

Table 3. Fatty acid compositions (%)[8,42]

Fatty Acids	Argan	Coconut	Cottonseed	Olive	Sweet Almond	Wheat Germ
Caproic (C6)		0.008 – 1.2				
Caprylic (C8)		3.4 – 15				
Capric (C10)		3.2 – 15				
Lauric (C12)		41 – 51.3				
Myristic (C14)		13 – 23	2		1	
Palmitic (C16)	10 – 15	4.2 – 18	21	7.5 – 20	4 – 9	11 – 16
Heptadecanoic (C17)					0.2	
Stearic (C18)	5 – 6.5	1.6 – 4.7	trace	0.5 – 3.5		1 – 6
Oleic (C18:1)	45 – 55	3.4 – 12	30	53 – 86	62 – 86	8 – 30
Linoleic (C18:2)		0.9 – 3.7	45	3.5 – 20	20 – 30	44 – 65
Arachidic (C20)		1.03	trace		0.2	
Palmitoleic (C16:1)				0.3 – 3.5	0.8	4 – 10
Stearic (C18)					2 – 3	
Linolenic (C18:3)	28 – 36			0 – 1.5	0.4	
Eicolenoic (C20:1)					0.3	
Behenic (C22)					0.2	
Erucic (C22:1)					0.1	
Other					< C16 = 0.1	0 – 1.2 (C20 – C22 saturated acids)

Table 4. Tradename mixtures of amphoacetates

Ingredient	Composition	Reference
Disodium Cocoamphodiacetate	47.5-52.5% Disodium Cocoamphodiacetate, 37.5-40% water, 11-12.5% sodium chloride, 0.02% dichloroacetic acid, and 0.01% chloroacetic acid	[43]
Disodium Cocoamphodipropionate	30-40% Disodium Cocoamphodipropionate, 60-70% water, <0.1% other components (not specified)	[44]
Disodium Lauroamphodiacetate	30-60% Sodium Lauroamphoacetate and < 0.1% dichloracetic acid (remaining components not stated)	[45]
Sodium Cocoamphoacetate	30% pure active surfactant, 59% water, 7% sodium chloride, 1-2% glycolic acid, <1% fatty acid, < 0.6% diamide, 0.5% amidoamine , < 10 ppm dichloroacetic acid, and < 5 ppm monochloroacetic acid	[46]
Sodium Lauroamphoacetate	30 – 32% Sodium Lauroamphoacetate, 1-5% amidoamine, 1-5% glycolate, <70% water/inert materials	[7]

Table 5. Frequency (2023) and concentration (2021) of use according to likely duration and exposure and by product category[13,14,47]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Disodium Lauroamphodiacetate		Disodium Wheatgermamphodiacetate		Sodium Arganamphoacetate		Sodium Cottonseedamphoacetate	
Totals	**10**	**0.18 – 5.4**	**NR**	**0.93**	**1**	**NR**	**1**	**NR**
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	*1*	*1.6 – 5.4*	*NR*	*NR*	*1*	*NR*	*NR*	*NR*
Rinse-Off	*9*	*0.18 – 1.3*	*NR*	*0.93*	*NR*	*NR*	*1*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type*								
Eye Area	2	0.18	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	1[a]	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	1[a]	NR	NR	NR
Dermal Contact	9	0.18 – 1.6	NR	NR	1	NR	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	1	1.3 – 5.4	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	0.93	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	1	NR
Baby Products	1	1.3 – 1.6	NR	NR	NR	NR	NR	NR
as reported by product category								
Baby Products								
Baby Shampoos	NR	1.3						
Baby Lotions/Oils/Powders/Creams								
Other Baby Products	1	1.6						
Bath Preparations (diluted for use)								
Bubble Baths								
Other Bath Preparations								
Eye Makeup Preparations								
Eye Makeup Remover	2	0.18						
Other Eye Makeup Preparations								
Fragrance Preparations								
Perfumes								
Hair Preparations (non-coloring)								
Hair Conditioner								
Hair Spray (aerosol fixatives)								
Hair Straighteners								
Permanent Waves								
Shampoos (non-coloring)	1	NR						
Tonics, Dressings, and Other Hair Grooming Aids								
Other Hair Preparations	NR	5.4						
Hair Coloring Preparations								
Hair Dyes/Colors (all types requiring caution statements and patch tests)			NR	0.93				
Hair Shampoos (coloring)								
Other Hair Coloring Preparations								
Makeup Preparations								
Other Makeup Preparations								
Manicuring Preparations (Nail)								
Other Manicuring Preparations								

Table 5. Frequency (2023) and concentration (2021) of use according to likely duration and exposure and by product category[13,14,47]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Personal Cleanliness Products								
Bath Soaps and Detergents								
Douches								
Feminine Deodorants								
Other Personal Cleanliness Products							1	NR
Shaving Preparations								
Preshave Lotions (all types)								
Shaving Cream								
Skin Care Preparations								
Cleansing	6	0.2						
Face and Neck (exc shave)					1	NR		
Body and Hand (exc shave)								
Moisturizing								
Paste Masks (mud packs)								
Other Skin Care Preparations								
	Sodium Lauroamphoacetate		**Sodium Olivamphoacetate**		**Sodium Sweetalmondamphoacetate**			
Totals	**202**	**0.46 – 9.9**	**25**	**NR**	**15**	**NR**		
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	*17*	*0.8 – 1.1*	*NR*	*NR*	*NR*	*NR*		
Rinse-Off	*183*	*0.46 – 9.9*	*25*	*NR*	*15*	*NR*		
Diluted for (Bath) Use	*2*	*0.72 – 1.3*	*NR*	*NR*	*NR*	*NR*		
Exposure Type*								
Eye Area	3	1.3	NR	NR	NR	NR		
Incidental Ingestion	NR	NR	NR	NR	NR	NR		
Incidental Inhalation-Spray	1; 1[b]	NR	NR	NR	NR	NR		
Incidental Inhalation-Powder	1[c]	NR	NR	NR	NR	NR		
Dermal Contact	183	0.46 – 9.9	15	NR	15	NR		
Deodorant (underarm)	NR	NR	NR	NR	NR	NR		
Hair - Non-Coloring	17	0.75 – 4.4	10	NR	NR	NR		
Hair-Coloring	2	NR	NR	NR	NR	NR		
Nail	NR	NR	NR	NR	NR	NR		
Mucous Membrane	112	0.72 – 5.3	15	NR	15	NR		
Baby Products	8	0.8 – 1.1	NR	NR	NR	NR		
as reported by product category								
Baby Products								
Baby Shampoos	2	0.8						
Baby Lotions/Oils/Powders/Creams	1	1.1						
Other Baby Products	5	0.8						
Bath Preparations (diluted for use)								
Bubble Baths	NR	0.72						
Other Bath Preparations	2	1.3						
Eye Makeup Preparations								
Eye Makeup Remover	2	1.3						
Other Eye Makeup Preparations	1	NR						
Fragrance Preparations								
Perfumes	1	NR						
Hair Preparations (non-coloring)								
Hair Conditioner			1	NR				
Hair Spray (aerosol fixatives)								

Table 5. Frequency (2023) and concentration (2021) of use according to likely duration and exposure and by product category[13,14,47]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Hair Straighteners	1	0.75						
Permanent Waves								
Shampoos (non-coloring)	13	0.8 – 4.4	9	NR				
Tonics, Dressings, and Other Hair Grooming Aids	1	NR						
Other Hair Preparations								
Hair Coloring Preparations								
Hair Dyes/Colors (all types requiring caution statements and patch tests)								
Hair Shampoos (coloring)	2	NR						
Other Hair Coloring Preparations								
Makeup Preparations								
Other Makeup Preparations								
Manicuring Preparations (Nail)								
Other Manicuring Preparations								
Personal Cleanliness Products								
Bath Soaps and Detergents	107	0.8 – 5.3	15	NR	15	NR		
Douches								
Feminine Deodorants								
Other Personal Cleanliness Products	3	0.8 – 2.8						
Shaving Preparations								
Preshave Lotions (all types)								
Shaving Cream								
Skin Care Preparations								
Cleansing	53	0.46 – 9.9						
Face and Neck (exc shave)								
Body and Hand (exc shave)								
Moisturizing								
Paste Masks (mud packs)	NR	1.2						
Other Skin Care Preparations	8	NR						

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

**Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

[b] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 6. Current and historical frequency and concentration of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[13]	**2005[2]**	**2022[14]**	**2006[2]**	**2023[13]**	**2005[2]**	**2022[14]**	**2006[2]**	**2023[13]**	**2005[2]**	**2022[14]**	**2006[2]**	**2023[13]**	**2005[2]**	**2022[14]**	**2006[2]**
	Disodium Cocoamphodiacetate				**Disodium Cocoamphodipropionate**				**Sodium Cocoamphoacetate**				**Sodium Cocoamphopropionate**			
Totals	**220**	**194**	**0.1 - 20**	**0.0006 – 12**	**73**	**72**	**0.8 – 1.8**	**0.008 - 15**	**121**	**46**	**0.03 – 4.5**	**0.09 – 18**	**21**	**7**	**0.84 – 7.5**	**0.3 – 10**
summarized by likely duration and exposure*																
Duration of Use																
Leave-On	*40*	*18*	*0.1 – 3.4*	*0.0006 – 10*	*29*	*20*	*NR*	*0.8 - 1*	*20*	*NR*	*0.56 – 0.93*	*0.1 – 4*	*15*	*4*	*NR*	*NR*
Rinse-Off	*179*	*168*	*0.1 – 20*	*0.005 – 12*	*40*	*52*	*0.8 – 1.8*	*0.008 – 15*	*101*	*42*	*0.03 – 4.5*	*0.7 – 18*	*6*	*3*	*0.84 – 7.5*	*0.3 – 8*
Diluted for (Bath) Use	*1*	*8*	*1.2*	*4 – 8*	*4*	*NR*	*NR*	*NR*	*NR*	*4*	*NR*	*0.09*	*NR*	*NR*	*NR*	*10*
Exposure Type*																
Eye Area	3	15	NR	0.005 – 0.8	3	NR	NR	NR	3	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	6[a]; 22[b]	5[a]; 3[b]	2.3 – 2.7[a]	0.004 – 0.06[a]; 0.03 – 0.2[b]	2[a]	4[a]	NR	1; 0.8[a]	4[a]; 13[b]	NR	0.56[a]	0.1[a]	NR	2[a]	NR	NR
Incidental Inhalation-Powder	22[b]	3[b]	3.4[c]	0.03 – 0.2[b]	NR	NR	NR	NR	13[b]	NR	0.93[c]	NR	NR	NR	NR	NR
Dermal Contact	141	97	0.1 – 20	0.0006 – 12	10	9	0.8 – 1.8	0.5 – 8	81	29	0.93 – 4.5	0.09 – 18	17	22	2	10
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	64	92	0.9 – 6.9	2 – 8	61	60	NR	0.2 – 15	40	15	0.03 – 4.5	0.1 – 6	4	6	0.84 – 7.5	0.3 – 8
Hair-Coloring	2	5	NR	5	2	3	NR	0.008	NR	2	2.1	0.7	NR	NR	2.4	NR
Nail	1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	60	20	0.1 – 3.3	0.05 – 9	5	3	NR	0.5 – 8	21	26	3.3	0.09 – 18	NR	2	NR	10
Baby Products	7	8	0.56 – 5.4	2 - 7	NR	1	NR	NR	6	NR	2.8	4	NR	NR	NR	NR
as reported by product category																
Baby Products																
Baby Shampoos	4	NR	0.9 – 5.4	NR					5	NR	2.8	NR				
Baby Lotions/Oils/Powders/Creams																
Other Baby Products	3	NR	0.56	4	NR	1	NR	NR	1	NR	NR	4				
Bath Preparations (diluted for use)																
Bubble Baths	NR	4	1.2	0.09					NR	4	NR	0.09				
Other Bath Preparations	1	NR	NR	NR	4	15	NR	NR					NR	NR	NR	10
Eye Makeup Preparations																
Eye Makeup Remover	2	NR	NR	NR	1	NR	NR	NR	3	NR	NR	NR				
Other Eye Makeup Preparations	1	NR	NR	NR	2	NR	NR	NR								
Fragrance Preparations																
Perfumes																
Hair Preparations (non-coloring)																
Hair Conditioner	3	3	NR	2	15	14	NR	0.2	1	3	1	2	NR	NR	2 – 7.5	3 - 5
Hair Spray (aerosol fixatives)					NR	NR	NR	1								
Hair Straighteners																
Permanent Waves	NR	1	NR	NR					NR	1	NR	NR	NR	NR	0.84	0.3
Shampoos (non-coloring)	55	11	1.4 – 6.9	1 – 6	19	27	NR	15	30	11	0.03 – 4.5	1 – 6	4	3	2.4	8
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	2.3 – 2.7	0.1	2	4	NR	0.8	3	NR	0.56	0.1	NR	2	NR	NR
Other Hair Preparations	2	NR	NR	NR	25	NR	NR	NR	1	NR	NR	NR	NR	2	NR	0.3 – 10
Hair Coloring Preparations																
Hair Dyes and Colors (all types requiring caution statements and patch tests)	2	NR	NR	0.7	NR	3	NR	0.008	NR	NR	NR	0.7				
Hair Shampoos (coloring)									NR	NR	2.1	NR	NR	NR	2.4	NR
Other Hair Coloring Preparation	NR	2	NR	NR	2	NR	NR	NR	NR	2	NR	NR				

Table 6. Current and historical frequency and concentration of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[13]	**2005[2]**	**2022[14]**	**2006[2]**	**2023[13]**	**2005[2]**	**2022[14]**	**2006[2]**	**2023[13]**	**2005[2]**	**2022[14]**	**2006[2]**	**2023[13]**	**2005[2]**	**2022[14]**	**2006[2]**
Makeup Preparations																
Other Makeup Preparations	NR	NR	NR	3					1	NR	NR	3				
Manicuring Preparations (Nail)																
Other Manicuring Preparations	1	NR	NR	NR												
Personal Cleanliness Products																
Bath Soaps and Detergents	22	4	2.1	3 – 18	NR	3	NR	8	15	4	3.3	3 – 18				
Douches	12	NR	NR	0.8 – 2					NR	NR	NR	0.8 – 2				
Feminine Deodorants	1	NR	NR	NR												
Other Personal Cleanliness Products	24	18	0.1 – 3.3	NR	1	NR	NR	0.5	6	18	NR	NR				
Shaving Preparations																
Preshave Lotions (all types)					NR	NR	1.8	NR					NR	NR	2	NR
Shaving Cream	3	NR	0.99	NR					1	NR	NR	NR				
Skin Care Preparations																
Cleansing	52	3	0.77 - 20	2 – 5	2	5	0.8	7	38	3	1.6 – 4.5	2 - 5	2	NR	NR	NR
Face and Neck (exc shave)	3	NR	3.4 (not spray)	NR					8	NR	0.93 (not spray)	NR				
Body and Hand (exc shave)	18	NR	NR	NR					5	NR	NR	NR				
Moisturizing	6	NR	NR	NR					1	NR	NR	NR				
Paste Masks (mud packs)									2	NR	1.5	NR				
Other Skin Care Preparations	5	NR	0.1	NR									15	NR	NR	NR

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

**Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.

[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

Table 7. Acute oral toxicity studies on Sodium Lauroamphoacetate[4]

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/ Results
Sodium Lauroamphoacetate (water and sodium chloride)	No vehicle	Carworth mice (10/group; sex not specified)	100%; 10, 12.5, 15 ml/kg bw	OECD TG 401; gavage administration; 5 d observation period	One, four, and eight animals died in groups given 10, 12.5, and 15 ml/kg bw of the test substance, respectively. The LD_{50} was determined to be 12.7 ml/kg for the aqueous solution. This corresponds to 14,224 mg/kg for the aqueous solution and 6116 mg/kg for the undiluted test substance.
Sodium Lauroamphoacetate (50% solids; water and sodium chloride)	Water and 0.5% carboxymethylcellulose	Hsd: Sprague-Dawley rats (3/sex)	20%; 10 ml/kg	OECD TG 423; gavage administration; 14 d observation period	LD_{50} > 10 ml/kg (corresponding to 2000 mg/kg bw)
Sodium Lauroamphoacetate (35% solids; water, sodium chloride, sodium glycolate)	Water	Wistar rats (5/sex)	20% aqueous dilution; 10 ml/kg	OECD TG 401; gavage administration; 14 d observation period	LD_{50} > 10 ml/kg (corresponding to 2000 mg/kg bw)
Sodium Lauroamphoacetate (50% solids; water and sodium chloride)	Water	Charles River rats (5/sex/group)	50% aqueous dilution; 5, 5.5, 6.25, and 6.5 ml/kg bw;	OECD TG 401; gavage administration; 7 d observation period	One and 3 animals died in groups given 5 and 5.5 ml/kg bw test substance, respectively. Seven animals died in the group receiving 6.25 ml/kg test substance, and 7 animals died in the group receiving 6.5 ml/kg bw test substance. The acute oral LD_{50} was calculated to be 5.85 ml/kg. This corresponds to 6844 mg/kg for the aqueous solution and 3422 mg/kg for the undiluted test substance.
Sodium Lauroamphoacetate (50% solids; water, and sodium chloride)	Water	Sprague-Dawley rats (5/sex)	50% aqueous dilution; 15 ml/kg bw	OECD TG 401; gavage administration; 14 d observation period	LD_{50} determined to be > 15 ml/kg; corresponds to an LD_{50} > 7500 mg/kg for the undiluted test substance.

LD_{50} = median lethal dose; OECD TG: Organisation for Economic Cooperation and Development Test Guidelines

Table 8. Oral reproductive and developmental toxicity studies[4]

Test Article	Vehicle	Animals/Group	Dose	Procedure	Results
Disodium Cocoamphodiacetate	Water	female Wistar Han rats (22/group)	0, 100, 300, or 1000 mg/kg bw/d	OECD TG 414; animals treated via gavage on days 6-20 post-coitum; animals killed on day 21; control animals treated with water only; clinical observations performed throughout study; reproductive organs evaluated post-mortem (gravid uterine weight, number of corpora lutea, implantations, early and late resorptions); fetal examinations included external, soft tissue, skeletal, and head examinations, anogenital distance, body weights, survival rate, sex ratio, developmental variations	No treatment-related mortality or adverse effects in dams were observed. Visceral examination of fetuses revealed severe cardiovascular malformations in all test groups (non-dose-dependent; not including control group). In the 1000 mg/kg bw/d group, one fetus had a right-sided aortic arch, ventricular septum defect, and no eyes. At 300 mg/kg bw/d, one fetus had a ventricular septum defect, absence of the ductus arteriosus, situs inversus, and abnormal lung lobation. At 100 mg/kg bw/d, two fetuses were viscerally malformed; one fetus had abnormal lung lobation and transposition of the great vessels, and the other fetus presented with situs inversus, abnormal lung lobation, interrupted aortic arch, retroesophageal ductus arteriosus, and ventricular septum defect. Mean litter incidences of a 7th cervical ossification site were 1.5, 5.3, 4.6, and 11.3% per litter in the 0, 100, 300, and 1000 mg/kg bw/d groups, respectively. No other adverse effects relating to developmental parameters evaluated were observed. The maternal NOAEL was determined to be 1000 mg/kg bw/d. A developmental NOAEL could not be determined as severe cardiovascular malformations were observed at all doses tested, in a non-dose-dependent manner.
Disodium Cocoamphodiacetate	Water	Wistar Han rats (10/sex/group)	0, 100, 300, or 1000 mg/kg bw/d	OECD TG 422; animals treated via gavage; control animals treated with water only; males treated for 29 d (2 wk prior to mating, during mating, and up to necropsy); females treated for 50-54 d (2 wk prior to mating, during mating, post-coitum, and 14-16 d of lactation); females without offspring were treated for 41 d; animals were observed for mortality, estrous cycle lengths, sperm parameters, mating index, fertility index, gestation index, precoital time, and duriation of gestation, and histopathology of reproductive organs; offspring viability indices evaluated include the post-implantation index, live birth index, sex ratio, and lactation index	Treatment with the test substance did not cause any adverse morphological effects in reproductive organs. No adverse effects were noted in any of the parameters evaluated. A high mortality rate was observed in females (4/10), and one death was reported in males. These deaths were concluded to be related to regurgitation, and thus, secondary to the test item; however, it is possible that the physical/chemical properties of the test item solution in combination with the route of administration could have resulted in these deaths. No treatment related abnormalities were observed in the F1 generation. Because the mortalities reported, the NOAEL was determined to be 300 mg/kg bw/d and the reproductive NOAEL was determined to be 1000 mg/kg bw/d.
Disodium Cocoamphodiacetate (47.2 – 48% solids)	Water	Wistar Han rats (10/sex/group)	0, 100, 300, or 1000 mg/kg bw/d	OECD TG 408; animals treated via gavage for 90 d; estrous cycle length, spermatogenesis, and weight/ appearance/histopathology of reproductive organs evaluated	No adverse effects relating to the parameters evaluated were observed.

Table 8. Oral reproductive and developmental toxicity studies[4]

Test Article	Vehicle	Animals/Group	Dose	Procedure	Results
Sodium Cocoamphoacetate	Water	Wistar Han rats (10/sex/group)	0, 100, 300, or 1000 mg/kg bw/d	OECD TG 422; animals treated via gavage; control animals treated with water only; males treated for 29 d (2 wk prior to mating, during mating, and up to and including the day before necropsy); females treated for 50-56 d (14 d prior to mating, the time to conception, duration of pregnancy, and 13 or 15 d after delivery, up to and including the day before necropsy); females without offspring were treated for 53 d (no evidence of mating) or 42-43 d (not pregnant or implantation site only); animals were observed for mortality, estrous cycle lengths, sperm parameters, mating index, fertility index, gestation index, precoital time, and duriation of gestation, and histopathology of reproductive organs; offspring viability indices evaluated include the post-implantation index, live birth index, sex ratio, and lactation index	No test-item related abnormalities in estrous cycle length and regularity were observed. One male at 300 mg/kg bw/d showed tubular atrophy in the testes and reduced luminal sperm with luminal cell debris in the epididymis. No treatment-related effects in the F1 generation were observed. The reproductive NOAEL was determined to be 1000 mg/kg bw/d.
Sodium Lauroamphoacetate	Water	female Wistar Han (22/group)	0, 100, 300, and 1000 mg/kg bw/d	OECD TG 414; animals treated from day 6 to day 20 post-coitum via gavage; animals killed on day 21; control animals treated with water only; clinical observations performed throughout study; reproductive organs evaluated post-mortem (gravid uterine weight, number of corpora lutea, implantations, early and late resorptions); fetal examinations included external, soft tissue, skeletal, and head examinations, anogenital distance, body weights, survival rate, sex ratio, developmental variations	Abnormal breathing sounds, temporary slight weight loss and decreased food consumption, and salivation were observed in dams dosed with 300 and 1000 mg/kg bw/d. Body weight and food intake recovered throughout dosing. A statistically significant decrease of T3 (thyroid hormone) blood concentration was observed in dams dosed with 1000 mg/kg bw/d; however, values were within the historical control database values of the laboratory. Irregular surface of the non-glandular stomach was noted in 12/22 females treated with 1000 mg/kg bw/d. Dark red foci on the glandular stomach were observed in 1 animal in this group. No other adverse effects relating to maternal parameters investigated were observed (uterine content, gravid uterine weight, corpora lutea, implantation sites, pre-/post-implantation loss). No adverse effects relating to developmental parameters were observed in fetuses. The maternal and developmental NOAEL was determined to at least 1000 mg/kg bw/d.

NOAEL = no-observed-adverse-effect-level; OECD TG = Organisation for Economic Cooperation and Development test guidelines

Table 9. Genotoxicity studies[4]

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results
Sodium Lauroamphoacetate (35% solids; water, sodium chloride, and sodium glycolate)	Water	Experiment 1: 7, 35, 175, 875 and 4375 µg/plate Experiment 2: 5.5, 21.9, 87.5, 350 and 1400 µg/plate	*S. typhimurium* TA1535, TA1537, TA1538, TA98, and TA100	OECD TG 471; Ames assay performed with and without metabolic activation; 2-part experiment; Experiment 1 conducted on *S. typhimurium* strains TA1535, TA1537, and TA100; Experiment 2 conducted on *S. typhimurium* strains TA1538 and TA98; positive (sodium azide, 9-aminoacride, 4-nitro-o-phenyldiamine, or 2-aminoanthracene) and negative controls (water) were used in both experiments	Non-genotoxic; valid controls
Sodium Lauroamphoacetate (water and sodium chloride)	Water	Experiment 1 and 2: 313, 625, 1250, 2500 and 5000 µg/plate (TA1535, TA1537, TA98 and WP2 uvrA) and 156, 313, 625, 1250 and 2500 µg/plate (TA100) Experiment 3: 39.1, 78.1, 156, 313, 625 and 1250 µg/plate (TA1535 and TA1537) and 39.1, 78.1, 156, 313 and 625 µg/plate (TA100 without S9-mix)	*S. typhimurium* TA1535, TA1537, TA98, and TA100; *E. coli* WP2 uvr A	OECD TG 471; Ames assay performed with and without metabolic activation; 3-part experiment; 1st experiment conducted using a plate-incorporation method; 2nd experiment conducted with a pre-incubation step; 3rd experiment conducted with pre-incubation step at lower test concentrations; positive (substance not stated) and negative controls (water) were used in all experiments	Non-genotoxic; valid controls
Sodium Lauroamphoacetate (water, sodium chloride, and sodium glycolate)	Water	Experiment 1: 30, 65, 130, 146, 162, 190, 200 and 250 µg/ml Experiment 2: 30, 65, 125, 140, 155, 170, 185, and 200 µg/ml	Human peripheral blood lymphocytes	OECD 473; in vitro mammalian chromosome aberration assay performed with and without metabolic activation; 2-part experiment; in the 1st experiment, cells were treated for 4 h (with and without metabolic activation) and for 20 h (without metabolic activation); in the 2nd experiment, cells were treated for 4 h (with metabolic activation) at lower test concentrations; positive (substance not stated) and negative controls (water) were used in both experiments	Non-clastogenic; valid controls

OECD TG = Organisation for Economic Cooperation and Development test guidelines

Table 10. Dermal irritation and sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
Animal						
Sodium Lauroamphoacetate (35% solids; water, sodium chloride, and sodium glycolate)	No vehicle	100%; 0.5 ml	3 male Chbb:Hm rabbits	OECD TG 404; semi-occlusive dressing; single patch application for 4 h; evaluation 1, 24, 48, and 72 h after patch removal	Non-irritating	[4]
Sodium Lauroamphoacetate (50% solids; water and sodium chloride)	No vehicle	100%; 0.5 g	3 female New Zealand white rabbits	OECD TG 404; semi-occlusive dressing; single patch application for 4 h; evaluation 1, 24, 48, and 72 h after patch removal	Non-irritating; very slight erythema observed 24 h after patch removal, fully reversed within 72 h	[4]
Trade name mixture consisting of Sodium Lauroamphoacetate, sodium trideceth sulfate, isopropyl alcohol (2%), and water (67.9%) (concentration of Sodium Lauroamphoacetate and sodium trideceth sulfate combined: 30.1%)	No vehicle	100%; 0.5 ml	3 New Zealand albino rabbits (sex not specified)	Test substance placed on abraded and intact skin under 2.5 cm^2 gauze patches; occlusive conditions; patches left on for 24 h; sites evaluated 24 and 72 h after patch removal	severe primary irritant in intact and abraded skin; primary irritation score of 6.75 (score of > 5.1 indicates severe irritation)	[21]
Trade name mixture containing Sodium Lauroamphoacetate (36%) and water (64%)	No vehicle	100%; 0.5 ml	3 New Zealand albino rabbits (sex not specified)	Test substance placed on abraded and intact skin under 2.5 cm^2 gauze patches; occlusive conditions; patches left on for 24 h; sites evaluated 24 and 72 h after patch removal	severe primary irritant in intact and abraded skin; primary irritation score of 5.84 (score of > 5.1 indicates severe irritation)	[22]
Human						
Disodium Cocoamphodiacetate	Water	0.5%; 40 µl	105 subjects	The test substance as applied to the skin under occlusive conditions for 48 h; readings were performed 15 min and 24 h after patch removal; parameters measured include erythema and edema	Non-irritating	[24]
Disodium Cocoamphodiacetate	Water	1%; 100 µl	22 subjects	Soap chamber test; test substance applied to forearm under occlusive conditions; repeated patching was performed for 24 h, followed by a 6 h patch period per day, for the next 4 d; first assessment occurred 15 min after patch removal on day 2; all other assessments were performed prior to reapplication on days 3-5, and on day 8	Non-irritating; total irritation score: 4.42 (score ≤ 10 indicates very slightly or not irritating)	[16]
Disodium Cocoamphodiacetate	Water	2%; 75 µl	20 subjects	Epicutaneous patch test; test substance applied to back under occlusive conditions; patches removed after 24 h; sites evaluated 6, 24, 48, and 72 h after removal	Slightly irritating; total irritation score: 14.14 (score of 10 - ≤ 25 indicates slightly irritating)	[16]
Disodium Cocoamphodiacetate	NR	5%	8 subjects	Test areas (approximately 3 cm^2 each) were marked on the forearm. Three successive washings were performed. For each wash, a technician poured 4 ml of 1 surfactant solution into both palms, rubbed solution into the hands, and used three fingers in a to rub the solution into the predesignated test area for 1 min with the lather. The area was then rinsed for 15 sec, followed by a 30-min rest period. This process was repeated 2 additional times. The degree of irritation was evaluated at baseline and after each washing. A water washing control and non-treatment site were used for comparison. Erythema was quantified by skin color reflectance measurements using a colorimeter.	Clinical scores did not reveal any significant differences between treated and untreated sites.	[23]
Sodium Cocoamphoacetate	Water	1%; 100 µl	21 subjects	Soap chamber test; test substance applied to forearm under occlusive conditions; repeated patching was performed for 24 h, followed by a 6 h patch period per day, for the next 4 d; first assessment occurred 15 min after patch removal on day 2; all other assessments were performed prior to reapplication on days 3-5, and on day 8	Slightly irritating; total irritation score: 13.46 (score of 10 - < 15 indicates slightly irritating)	[16]

Table 10. Dermal irritation and sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Sodium Cocoamphoacetate	Water	2%; 75 µl	20 subjects	Epicutaneous patch test; test substance applied to back under occlusive conditions; patches removed after 24 h; sites evaluated 6, 24, 48, and 72 h after removal	Non-irritating; total irritation score: 8.51 (score ≤ 10 indicates very slightly or not irritating)	[16]
Sodium Cocoamphoacetate	NR	5%	8 subjects	Test areas (approximately 3 cm^2 each) were marked on the forearm. Three successive washings were performed. For each wash, a technician poured 4 ml of 1 surfactant solution into both palms, rubbed solution into the hands, and used three fingers in a to rub the solution into the predesignated test area for 1 min with the lather. The area was then rinsed for 15 sec, followed by a 30-min rest period. This process was repeated 2 additional times. The degree of irritation was evaluated at baseline and after each washing. A water washing control and non-treatment site were used for comparison. Erythema was quantified by skin color reflectance measurements using a colorimeter.	Clinical scores did not reveal any significant differences between treated and untreated sites.	[23]
Sodium Cocoamphoacetate	Citrate buffer (diluted to citrate concentration of 5 mM; pH 6 ± 0.5)	10% (274 mM); 50 µl	12 subjects	48-h occlusive patch test; Finn chambers were applied to the volar forearm; applications sites were evaluated 1 h, 24 h, 5 d, 9 d, and 14 d after patch removal for erythema (on a scale of 1 (slight redness) to 4 (fiery red with edema)) and scaling (on a scale of 1 (fine) to 3 (severe with large flakes)). SLS (2%) was included in the study for comparison. Citrate buffer (10 mM) served as the negative control.	At 1 h after patch removal, the visual erythema score (as % of total) was 33; the scores were 10, 4, 0, and 4 at 24 h and 5, 9, and 14 d after patch removal, respectively. Scaling scores (as % of total) were 0, 3, 22, 22, and 14 at 1 h, 24 h, and 5, 9, and 14 d after patch removal, respectively. For SLS, erythema scores ranged from 58 at 1 h to 17 at 14 d after patch removal, and scaling scores ranged from 0 after 1 h to 22 at 14 d, with a max of 47 at 5 d after patch removal.	[25]
Sodium Lauroamphoacetate	Water	1%; 100 µl	21 subjects	Soap chamber test; test substance applied to forearm under occlusive conditions; repeated patching was performed for 24 h, followed by a 6 h patch period per day, for the next 4 d; first assessment occurred 15 min after patch removal on day 2; all other assessments were performed prior to reapplication on days 3-5, and on day 8	Irritating; total irritation score: 20.93 (score of 20 - < 30 indicates irritating)	[16]
Sodium Lauroamphoacetate	Water	2%; 75 µl	20 subjects	Epicutaneous patch test; test substance applied to back under occlusive conditions; patches removed after 24 h; sites evaluated 6, 24, 48, and 72 h after removal	Moderately irritating; total irritation score: 27.19 (score of 25 - < 50 indicates moderately irritating)	[16]
Sodium Lauroamphoacetate (35% solids; water, sodium chloride, and sodium glycolate)	Water	50 and 100%; dose not reported	20 subjects	The test substance was applied to the skin, under open conditions, every 30 sec for 30 min. All applications occurred under open conditions.	Non-irritating	[4]

Table 10. Dermal irritation and sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
SENSITIZATION						
Animal						
Sodium Cocoamphoacetate (water, sodium chloride, and sodium glycolate)	Water	Intradermal induction: 5% (% solids not stated) Epicutaneous induction: 75% (% solids not stated) Challenge exposure: 1% (0.394% solids)	female Himalayan spotted guinea pigs (control: 5/group; test: 10/group)	-Guinea pig maximization test performed according to OECD TG 406 -Intradermal injections of adjuvant and physiological saline, test substance diluted to 5% in water, and the test substance diluted to 5% by emulsion in a mixture of adjuvant and physiological saline (control groups given mixtures of adjuvant and physiological saline or water) -Topical application on day 7 for epicutaneous induction, aqueous dilutions, under occlusive conditions, for 48 h (control animals treated with water only) -Challenge exposure on day 21, aqueous dilution, under occlusive conditions, for 24 h	Non-sensitizing	4
Sodium Lauroamphoacetate (water and sodium chloride)	Propylene glycol	1, 3, 6, 12, and 30% (experiment 1); 30, 40, and 50% (experiment 2)	4 female CBA/J mice/group	-Local lymph node assay performed according to OECD TG 429 -First experiment: animals treated with the test substance in dilutions of 1, 3, 6, 12, and 30% in propylene glycol (25 µl); animals received this treatment for 3 consecutive days, on one ear -Second experiment: animals treated with the test substance in dilutions of 30, 40, and 50% in propylene glycol; animals received this treatment for 3 consecutive days, on one ear -First and second experiments utilized a positive (hexylcinnamaldehyde) and negative (propylene glycol) group -On day 6, animals received an injection of 0.9% sodium chloride containing 20 µCi of 3H-TdR via the tail vein -Animals were killed 5 h after injection, lymph nodes were pooled, and proliferation evaluated -Ear thickness and local reactions were observed on days 1, 2, and 3 (before application), and on day 6 (after animals were killed)	No adverse effects or lymphoproliferation was observed in experiment 1. In experiment 2, an 11.34% increase in ear thickness was observed after treatment with the test substance at 50%. The test substance was found to induce delayed contact hypersensitivity at concentrations of 50%. The result was considered to be inconclusive as surfactants have clear irritating effects, and may lead to false positives.	4
Sodium Lauroamphoacetate (0.18 – 17.5% solids; water, sodium chloride, and sodium glycolate)	Physiological saline	Intradermal induction: 0.5% (0.18% solids) Epicutaneous induction: 50% (17.5 % solids) Challenge exposure: 20% (7% solids)	2-3 female Pirbright white guinea pigs/group	-Guinea pig maximization test performed according to OECD TG 406 -Intradermal injections of adjuvant and physiological saline, test substance diluted to 5% in physiological saline, and the test substance diluted to 5% by emulsion in a mixture of adjuvant and physiological saline (control groups given mixtures of adjuvant and physiological saline or water) -Topical application on day 7 of the test substance diluted to 50% in physiological saline, under occlusive conditions, for 48 h (control animals treated with water only) -Challenge exposure on day 21 with test substance diluted to 20% in physiological saline, under occlusive conditions, for 24 h	Positive reactions were observed in 5 of 20 test animals during challenge. The test substance was classified to be non-sensitizing.	4
Human						
Sodium Lauroamphoacetate (0.15% solids)	Water	0.5%; 200 µl	99 subjects	HRIPT -9 total induction exposures; 24 h induction periods -2-wk rest period followed by a challenge exposure -all exposures were performed under occlusive conditions	Non-irritating and non-sensitizing	4

HRIPT = human repeated-insult patch test; NR = not reported; OECD TG = Organisation for Economic Cooperation and Development test guidelines; SLS = sodium lauryl sulfate

Table 11. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
			IN VITRO			
Disodium Cocoamphodiacetate	Water	0.6%	3 skin samples	30 µl of test substance applied to reconstituted human corneal epithelial tissues and incubated; cell viability evaluated via MTT assay	Non-irritating	[16]
Disodium Cocoamphodiacetate	Water	1%	3 trials	Red blood cell test (evaluates hemolysis and protein denaturation in porcine erythrocytes)	Moderately irritating; H_{50}/DI = 7.77 (score of 1 - ≤ 10 indicates moderately irritating)	[16]
Disodium Cocoamphodiacetate	Water	3%	6 eggs	HET-CAM assay	Slightly irritating; irritation quotient = 0.63 (quotient ≤ 0.8 indicates slightly irritating)	[16]
Disodium Cocoamphodiacetate	Water	50%	6 eggs	EpiOcular™ assay; tissues treated with 100 µl of test article and incubated; MTT assay following incubation	Severe/extreme ocular irritant; ET_{50} < 2 (score < 3 indicates severely/extremely irritating)	[26]
Sodium Cocoamphoacetate	Water	0.6%	3 skin samples	30 µl of test substance applied to reconstituted human corneal epithelial tissues and incubated; cell viability evaluated via MTT assay	Slightly irritating	[16]
Sodium Cocoamphoacetate	Water	1%	3 skin samples	Red blood cell test	Non-irritating; H_{50}/DI = 102.40 (score > 100 indicates non-irritating)	[16]
Sodium Cocoamphoacetate	Water	3%	6 eggs	HET-CAM assay	Slightly irritating; irritation quotient = 0.42 (quotient ≤ 0.8 indicates slightly irritating)	[16]
Sodium Lauroamphoacetate	Water	1%	3 trials	Red blood cell test	Non-irritating; H_{50}/DI = 222.13 (score > 100 indicates non-irritating)	[16]
Sodium Lauroamphoacetate	Water	3%	6 eggs	HET-CAM assay	Slightly irritating; irritation quotient: 0.79 (quotient ≤ 0.8 indicates slightly irritating)	[16]
Sodium Lauroamphoacetate	Water	40%	6 eggs	HET-CAM assay	Severely irritating; irritation quotient: 3.41 (quotient ≥ 2 indicates severely irritating)	[27]
			ANIMAL			
Sodium Lauroamphoacetate (10% solids: water and sodium chloride; 10% aqueous dilution)	No vehicle	100%; 0.1 ml	3 rabbits (strain and sex not specified)	The test material was placed in one eye of each animal in an amount of 0.1 ml. The left eye served as a control. Eyes were evaluated 24, 48, and 72 h after test substance administration. Eyes were also evaluated on day 7 after administration. OECD TG 405.	The test substance was not considered to be an ocular irritant based on CLP criteria. Mean corneal opacity, iris, conjunctivae irritation and chemosis scores were 0/4, 0/2, 0.2/3, and 0/4, respectively. The slight conjunctival irritation was fully reversed by day 7.	[4]
Sodium Lauroamphoacetate (15% solids; water and sodium chloride; 30% aqueous dilution)	No vehicle	100%; 0.1 ml	3 rabbits (strain and sex not specified)	Assay performed according to the same procedure as above.	The test substance was not considered to be an ocular irritant based on CLP criteria. Mean corneal opacity, iris, conjunctivae irritation and chemosis scores were 0/4, 0/2, 0.7/3, and 1.1/4, respectively. All effects were fully reversible within 7 d.	[4]
Sodium Lauroamphoacetate (50% solids; water and sodium chloride; 50% aqueous dilution)	No vehicle	100%; 0.1 ml	3 female New Zealand White rabbits	Assay performed according to the same procedure as above.	The test substance was considered to be a Category 2 irritant based on CLP criteria. Mean corneal opacity, iris, conjunctivae irritation and chemosis scores were 1.2/4, 0/2, 1.7/3, and 0/4, respectively. All effects were fully reversible within 7 d.	[4]

Table 11. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Sodium Lauroamphoacetate (50% solids; water and sodium chloride; 50% aqueous dilution)	No vehicle	100%; 0.1 ml	6 female New Zealand White rabbits	Assay performed according to the same procedure as above, with the exception that a day 7 evaluation was not performed.	The test substance was not considered to be an irritant based on CLP criteria. Mean corneal opacity, iris, conjunctivae irritation and chemosis scores were 0.06/4, 0.1/2, 0.7/3, and 0.6/4, respectively. All effects were fully reversible within 72 h.	[4]
			HUMAN			
Micellar water cleanser containing 0.4% Disodium Cocoamphodiacetate and 3% poloxamer 184 (remaining product composition not stated)	No vehicle	100%	10	Subjects instructed to use each product once a day (as an eye makeup remover) for 21 d; reaction responses evaluated at 24 h, 7, and 21 d	No symptoms of eye irritation or adverse effects were noted.	[28]
Micellar water cleanser containing 1.2% Disodium Cocoamphodiacetate and 1% cetearyl alcohol (remaining product composition not stated)	No vehicle	100%	10	Subjects instructed to use each product once a day (as an eye makeup remover) for 21 d; reaction responses evaluated at 24 h, 7, and 21 d	No symptoms of eye irritation or adverse effects were noted.	[28]

CLP = Classification, Labeling, and Packaging; DI = denaturation index: ET_{50} = effective time of exposure to reduce tissue viability to 50%; H_{50} = half-maximal effective concentration for hemolysis; HET-CAM = hen's egg test-chorioallantoic membrane; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; OECD TG = Organisation for Economic Cooperation and Development test guidelines

REFERENCES

1. Andersen F.A. (ed). Final Report on the Safety Assessment of Cocoamphoacetate, Cocoamphopropionate, Cocoamphodiacetate, and Cocoamphodipropionate. *J Am Coll Toxicol*. 1990;9(2):121-142.

2. Andersen F.A. (ed). Annual Review of Cosmetic Ingredient Safety Assessments: 2005/2006. Cocoamphoacetate, Cocoamphopropionate, Cocoamphodiacetate, and Cocoamphodipropionate. *Int J Toxicol*. 2008;27(Suppl 1):98-101.

3. Nikitakis J, Kowcz A. wINCI: *International Cosmetic Ingredient Dictionary and Handbook*. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated 2023. Accessed March 1, 2023.

4. European Chemicals Agency (ECHA). Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1-ethanol and sodium hydroxide. https://echa.europa.eu/en/registration-dossier/-/registered-dossier/13724. Last Updated 2021. Accessed August 19, 2021.

5. Andersen F.A. (ed). Final Report of the Safety Assessment of Glycolic Acid, Ammonium, Calcium, Potassium, and Sodium Glycolates, Methyl, Ethyl, Propyl, and Butyl Glycolates, and Lactic Acid, Ammonium, Calcium, Potassium, Sodium, and TEA-Lactates, Methyl, Ethyl, Isopropyl, and Butyl Lactates, and Lauryl, Myristyl, and Cetyl Lactates. *Int J Toxicol*. 1998;17(Suppl 1):1-241.

6. Fiume MM, Bergfeld WF, Belsito DV, et al. 40th Anniversary Overview and Rereview Summaries from 2011 to 2015. Alpha Hydroxy Acids. *Int J Toxicol*. 2017;36(Suppl 2):15S - 21S.

7. Parchem. Sodium Lauroamphoacetate: Safety Data Sheet. 2016. https://www.parchem.com/siteimages/Attachment/GHS%20Sodium%20Lauroamphoacetate%20MSDS.pdf. Accessed November 30, 2022.

8. Burnett CL, Bergfeld WF, Belsito DV, et al. Final Report of the Cosmetic Ingredient Review Expert Panel on the Safety Assessment of Cocamidopropyl Betaine (CAPB). *Int J Toxicol*. 2012;31(Suppl 1):77S-111S.

9. Zhang Y, Xu H, Lu S. Preparation and application of layered double hydroxide nanosheets. *RSC Adv*. 2021;11:24254-24281.

10. Farn RJ. Chemistry and Technology of Surfactants. In: Oxford, UK: Blackwell Published Ltd.; 2006. 175.

11. Foti C, Bonamonte D, Mascolo G, Tiravanti G, Rigano GA. Aminoethylethanolamine: a new allergen in cosmetics? *Contact Dermatitis*. 2001;45(3):129-133.

12. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Full Public Report: Disodium Wheatgermamphodiacetate. 1998. https://www.industrialchemicals.gov.au/sites/default/files/NA601%20Public%20Report%20PDF.pdf. Accessed March 24, 2023.

13. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). 2023. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2023; received February 2, 2023).

14. Personal Care Products Council. 2021. Concentration of Use by FDA Product Category: Additions to Sodium Lauroamphoacetate Report. Unpublished data submitted by Personal Care Products Council on January 10, 2022.

15. EUR-Lex. Access to European Union Law. Last Updated 2023. Accessed 03/29/2023.

16. Mehling A, Kleber M, Hensen H. Comparative studies on the ocular and dermal irritation potential of surfactants. *Food Chem Toxicol*. 2007;45(5):747-758.

17. U.S. Food and Drug Administration. Inactive Ingredient Search for Approved Drug Products. https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm. Last Updated 2022. Accessed November 10, 2022.

18. Wu J, Wu T, Zheng S, Huang Y, Wang L. Low-dose repeated exposure to chemical surfactant impairs corneal epithelium: When personal cleaning products entering the eye. *Exp Eye Res*. 2021 Sep;210:108696.

19. Grey KR, Hanson JL, Hagen SL, Hylwa SA, Warshaw EM. Epidemiology and Co-Reactivity of Novel Surfactant Allergens: A Double-Blind Randomized Controlled Study. *Dermatitis*. 2016;27(6):348-354.

20. Santucci B, Cannistraci C, Lesnoni I, et al. Cutaneous response to irritants. *Contact Dermatitis*. 2003;48(2):69-73.

21. Rhone-Poulenc Inc. Initial Submission: Internal Research Screening Skin Irritation Study of Miranol MHT in Rabbits with Cover Letter Dated 061992. Prod Safety Labs; 1992. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0541043.xhtml. Accessed March 24, 2023.

22. Rhone-Poulenc Inc. Initial Submission: Internal Research Screening Skin Irritation Study with Sodium Lauroamphoacetate in Rabbits with Cover Letter Dated 060892. Product Safety Labs; 1992. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0540016.xhtml. Accessed March 24, 2023.

23. Zhai H, Fautz R, Fuchs A, Branco N, Maibach HI. Assessment of the subclinical irritation of surfactants: a screening open assay model. *Exog Dermatol*. 2002;1:238-241.

24. Corazza M, Lauriola MM, Bianchi A, Zappaterra M, Virgili A. Irritant and sensitizing potential of eight surfactants commonly used in skin cleansers: an evaluation of 105 patients. *Dermatitis*. 2010;21(5):262-268.

25. Bárány E, Lindberg M, Lodén M. Biophysical characterization of skin damage and recovery after exposure to different surfactants. *Contact Dermatitis*. 1999;40(2):98-103.

26. Stern M, Klausner M, Alvarado R, Renskers K, Dickens M. Evaluation of the EpiOcular((TM)) Tissue Model as an Alternative to the Draize Eye Irritation Test. *Toxicol In Vitro*. 1998;12(4):455-461.

27. Scheel J, Kleber M, Kreutz J, et al. Eye irritation potential: usefulness of the HET-CAM under the Globally Harmonized System of classification and labeling of chemicals (GHS). *Regul Toxicol Pharmacol*. 2011;59(3):471-492.

28. Rivero MN, Lenze M, Izaguirre M, et al. Comparison between HET-CAM protocols and a product use clinical study for eye irritation evaluation of personal care products including cosmetics according to their surfactant composition. *Food Chem Toxicol*. 2021 Jul;153:112229.

29. de Groot AC, Weijland W. Contact allergy to disodium cocoamphodipropionate. *Contact Dermatitis*. 1996;35(4):248-249.

30. Hanson JL, Warshaw EM. Contact allergy to surfactants in a hypoallergenic liquid cleanser. *Dermatitis*. 2015;26(6):284-286.

31. Goossens A, Bruze M, Gruvberger B, Gielen K, Stoskute L. Contact allergy to sodium cocoamphoacetate present in an eye make-up remover. *Contact Dermatitis*. 2006;55(5):302-304.

32. Skudlik C, Markthaler M, John SM. Occupational contact allergy to sodium cocoamphopropionate in a skin protection cream. *Contact Dermatitis*. 2018;78(4):295-296.

33. Pesonen M, Suomela S, Kuuliala O, Aalto-Korte K. Occupational contact allergy to sodium cocoamphopropionate in a hand cleanser. *Contact Dermatitis*. 2016;74(4):246-248.

34. Hagvall L, Brared-Christensson J, Inerot A. Occupational contact dermatitis caused by sodium cocoamphopropionate in a liquid soap used in fast-food restaurants. *Contact Dermatitis*. 2014;71(2):122-124.

35. ATAMAN Chemicals. Disodium Cocoamphodiacetate. https://www.atamanchemicals.com/disodium-cocoamphodiacetate_u24965/. Last Updated 2020. Accessed October 28, 2021.

36. PubChem. beta-Alanine, N-(2-aminoethyl)-N-(2-(2-carboxyethoxy)ethyl)-, N-coco acyl derivs., disodium salts. https://pubchem.ncbi.nlm.nih.gov/compound/6455953. Last Updated 2021. Accessed October 28, 2021.

37. Stepan. AMPHOSOL® 2CSF. https://www.stepan.com/content/stepan-dot-com/en/products-markets/product/AMPHOSOL2CSF.html. Last Updated 2021. Accessed October 28, 2021.

38. European Chemicals Agency (ECHA). β-Alanine, N-(2-aminoethyl)-N-[2-(2-carboxyethoxy)ethyl]-, N-coco acyl derivs., disodium salts. https://echa.europa.eu/da/registration-dossier/-/registered-dossier/27215/4/2. Last Updated 2022. Accessed February 23, 2022.

39. PubChem. Disodium 1-[2-(carboxymethoxy)ethyl]-1-(carboxymethyl)-4,5-dihydro-2-undecyl-1H-imidazolium hydroxide. https://pubchem.ncbi.nlm.nih.gov/compound/84372. Last Updated 2021. Accessed October 28, 2021.

40. PubChem. Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-1H-imidazole-1-ethanol 2-norcoco alkyl derivs. and sodium hydroxide. https://pubchem.ncbi.nlm.nih.gov/compound/155132. Last Updated 2021. Accessed October 28, 2021.

41. PubChem. Sodium lauroamphoacetate (Compound). https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-lauroamphoacetate#section=Chemical-and-Physical-Properties. Last Updated 2021. Accessed August 20, 2021.

42. Burnett CL, Fiume MM, Bergfeld WF, et al. Safety Assessment of Plant-Derived Fatty Acid Oils. *Int J Toxicol*. 2017;36(Suppl 3):51S-129S.

43. Parchem. Safety Data Sheet: Disodium Cocoamphodiacetate. 2015. https://www.parchem.com/siteimages/Attachment/GHS%20Disodium%20Cocoamphodiacetate%20MSDS.pdf. Accessed November 29, 2022.

44. Stepan. Safety Data Sheet: Amphosol 2CSF-AF. 2018. https://pt.stepan.com/content/dam/stepan-dot-com/webdam/website-product-documents/safety-data-sheets/sds-us/en/01005600.pdf. Accessed November 30, 2022.

45. Colonial Chemical. Safety Data Sheet: Cola®Teric J49. 2017. https://colonialchem.me/wp-content/uploads/2020/10/ColaTeric-J49-SDS-US.pdf. Accessed November 30, 2022.

46. Leidreiter HI, Gruning B, Kaseborn D. Amphoteric surfactants: processing, product composition and properties. *Int J Cosmet Sci*. 1997;19(5):239-253.

47. Personal Care Products Council. 2021. Concentration of Use by FDA Product Category: Sodium Lauroamphoacetate. Unpublished data submitted by Personal Care Products Council on September 8, 2021.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 26, 2023

SUBJECT: Amphoacetates

Alkylamphoacetate Consortium. 2023. Analogue Approach for REACH Registration of Alkylamphoacetates-version December 2022.

DeSesso JM and Lavin Williams A. 2023. Expert Review of Available Repeat-Dose and Developmental and Reproductive Toxicity (DART) Studies for Amphoacetates.

charles river

Test Facility Study No. 20342906

Analogue Approach for REACH Registration of ALKYLAMPHOACETATES- version December 2022

SPONSOR
(on behalf of the alkylamphoacetates consortium):
CRB-Chemie Revisions- und Beratungsgesellschaft mbH
Wirtschaftsprüfungsgesellschaft. Rudolf-Breitscheid-Strasse 21
90762 Fürth
Germany

TEST FACILITY:
Charles River Laboratories Den Bosch BV
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

26 May 2023
(revised version of Charles River report finalized on April 29, 2019)

TABLE OF CONTENTS

LIST OF FIGURES 3
LIST OF TABLES 4
LIST OF APPENDICES 5
1. RESPONSIBLE PERSONNEL 6
1.1. Test Facility 6
1.2. Sponsor 6
2. SUMMARY 7
3. INTRODUCTION 8
4. ANALOGUE DEFINITION 9
4.1. Definition of analogues 9
4.1.1. Read-across hypothesis 9
4.1.2. Applicability domain (AD) of the analogues 13
4.1.3. List of endpoints covered 13
4.2. Analogues 13
4.3. Purity/Impurities 17
5. READ-ACROSS JUSTIFICATION 18
5.1. Physico-chemical properties 18
5.2. Environmental fate and eco-toxicological properties 19
5.3. Toxicological properties 25
6. DATA MATRIX 34
7. RETENTION OF RECORDS 42
8. CLASSIFICATION AND LABELLING (C&L) AND PBT PROPERTIES 42
9. REFERENCES 43
APPENDICES 45

LIST OF FIGURES

Figure 1: Chemistry of the manufacture of alkylamphoacetates 9

Figure 2: General structures of the main constituents of the alkylamphoacetates fraction of the substances 11

LIST OF TABLES

Table 1: Alkylamphoacetate analogues 8
Table 2 Substance identifiers for all analogues 14
Table 3 Acute *Daphnia* toxicity of Amphoacetates C8-C18 and Amphoacetates C12-C14 tested in 2017 20
Table 4 Data Matrix, Physico-chemical Properties for the Alkylamphoacetates 35
Table 5 Data Matrix, Environmental Fate/Toxicity for the Alkylamphoacetates 37
Table 6 Data Matrix, Toxicological endpoints for the Alkylamphacteates 39
Table 7 Classification and labelling for the Alkylamphoacetates 42

LIST OF APPENDICES

Appendix I: Overview alkyl chain length distribution of the Alkylamphoacetate analogues....45
Appendix II: Chemical structures evaluated by DEREK NEXUS (version 5.0.2)....46
Appendix III: Overview of available ecotoxicity studies....48
Appendix IV: Overview of available thyroid-related data....50
Appendix V: Overview of available toxicity studies....51

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Test Facility	Charles River Laboratories Den Bosch BV Hambakenwetering 7 5231 DD ‘s-Hertogenbosch The Netherlands

1.2. Sponsor

Sponsor	(on behalf of the Amphoacetates consortium): CRB-Chemie Revisions- und Beratungsgesellschaft mbH Wirtschaftsprüfungsgesellschaft. Rudolf-Breitscheid-Strasse 21, 90762 Fürth Germany

2. SUMMARY

This report describes the results of an *analogue approach* applied for the REACH registration of alkylamphoacetates with varying alkyl chain lengths covering volume bands of 100-1000 or >1000 tonnes/year (Annex VII, VIII, IX and Annex X data requirements), as summarized below:

Substance name	EC no.	Highest tonnage band in the SIEF
Amphoacetates C8-C18	931-291-0	>1000
Amphoacetates C12-C14	938-645-3	100-1000
Amphoacetates C12	271-794-6	100-1000

The analogues were identified based on comparable manufacturing processes, structural similarity (shared core structure, main components C12 and/or C14 linear alkyl chain derivatives) and resulting similar physico-chemical and (eco)toxicological properties.

The current update was initiated following ECHA decisions on testing proposals on the three analogues. The testing outline followed was chosen to strengthen the Read-Across hypothesis, by substantiating the data-set with new test data and by confirming the scientific validity of the historic data-set. The strategy followed was outlined in the document "Update on category approach and testing strategy for REACH registration of ALKYLAMPHOACETATES" (06 October 2016).

Alkylamphoacetates can be divided into two forms: the mono-acetate form in which mainly the mono-acetate molecules are present (>80%); and the di-acetate form, in which both mono-acetates and di-acetates are present at approximately 50% (see figure 1). Attempts were made to isolate the mono- and diacetate forms of a relatively narrow C-chain distribution amphoacetate by aid of preparative chromatography, and to use the isolated forms as standard in further HPLC-tests to gain insights a.o. on elution order and (UV) response factors. These attempts have been more successful than they were in the past and more in-depth investigations are ongoing.

The additional data include physico-chemical parameters (vapour pressure and CMC), ecotoxicological tests (algae tests, acute *Daphnia* and fish tests, a *Daphnia* reproduction test and a fish early life stage test). The conclusions of the new studies, which were done including analytical verification of the test concentrations, were comparable to the conclusions of the old studies (effect concentrations based on nominal test concentrations). The test work was planned in a step-wise approach: chronic test work was performed with the analogue that showed the highest toxicity in the acute tests. A step-wise approach was also followed for human toxicity testing. Twenty-eight day repeated dose studies were performed with the mono- and the diacetate forms of alkylamphoacetates C8-C18, the analogue in which all alkyl lengths covering the spectrum/chemical space of the category are present. These studies (in which no adverse effects were seen apart from a secondary effect (regurgitation)) were followed up by a 90-day repeated dose study and a prenatal developmental study on the C8-C18 alkylampho(di)actetates. No adverse effects were observed after sub-chronic exposure up to and including the highest dose tested, resulting in a NOAEL of 1000 mg/kg bw/day. In the prenatal developmental toxicity study, adverse effects were observed (cardiovascular/abdomen malformations in all dose groups and abnormal lung lobation in low and mid dose), but in absence of a dose-related response, it cannot be excluded if these effects were related to the test item. To get a better understanding of effect of alkyl chain length (distribution) and mono- and diacetate forms on toxicity, a 90-day repeated dose toxicity study and a prenatal developmental

toxicity study with C12-14 alkylampho(di)acetates were also conducted. In the 90-day study no adverse test-item related effects were seen at any dose level, the NOAEL for sub-chronic exposure was found to be 1000 mg/kg bw/day. Similarly, no maternal or developmental toxicity were observed up to the highest dose level tested in the prenatal developmental toxicity study with C12-14 alkylampho(di)acetate. The maternal and developmental NOAELs were both established as being at least 1000 mg/kg bw/day. An additional prenatal developmental toxicity study has been conducted with C12 alkylampho(mono)acetates, in this study maternal and developmental toxicity were not observed up to the highest dose level tested, and the maternal and developmental NOAELs were all established as being at least 1000 mg/kg/day.

It is of note that additional test work is planned in order to strengthen the read-across analogue approach and to elucidate the observations in tests that have been recently performed (test proposals included in dossiers; analytical work ongoing).

3. INTRODUCTION

The aim of this document is to provide the scientific basis and rationale for a read-across analogue approach used for the REACH registration of three alkylamphoacetate surfactant substances. The rationale was created based on the ECHA Guidance for the implementation of REACH, Guidance on information requirements and chemical safety assessment, Chapter R.6 (reporting format for a chemical category) and the Read-Across Assessment Framework (RAAF).[1,2,3] A substance-based structural and compositional analogue approach for read-across was followed, meaning that results are obtained with the source substance as such, and the result of the tests are used to predict the properties for the target substance(s).

The following substances are currently considered as analogues:

Table 1: Alkylamphoacetate analogues

Substance name	EC no.	Highest tonnage band in the SIEF
Amphoacetates C8-C18	931-291-0	>1000
Amphoacetates C12-C14	938-645-3	100-1000
Amphoacetates C12	271-794-6	100-1000

[1] Guidance on information requirements and chemical safety assessment, Chapter R.6, May 2008; http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r6_en.pdf?vers=20_08_08
[2] Read-Across Assessment Framework (RAAF), ECHA-17-R-01-EN, March 2017
[3] Read-Across Assessment Framework (RAAF)_Considerations on multi-constituent substances and UVCBs, ECHA-17-R-04-EN, March 2017

4. ANALOGUE DEFINITION

4.1. Definition of analogues

4.1.1. Read-across hypothesis

The read-across hypothesis is that the organism is not exposed to common compounds (metabolites/degradation products) but rather, as a result of structural similarity, that different compounds have similar (eco)toxicological and fate properties. The properties investigated in a study conducted with one source substance are used to predict properties that would be observed in a study with the target substance if it were to be conducted. Qualitatively similar properties or absence of effect are predicted. The predicted property may be similar or based on a worst-case approach.

The analogues are alkylamphoacetates, which are amphoteric surfactants. The analogues are manufactured, marketed and used in aqueous solutions. The solid(s) content in a manufactured commercial product corresponds to the substance to be registered in accordance with REACH Article 3(1), in case water can be removed from the products without affecting or impacting upon the stability of the substance and/or its composition. For some alkylamphoacetate analogues the water cannot be removed and is thus part of the registered substance. The solid concentration in the commercial products generally ranges from 30 to 95%.

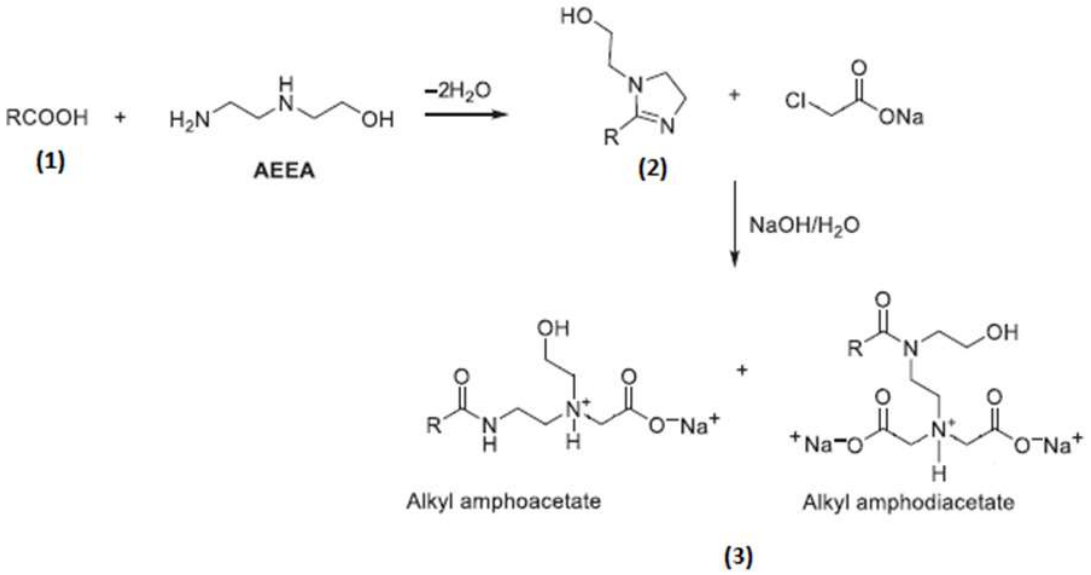
The analogues in this read-across justification report are identified and grouped based on the following characteristics: similarities in the general manufacturing process (including identical and/or comparable starting materials), functional groups, and general composition. The main variable resides in the alkyl chain distribution present in the raw starting materials.

1) Chemistry

Synthesis

The general chemistry of the manufacture of alkylamphoacetates is depicted in Figure 1 below.

Figure 1: Chemistry of the manufacture of alkylamphoacetates



Whereby:

AEEA is Aminoethylethanolamine (2-(2-aminoethylamino)ethanol; CAS: 111-41-1)
R is the alkyl chain distribution derived from fatty acids or oils (see Table 2 and Appendix I)

The substances are manufactured in a batch-wise process, under similar reaction conditions. The imidazoline intermediate (2) (1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(Cx-y odd-numbered, alkyl) derivatives) is synthesised from the raw material fatty alkyl carboxylic acids (1) with aminoethylethanolamine and is often isolated.

The alkylampho(di)acetates (3) are subsequently synthesised in water, at ambient pressure and typically at a temperature of 80°C (cooled). The imidazoline intermediate (2) is reacted with chloroacetic acid in the presence of sodium hydroxide (alternatively, sodium chloroacetate can be used) and water. The amount of sodium hydroxide is as much as needed to have the pH well above 7, to start the exothermic reaction between the anion of chloroacetic acid that is formed in the water and the intermediate (2). The molar ratio between the intermediate and chloroacetic acid ranges from 1:1 to 1:2. The 1:1 molar ratio results in a monoacetate, while an excess of sodium chloroacetate/chloroacetic acid in the 1:2 molar ratio favours the formation of the diacetate. As a by-product, hydrochloric acid is formed during the reaction, that is neutralized via the addition of sodium hydroxide (pH is monitored and remains above 7 to keep the reaction going). The reaction terminates with complete consumption of chloroacetic acid resp. its anion. The resulting reaction mixture is neutralized to a pH of 9 or lower, by adding any acid (e.g. hydrochloric acid). The by-product sodium chloride is formed from the reaction of sodium hydroxide with hydrochloric acid.

Functional groups

Figure 2 presents the structural information of the alkylampho(di)acetates. The common structural features present in the surfactant are an amide bond and a hydroxyl group both originating from the reaction of the carboxylic acids and the AEEA and the presence of aminoglycinate function(s) (or “acetate”) originating from the reaction of the imidazole intermediate with the chloroacetic acid.

The upper two structures of Figure 2 are representative for the alkylampho(mono)acetates and the lower two structures the alkylampho(di)acetates. These are theoretical structures based on the knowledge of the chemistry (Uphues, 1998; Behler et al., 2001). The NMR spectra show peaks that are characteristics of these structures but the difficulty in identifying the precise structures present has been discussed in the document “interpretation of NMR spectra” attached to the Amphoacetates C8-18 and Amphoacetates C12 submissions (section 1.4). Their precise structure (i.e. positioning of the acetate and hydroxyl groups) and respective percentages are variable and cannot be analytically determined due to the lack of a suitable analytical method for these UVCB substances.

Attempts were undertaken to isolate the mono- and diacetate forms of a relatively narrow C-chain distribution amphoacetate by aid of preparative chromatography, and to use the isolated forms as standard in further HPLC-tests to gain insights amongst others on elution order and (UV) response factors. Although the separation and isolation appeared to be reasonably successful based on chromatograms, further attempts to crystallize and identify the collected fractions were less successful. Evaporation of preparative chromatography solvents from the collected fractions caused amongst others foaming and never yielded dry residues, which possibly could have been re-crystallized to yield purer acetate forms. In a best case, an amorphous monoacetate solid could be isolated (confirmed by 1H-NMR analysis), but it proved

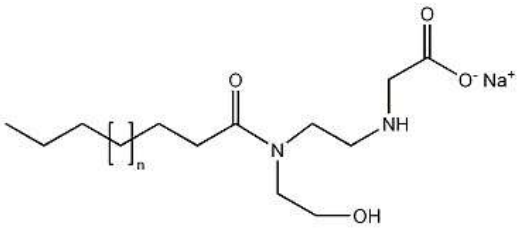
impossible to isolate a diacetate solid. Attempts are currently being undertaken to obtain further insight and details into the mono- and diacetate ratios of the registered alkylamphoacetates.

Composition

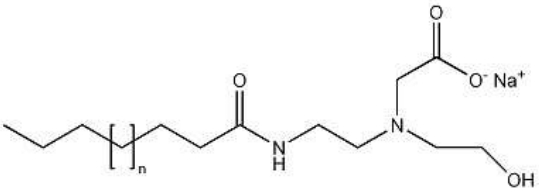
The alkylamphoacetates are UVCB substances and contain multiple constituents. The substances contain an alkylamphoacetate fraction, which consists of a group of various alkyl derived constituents bearing aminoglycinate functional group(s) (the "active surfactant fraction") and the by-product sodium chloride. The substances also contain some other constituents, such as residual water and the by-product sodium glycolate ($C_2H_4O_3Na$). The typical compositions of the analogues are reported in Table 2 (section 4.2).

Figure 2: General structures of the main constituents of the alkylamphoacetates fraction of the substances

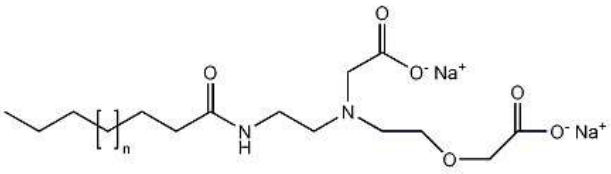
Alkyl-monoacetate 1



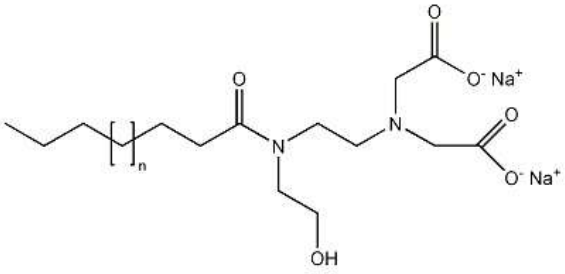
Alkyl-monoacetate 2



Alkyl-diacetate 1



Alkyl-diacetate 2



Variability/differences

An important difference is the use of various types of raw materials, differing mainly in the C-Chain length of the linear alkyl carboxylic acid starting material. UVCB-type substances derived from oleochemicals consist as mixtures of various alkyl-chain lengths at varying

concentrations (OECD 193). The amount of each chain length depends on the source of fatty acids, which usually originates from natural fats and oils (containing for example the alkyl chain range from C8 to C18), but can also be from synthetic origin. As it is in general derived from a natural origin, the C8-18 alkyl distribution is variable, and can only be given as a range of chain lengths with the main constituents being C12 and C14. Fractionation can increase the concentration of a specific C-chain length cut (for example, to > 90% C12-alkyl for Alkylamphoacetates C12).

All analogue substances contain mono- and diacetate structures and are mainly comprised of the C12 and C14 forms. The ratio of mono- and diacetate constituents differ as a consequence of the relative amount of chloroacetic acid used in the manufacturing process as described above. The more chloroacetic acid is used, the more diacetate constituents will be present in the resulting product.

Besides differences in the number of incorporated carboxymethyl-groups (mono- and diacetates) and in their alkyl constituents, differences in the position of the acetate and hydroxyl groups may give rise to substructures 1 and 2 (see figure 2). While mono- and diacetates with substructure 2 predominate, mono- and diacetates with substructure 1, cannot be ruled out.

The ratio of the (potential) structures contained in the surfactant part of the substance have been found to influence the (eco)toxicological properties of the substances. For certain endpoints this influence is addressed, and follow-up testing (where applicable) is carried out, using the worst-case approach (i.e. the analogue with the least favourable properties will be tested and/or considered the source). Furthermore all analogous structures have the same functional groups, i.e. one or two aminoglycinate (-NH-CH_2-COONa) functions (i.e. terminal acetate) and hydroxyl, linked to a fatty chain by an amide bond. The structural and compositional similarity is expected to result in similar behaviour of the analogues upon exposure to eco-system/environment and exposure to and uptake in the human body.

All analogue substances contain a main alkylamphoacetate ‘active surfactant’ fraction, as well as sodium chloride, sodium glycolate and residual water as impurities/by-products, all in comparable amounts (see Table 2). Because of the decreasing proportion of other alkyl chains, the Amphoacetates C12-C14 and Amphoacetates C12 have an increasing content in the C12 alkyl structures compared to the Amphoacetates C8-18.

2) Physicochemical properties and distribution

The alkylamphoacetate analogues are designed to be surface active, exhibit low vapour pressure (due to their relatively high molecular weight and presence of polar groups) and a high water solubility (amphiphilic and polar groups). As the alkylamphoacetates are mainly present as sodium carboxylates at environmental pH (pKa of carboxylic acids is approx. 4 – 5), these constituents are expected to partition predominantly to the aquatic compartment and minimally adhere to organic matter. As the vapour pressure is expected to be low, the substances do not volatilize. Based on their amphiphilic structure together with a high water solubility and moderate lipophilic character, the amphoacetates are expected to be systemically absorbed to some extent by the oral or dermal route (REACH guidance R7c, 2017). However, the presence of charged functional groups in substances has been shown to reduce dramatically the passage across the skin (Schaefer et al., 1996). As produced or under the use conditions the surfactant part of the substances will be either as a sodium salt, or as an amphoteric (zwitterionic) form with positive and negatively charged functional groups present.

It is concluded that based on the compositional and structural similarity of the components present and their respective water solubility, partition coefficient, vapour pressure and surface activity, the alkylamphoacetate analogues will be distributed similarly upon exposure to environment and in the human body and are expected to exhibit similar (eco)toxicological properties.

4.1.2. Applicability domain (AD) of the analogues

The alkylamphoacetates are defined as amphoteric surfactants. The proportion of alkyl chain lengths which comprise the substances can vary between C8 and C18. The alkylamphoacetate analogues all share the same key functional groups, i.e. one or two aminoglycinate (-NH-CH_2-COONa) functionalities (i.e. terminal acetate) and a single N-hydroxyethyl, linked to a fatty acid chain by an amide bond. In one of the diacetate structure forms the hydroxyethyl group is converted to an ether bond after reaction with a second chloroacetate molecule. In view of their potential chemical reactivity these structural features are considered to define the toxicological profile to a higher extent than the alkyl chain length and/ or presence of mono- or diacetate forms.

It is obvious that a more detailed determination and discrimination of the compositional profile of the individual substances which comprise the alkylamphoacetate analogues would strengthen the read across hypothesis. To this end, new analytical data fulfilling the requirements of ECHA's Advice on using read-across for UVCB substances[4] is being commissioned by the alkylamphoacetate consortium at the moment.

4.1.3. List of endpoints covered

An analogue approach (read-across) was applied to the following endpoints (nb: read across can differ per endpoint for each analogue):

- self-ignition temperature;
- biodegradability;
- algae toxicity;
- acute toxicity to *Daphnia* and/ or fish;
- activated sludge respiration inhibition;
- *Daphnia* reproduction toxicity testing;
- fish chronic testing;
- acute dermal toxicity;
- skin and eye irritation;
- skin sensitization;
- *in vitro* gene mutation in mammalian cells;
- sub-chronic repeated dose toxicity;
- toxicokinetic assessment.

4.2. Analogues

Substance identifiers for all alkylamphoacetate analogues are presented in Table 2. It should be noted that no molecular weight range can be accurately defined for these complex UVCB

[4] ECHA Advice on using read-across for UVCB substances, May 2022;
https://www.echa.europa.eu/documents/10162/11395738/advice_uvcb_read-across_en.pdf

substances containing multiple constituents. The molecular weight mentioned is the molecular weight used for the chemical safety assessment (CSA). In case of Amphoacetates C12-C14 and Amphoacetates C12, a possible C12 monoacetate constituent and in case of Amphoacetates C8-C18, a possible C12 diacetate constituent (representing a worst-case approach for the CSA for human health) were considered for calculation of molecular weights.

The solid(s) content corresponds to the substance to be registered in accordance with REACH Article 3(1), for some alkylamphoacetate analogues the water cannot be removed and is thus part of the registered substance .

The NaCl content was determined by the determination of chloride by titration with silver nitrate. Based on these results, the alkylamphoacetate derivatives (active surfactant) fraction and the solid content are determined (see also previous section). The percentual composition of the alkylamphoacetate derivatives fraction (or alkyl chain distribution) presented in table 2 is based on the known C-Chain distribution of the fatty acid starting material(s).

Table 2 Substance identifiers for all analogues

Identification: Amphoacetates C8-C18		
	Type of substance:	UVCB Monoacetate form (contains appr. 95% monoacetates and 5% diacetates) and diacetate form (contains appr. 40% monoacetates and 60% diacetates)
	IUPAC name:	Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C7-C17 odd-numbered, C17-unsatd. alkyl) derivs. and sodium hydroxide and chloroacetic acid
	CAS Number:	-
	Alternative CAS numbers[5]	68650-39-5; 68334-21-4; 68390-66-9; 61791-32-0; 90387-76-1; 68608-65-1
	EC/List Number:	931-291-0
	Molecular Weight (for the CSA):	446 g/mol
	Compositional information (as manufactured, w/w)	
	Water	47-64%
	Total solids:	36-53%
	Total alkylamphoacetate derivatives	27-43%
	NaCl	0-15%
	Sodium glycolate	0-6%
	Alkyl amidoamine	0-3%

[5] See Annex I for the SIEF merging justification document (as submitted with the registration of this substance)

<table>
<tr><th colspan="3">Identification: Amphoacetates C8-C18</th></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-600 ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-6 ppm</td></tr>
<tr><td></td><td>Compositional information (solvent free condition, w/w)</td><td></td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>65-86%[6]</td></tr>
<tr><td></td><td>Alkyl chain distribution, Cn</td><td>
<table>
<tr><th>Cn</th><th>Mono#</th><th>Di#</th><th>Total</th></tr>
<tr><td>C8</td><td>0-11%</td><td>0-2%</td><td>0-11%</td></tr>
<tr><td>C10</td><td>0.1-10%</td><td>0-2%</td><td>0-11%</td></tr>
<tr><td>C12</td><td>16-56%</td><td>0-36%</td><td>42-64%</td></tr>
<tr><td>C14</td><td>5-20%</td><td>0-15%</td><td>6-26%</td></tr>
<tr><td>C16</td><td>1-22%</td><td>0-8%</td><td>4-22%</td></tr>
<tr><td>C18</td><td>0.1-16%</td><td>0-7%</td><td>0.1-18%</td></tr>
<tr><td>C18:1 and/or C18:2 [7]</td><td>0-9%</td><td>0-12%</td><td>0-20%</td></tr>
</table>
</td></tr>
<tr><td></td><td>NaCl</td><td>0-26%</td></tr>
<tr><td></td><td>Sodium glycolate</td><td>0-12%[8]</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-6%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-1500ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-14ppm</td></tr>
</table>

Identification: Amphoacetates C12-C14		
	Type of substance:	UVCB Diacetate form only (contains appr. 40 to 45% monoacetate and 55 to 60 % diacetates)
	IUPAC name:	Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C11-C13 odd-numbered alkyl) derivs. and sodium hydroxide and chloroacetic acid
	CAS Number:	1689515-39-6
	Alternative CAS numbers[9]	66161-62-4; 68608-66-2
	EC/List Number:	938-645-3

[6] The lower range figure for the surfactant fraction is due to the greater difficulty in drying the C8-18 substance and residual water
[7] Number of unsaturations per C18 alkyl chain: 0.001 - 0.15
[8] Analysed as glycolic acid and converted to sodium glycolate as this is the form more likely present in the UVCB substance. Compositional information in registration dossiers may be given as glycolic acid (due to the analytical method) and/or can be also converted to the sodium salt.
[9] The SIEF merging justification document is submitted with the registration of this substance

<table>
<tr><th colspan="3">Identification: Amphoacetates C12-C14</th></tr>
<tr><td></td><td>Molecular Weight (for the CSA):</td><td>367 g/mol</td></tr>
<tr><td></td><td>Compositional information (as manufactured, w/w)</td><td></td></tr>
<tr><td></td><td>Water</td><td>50-51%</td></tr>
<tr><td></td><td>Total solids:</td><td>49-50%</td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>≥39%</td></tr>
<tr><td></td><td>NaCl</td><td>0-10%</td></tr>
<tr><td></td><td>Sodium glycolate</td><td>2-4%</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-2%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-65 ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-5 ppm</td></tr>
<tr><td></td><td>Compositional information (solvent free condition, w/w)</td><td></td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>≥78%</td></tr>
<tr><td></td><td>Alkyl chain distribution, Cn</td><td><table><tr><th>Cn</th><th>mono</th><th>di</th><th>total</th></tr><tr><td>C8</td><td>n.d.[10]</td><td>n.d.</td><td>n.d.</td></tr><tr><td>C10</td><td>≤2%</td><td>≤2%</td><td>≤4%</td></tr><tr><td>C12</td><td>26-37%</td><td>36-49%</td><td>67-80%</td></tr><tr><td>C14</td><td>7-16%</td><td>10-20%</td><td>20-32%</td></tr><tr><td>C16</td><td>≤2%</td><td>≤2%</td><td>≤4%</td></tr><tr><td>C18</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></tr><tr><td>C18:1 and/or C18:2</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></tr></table></td></tr>
<tr><td></td><td>NaCl</td><td>0-20%</td></tr>
<tr><td></td><td>Sodium glycolate[11]</td><td>6 -11%</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-6%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-130ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-14ppm</td></tr>
</table>

<table>
<tr><th colspan="3">Identification: Amphoacetates C12</th></tr>
<tr><td></td><td>Type of substance:</td><td>UVCB
Monoacetate form only (contains appr. 75 to 100% monoacetate and 0 to 25% diacetates)</td></tr>
</table>

[10] n.d – Not determined.

[11] Analysed as glycolic acid and converted to sodium glycolate as this is the form more likely present in the UVCB substance. Compositional information in registration dossiers may be given as glycolic acid (due to the analytical method) and/or can be also converted to the sodium salt.

<table>
<tr><th colspan="3">Identification: Amphoacetates C12</th></tr>
<tr><td></td><td>IUPAC name:</td><td>Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C11 alkyl) derivs. and sodium hydroxide and chloroacetic acid</td></tr>
<tr><td></td><td>CAS Number:</td><td>68608-66-2</td></tr>
<tr><td></td><td>EC Number:</td><td>271-794-6</td></tr>
<tr><td></td><td>Molecular Weight (for the CSA):</td><td>367 g/mol</td></tr>
<tr><td></td><td>Compositional information (as manufactured, w/w)</td><td></td></tr>
<tr><td></td><td>Water</td><td>60-70%</td></tr>
<tr><td></td><td>Total solids:</td><td>30-40%</td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>23-31%</td></tr>
<tr><td></td><td>NaCl</td><td>5-8%</td></tr>
<tr><td></td><td>Sodium glycolate</td><td>0.5-4%</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-0.3%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-5000 ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-4 ppm</td></tr>
<tr><td></td><td>Compositional information (solvent free condition, w/w)</td><td></td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>76-80%</td></tr>
<tr><td></td><td>Alkyl chain distribution, Cn</td><td><table><tr><th>Cn</th><th>mono</th><th>di</th><th>total</th></tr><tr><td>C12</td><td>61-93%</td><td>0.1-21%</td><td>80-99.9%</td></tr><tr><td>Unknown</td><td>-</td><td>-</td><td>0.1-20%</td></tr></table></td></tr>
<tr><td></td><td>NaCl</td><td>16-20%</td></tr>
<tr><td></td><td>Sodium glycolate</td><td>4-8%</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-0.5%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-9000ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-10ppm</td></tr>
</table>

4.3. Purity/Impurities

The water content of the registered substances is determined by Karl-Fischer titration after the drying procedure.

The NaCl content was determined by the determination of chloride by titration with silver nitrate.

5. READ-ACROSS JUSTIFICATION

5.1. Physico-chemical properties

The assumption/hypothesis that the properties of the analogues are similar was in the first instance verified with respect to the physico-chemical parameters (

Table 4).

The substances are structurally very similar, and are designed to exhibit surface active properties. The surface tension was measured for all analogues and found to be in the same range (29.1 – 35.4 mN). Since this property influences how the water solubility is interpreted, the Critical Micelle Concentration (CMC) was determined in GLP-compliant studies performed in accordance with OECD Guideline No. 115 for all of the analogues, by measuring the surface tension of test item solutions at different test item concentrations. The Critical Micelle Concentration (CMC) of the mono- and the diacetate forms were found to be in a similar range: 160/150 and 239/262 mg solids/L for the monoacetate/diacetate form of Amphoacetates C8-C18 and Amphoacetates C12-C14, respectively. The CMC of Amphoacetates C12 (mono-acetate) was also determined and found to be higher, at 718 mg solids/L. The bulk water solubility of the different Amphoacetates was determined in GLP-compliant studies performed in accordance with EC A.8 method and OECD Guideline No. 105, by means of visual observations; the water solubility of all analogues was high (> 1000g solids/L).

For all analogues, it was considered justified to study the n-octanol/water partition coefficient (log Pow) by the estimation method, as all other methods were assessed to be not adequate due to their surface active nature. However, based on the estimation method it was concluded that it is technically not possible to determine a reliable estimate of the log Pow for these complex and variable substances. Nevertheless the solubility in water was measured to be more than 1,000,000 mg solids/L and the solubility in octanol was found to be less than 82 mg/L. For these analogues, based on the complex and incompletely defined composition which contain variable alkyl chain lengths and a high concentration of NaCl and taking into account the tensio-active properties of the surfactant fraction and the fact that classical empirical methods cannot be used, it was deemed reasonable to assume a log Pow value of -1. This log Kow of -1 is justified on the basis of the experimentally determined solubility data in water and in octanol solvents, and on the basis of the calculated log Kow values of –0.64 to -4.19 for the main part of the surfactant fraction obtained with QSAR (US EPA Episuite KOWWIN v1.68).

All analogues were found to exhibit low vapour pressure. Measured vapour pressure values determined at 20 ºC for Amphoacetates C8-C18 was $1.4*10^{-7}$ Pa (monoacetate form) and < $8.4*10^{-7}$ Pa (diacetate form). For Amphoacetates C12-C14 the vapour pressure was $1.8*10^{-8}$ - $1.3*10^{-6}$ Pa (monoacetate form) and $2.6*10^{-6}$ Pa (diacetate form). For Amphoacetates C12 the vapour pressure was also very low with $1.5*10^{-7}$ Pa.

The density of the analogues in aqueous solutions is very similar and none of the analogues are highly flammable or exhibit pyrophoric or explosive properties.

All analogues decompose before reaching their boiling temperature. The differences observed in their melting point and decomposition temperatures can be explained by the difference in the extent of a more or less heterogeneous series of molecules present. The higher the content of molecules with a similar alkyl chain length (e.g. C12 alkyl), the easier the molecules can organize themselves to crystallize and melting at a specific temperature can be observed.

In view of the physico-chemical properties discussed above and the compositional and structural similarity of the components present in the analogues, it can be concluded that read-across between alkylamphoacetate analogues is justified and that they will be distributed similarly upon exposure to environment and in the human body and thus are expected to exhibit similar (eco)toxicological properties.

5.2. Environmental fate and eco-toxicological properties

The environmental properties of the analogues are presented in

Table 5. To allow comparison between the analogues, all concentrations mentioned in this chapter are expressed based on solids content (i.e. the pure active surfactant test item and salt, corrected for water content).

Aquatic toxicity: acute and chronic toxicity to invertebrates

Several older studies were available on the acute toxicity of alkylamphoacetates to the freshwater invertebrate *Daphnia magna* (see Appendix III). In short, the data indicated that the acute toxicity of Ampho(mono)acetates C12 to *Daphnia* (48h-EC_{50}'s: 89 – >100 mg/L) was lower than the toxicity of Amphoacetates C8-C18 (surrogate with unknown mono- to diacetate ratio) towards *Daphnia* (48h-EC_{50}'s: 2.5 – 18.5 mg/L). No data were available to address differences in toxicity profile between mono- and diacetate form of the analogues.

The historical tests were run without analytical verification of exposure concentrations, therefore ECHA concluded that the results were not adequate to fulfil the endpoint information requirement(s).

In order to provide more robust and high quality data to cover this endpoint, in 2017 four acute *Daphnia magna* toxicity studies were performed in parallel, which included analytical verification of the test concentrations. The substances tested were Alkylamphoacetates C8-C18 (monoacetate and the diacetate form) and Amphoacetates C12-C14 (monoacetate and the diacetate forms). Analytical monitoring data collected during the test period showed that all substances were stable in aqueous solution (>80% of nominal concentrations at test end in all test solutions relevant for calculation of effect concentrations). Therefore, nominal concentrations were used to express the effect parameters in the final tests.

The results of the acute *Daphnia* tests performed in 2017 are summarized in Table 3 below.

Table 3 Acute *Daphnia* toxicity of Alkylamphoacetates C8-C18 and Amphoacetates C12-C14 tested in 2017

Test item	48h-EC_{50} (*Daphnia*; concentration based on solid fraction)
Alkylamphoacetates C8-C18	
Monoacetate form	25.4 mg/L
Diacetate form	56.6 mg/L
Alkylamphoacetates C12-C14	
Monoacetate form	67.3 mg/L
Diacetate form	> 100 mg/L

The alkyl chain distribution of the alkylamphoacetate analogues can be found in Appendix I. Comparison of the EC_{50} values with the alkylchain distribution reveals that the acute toxicity to daphnids is slightly higher in presence of alkylderivatives with a greater proportion of the longer carbon chains. Furthermore, the daphnids appear to be slightly more sensitive to the monoacetate form in comparison to the diacetate form. By adopting a conservative approach, the lowest 48h-EC_{50} values are considered worst-case to fill this endpoint. Therefore, the results

of the monoacetate forms were used as the key study values to cover this endpoint/information requirement for both C8-C18 and C12-C14 analogues, respectively.

The results of the acute *Daphnia* studies performed in 2017 yielded slightly higher EC_{50} values (i.e. lower toxicity) for all test substances compared to the old data (all data are given in Appendix III). The results from the new acute daphnid toxicity studies with alkylamphoacetates are considered more relevant, accurate and reliable than the historical data. However the old data covers a relatively large range (2.5-18.5 mg/L) of which one data point falls nearly within the new data range.

As analytical verification of exposure concentrations in the more recent studies demonstrate the C12-C14 and C8-C18 alkylamphoacetate test substance concentrations were within 20% of nominal and stable throughout the exposure period, this new data validates the reliability of the available historical acute daphnia toxicity study result with Amphoacetates C12. The more recent data for the C8-C18 and C12-C14 analogues combined with historical data on C12, allow, in a weight-of-evidence approach, to conclude on this endpoint for the C12 analogue: 48h-EC_{50} = 89 mg/L.

Long-term toxicity to aquatic invertebrates was determined for the alkylamphoacetate analogue that caused the highest toxicity in the acute tests(Alkylampho(mono)acetates C8-C18). The C8-C18 alkylampho(mono) acetate substance was subject to *Daphnia magna* reproduction study in accordance with OECD 211 under GLP conditions.

Ten neonates (<24 h old) were individually exposed in a semi-static system to nominal test concentrations of 0.46, 1.0, 2.2, 4.6 and 10 mg/L for 21 days with test solutions renewed every 48 hours. Additionally, a blank control was included with 20 neonates. Parental mortality, number of living offspring, immobile young and appearance of unhatched (aborted) eggs were recorded and the lengths of the surviving parental daphnids were measured at the end of the test.

Samples taken at the beginning and the end of three 48-hour renewal intervals were analysed. The concentrations measured in the freshly prepared solutions ranged between 47 - 133% of nominal, with the majority of results being within 87 -120% of nominal. Since the concentrations appeared to be unstable during the refreshment periods, Time Weighted Average concentrations were calculated to be 0.075, 0.15, 0.45, 1.6 and 3.7 mg/L.

Mortality in the controls did not exceed 20%, mortality in the test item groups ranged from 10 to 40% but was not statistically different from the control treatment. An increase of reproduction, rather than a reduction was observed in all concentrations tested. The onset of reproduction was not delayed in any of the test concentrations when compared to the controls (onset at day 7), except at the highest treatment group (at day 8). The change in mean body length of parent daphnids ranged between -1.0 and 1.8% in the four lowest concentrations and was not dose-related. At the highest concentration, a statistically significant reduction in mean body length of 4.6% was observed.

The 21-d NOEC for reproduction of *Daphnia magna* exposed to Amphoacetates C8-C18 (monoacetate form) was set at 3.7 mg/L (TWA concentration). The 21-d NOEC for growth reduction was set at 1.6 mg/L.

This result is read across to the other analogues, as Amphoacetates C8-C18 (monoacetate form) was concluded to represent the worst-case for the analogues based on the acute toxicity studies, but similar toxicity was expected.

Aquatic toxicity: algae

Similar to acute toxicity testing with the freshwater invertebrate *Daphnia magna*, toxicity of various alkylamphoacetates to aquatic plants was previously tested in historical studies (see Appendix III). These studies revealed toxicity in the same concentration range for mono- and diacetate forms (72h ErC50 = 28.5 mg/L for C8-C18 alkylampho(mono)acetates and 72h ErC50 = 30 mg/L for C8-C18 alkylampho(di)acetates). Furthermore, the algal toxicity studies indicated that Amphoacetates C8-C18 (monoacetate form) caused the highest toxicity compared to the other analogues (72h ErC50 = 10 mg/l for C8-18 alkylampho(mono)acetates and 72h ErC50 = 14.8 mg/L for C12 alkylampho(mono)acetate).

New algal toxicity testing was initiated in 2018 to verify these data. Alkylampho(mono)acetates C8-C18 and alkylampho(mono)acetates C12 (monoacetate form) were subject to 72h algae toxicity studies conducted in accordance with OECD guideline 201 under GLP conditions.

For Alkylampho(mono)acetates C8-C18 (monoacetate form), freshwater algae (*Pseudokirchneriella subcapitata*) were exposed to individually prepared Water Accommodated Fractions (WAF) of the test item prepared at nominal loading rates of 1.0, 3.2, 10, 32, and 100 mg/L (3 replicates per concentration) and an untreated control (6 replicates). Measured concentrations were not stable throughout the exposure period, therefore Time Weighted Average concentrations were calculated to be 0.23, 0.87, 2.6, 22 and 81 mg/L in WAFs prepared at loading rates of 1.0, 3.2, 10, 32 and 100 mg/L. A concentration-related increase of growth rate inhibition was observed at all test concentrations. Statistically significant growth rate inhibition was observed at the four highest WAFs tested, but a biologically relevant growth rate inhibition was observed only in the three highest WAFs tested. The 72h ErC_{50} and ErC_{10} for growth rate inhibition were 13 and 1.7 mg/L, respectively. The 72h-NOEC was 0.23 mg/L based on statistical significance and 0.87 mg/L based on biological relevance.

For Alkylampho(mono)acetates C12, freshwater algae (*Pseudokirchneriella subcapitata*) were exposed to individually prepared Water Accommodated Fractions of the test item prepared at nominal loading rates of 1.0, 3.2, 10, 32, and 100 mg/L (3 replicates per concentration) and an untreated control (6 replicates). Measured concentrations were not stable throughout the exposure period, therefore Time Weighted Average concentrations were calculated to be 0.015, 0.0025, 0.27, 210 and 21000 µg/L in WAFs prepared at loading rates of 1.0, 3.2, 10, 32 and 100 mg/L. A concentration-related increase of growth rate inhibition was observed with increasing test concentration, resulting in 100% inhibition of growth rate at the highest test concentration. The analytical results indicated a rapid decrease (to undetectable levels) of test concentrations within the first 24 hours of the test. As this decrease was seen independent of the presence of algae, and also observed in pre-coated test vessels, this indicates that the absence of the substance in the test solutions was not caused by sorption to the test vessels. At this point, it appears to be the case that the algae medium is not compatible with the test item and that the measured concentrations are not a reliable base to determine the NOEC value. Instead, the effect parameters for growth rate inhibition based on nominal loading rate were considered to be more relevant. This is also considered justified based on the OECD Guidance document on aquatic toxicity testing of difficult substances and mixtures number 23 (2019), which states that effect parameters can be calculated from the loading rates of the entire UVCB/multi-constituent substances when WAFs are tested. The NOELR for growth rate was determined to be 10 mg/L (nominal), the ErL_{10} 7.3 mg/L (nominal) and the ErL_{50} 44 mg/L (nominal).

The 72h-ErC_{50} for Alkylampho(mono)acetates C8-C18 of 13 mg/L based on the TWA concentrations corresponds to a nominal loading rate 72h ErL50 value of 19.3 mg/L. This is in

the same order of magnitude as the 72h-ErL_{50} value of 44 mg/L (nominal) for Amphoacetates C12. The fact that the 72h ErL50 value for Alkylampho(mono)acetates C8-C18 is somewhat lower (i.e. slightly more toxic) than the result for Alkylampho(mono)acetates C12 is in line with the pattern of results from aquatic toxicity studies with other trophic level species. Therefore, it is reasonable to use the algal toxicity data for the C8-C18 Alkylampho(mono)acetate as read across to Alkylamphoacetates C12-C14. The 72h-ErC_{50}, 72h-ErC_{10} and 72h-NOErC values for the C8-C18 alkylampho(mono)acetate were determined to be 13, 1.7 and 0.87 mg/L, respectively. Since the NOErC is lower than the ErC_{10}, this value is used as a worst-case key read-across value for chemical safety assessment.

Aquatic toxicity: acute and chronic toxicity to fish

Several historical studies with Alkylampho(mono and di)acetates C8-C18 available covering acute toxicity to fish, and one historical, unreliable, study with Alkylampho(mono)acetates C12. An overview of these older studies can be found in Appendix III.

The acute toxicity of Alkylampho(mono)acetates C12 and Alkylampho(mono)acetates C8-C18 to the freshwater fish *Cyprinus carpio* were investigated in more recent studies in 2018. Both studies included analytical verification of test item concentrations and were performed in accordance with OECD 203 under GLP conditions.

In a semi-static acute toxicity test with Alkylampho(mono)acetates C12, carp were exposed for 96 hours to an untreated control and nominal test item concentrations of 5.0, 10, 20, 40 and 80 mg/L. Measured concentrations at the start and end of the first and last renewal periods were at 116 -154% of the nominal test concentrations. Based on these results, the effect parameters were expressed based on nominal exposure concentrations. No mortality or other effects were observed in the control and at the three lowest concentrations tested during the exposure period. All fish exposed to the highest concentration were found dead after the first 24 hours of exposure. All fish exposed to 40 mg/L were found dead after 48 hours of exposure. The 96h-LC_{50} for Alkylampho(mono)acetates C12 was determined to be 28 mg/L based on analytically confirmed nominal exposure concentrations.

In a semi-static test with Alkylampho(mono)acetates C8-C18 , carp were exposed for 96 hours to an untreated control and nominal test concentrations of 0.46, 1.0, 2.2, 4.6 and 10 mg/L. Measured test item concentrations were at the level of nominal concentrations (99 -115%) in freshly prepared test medium and at 95 - 142% of nominal in spent solutions. Because of the unknown reason for the observed increase in measured concentrations, the average nominal exposure concentrations were calculated to be 0.54, 1.1, 2.5, 5.1 and 11 mg/L. No mortality or other effects were observed in the control and at the three lowest concentrations tested during the exposure period. All fish exposed to the highest concentration were found dead after the first 24 hours of exposure. Six of the 7 fish exposed to 5.1 mg/L were found dead after 96 hours of exposure. The 96h-LC_{50} was determined to be 4.0 mg/L based on analytically confirmed average nominal exposure concentrations.

In line with the pattern observed with the acute toxicity testing on *Daphnia* and algae tests, Alkylampho(mono)acetates C8-C18 exhibited higher toxicity to fish than the C12 Alkylampho(mono)acetate analogue. Therefore, it is reasonable to use the acute fish toxicity data for C8-C18 Alkylampho(mono)acetate as read across to Amphoacetates C12-C14.

Following the conduct of a new suite of acute toxicity studies on fish, it was deemed appropriate to consider a flow-through fish early-life stage (ELS) toxicity test in order to assess possible

lethal and sub-lethal effects of alkylamphoacetate substances through exposure during embryonic and early larval development of the fathead minnow (*Pimephales promelas*). As Alkylampho(mono)acetates C8-C18 had been found to exhibit the highest acute toxicity to fish of all the alkylamphoacetate analogues, this substance was selected as a conservative worst-case representative substance from the category

The chronic fish study was conducted in accordance with OECD 210 and in compliance with GLP. Fertilized eggs (80 eggs per group, divided into four replicates) were exposed to an untreated control and the test item at mean measured concentrations of 0.035, 0.090, 0.22, 0.61 and 1.6 mg/L. Nominal concentrations were 0.05, 0.13, 0.31, 0.78 and 2.0 mg/L and were selected based on the results of a range-finding test (with target concentrations of 0.050, 0.50 and 5.0 mg solids/L) in which no mortality of the newly hatched larvae was observed in the control and 0.050 mg /L group, while mortality of 5.3% and 75% was observed at concentrations of 0.50 and 5.0 mg/L, respectively. Embryonic and larval survival was not affected at concentrations up to and including 1.6 mg/L; EC_{10} values were >1.6 mg/L (measured). It should be noted that consistent malformations of the caudal fin were observed in the highest test concentration of 1.6 mg/L (measured), and thus the NOEC was considered to be 0.61 mg/L (measured) based on malformation effects. EC_{10} values for growth reduction based on weight and length were 0.80 and 0.79 mg/L (measured), respectively. The NOEC for growth reduction based on weight and length was 0.61 mg/L (measured).

Since Alkylampho(mono)acetates C8-C18 exhibited higher toxicity than the other alkylamphoacetate analogues in all aquatic toxicity tests, it is considered both reasonable and scientifically justified to use these results as worst-case read across values to cover the chronic fish toxicity endpoint/information requirements for Amphoacetates C12-C14 and Amphoacetates C12.

Aquatic toxicity: microorganisms

With Amphoacetates C12 (monoacetate form) an activated sludge respiration inhibition study (OECD 209) has been performed. A NOEC of 560 mg/L was determined and this value is used as read across to the other C12-C14 and C8-C18 Alkylamphoacetate analogues.

Stability & biotic degradation

Amphoacetates C8-C18 and Amphoacetates C12 were tested for biodegradation (OECD 301 (mono- and diacetate form), OECD 302 (monoacetate form) and OECD 311 (mono- and diacetate form) for Amphoacetates C8-C18) (OECD 301 for Amphoacetates C12) and were found to be readily biodegradable. As Amphoacetates C12-C14 also contains mainly C12 and C14 mono- and diacetate constituents, similar to the tested substances, Amphoacetates C12-C14 are also considered to be ready biodegradable.

No hydrolysis data are available for any of the alkylamphoacetates. In accordance with column 2 of REACH Annex VIII, Hydrolysis as a function of pH does not have to be addressed in case the substances are readily biodegradable (study scientifically not necessary).

Bioaccumulation

As the analogues of the alkylamphoacetate consortium do not have a log Kow ≥4, the substances are considered to have a low potential for bioaccumulation. In accordance with column 2 of REACH Annex IX, the study bioaccumulation in aquatic species does not need to be conducted for any of the analogues.

Transport and distribution

As the screening study for adsorption and desorption behavior (OECD 121) is technically not feasible (due to the surface active nature of the alkylamphoacetates) and the adsorption/desorption using batch-equilibrium method (OECD 106) study has not been performed (due to the UVCB nature of alkylamphoacetates), the alternative option is to calculate the organic carbon-normalized sorption coefficient for soil and sediment (Koc) using an *in silico* (QSAR) approach. As the surfactant part of the analogues is present as sodium carboxylates at environmental pH, the substance is expected to partition predominantly in the aquatic compartment and to minimally adhere to organic matter. For the environmental CSA, the Koc of the substances has been calculated based on log Kow. The Koc of the substance has been calculated with EUSES version 2.1, based on log Kow, by using the in EUSES default QSAR for the chemical class non-hydrophobics:

$Koc = (10.47 \times Kow^{0.52})/1000$ (with Koc in m^3/kg)

For Amphoacetates C8, the Koc is thus 236.4 L/kg (based on a Kow of 401.2). The calculated Koc value for the other analogues is 3.16 L/kg (based on a Kow of 0.1).

Adsorption is thus considered to be negligible for the analogues of the alkylamphoacetate consortium. Modelling the distribution is not possible for this specific UVCB substance, but due to its extreme high water solubility, low vapour pressure and low LogKow, it can be concluded that the substance will predominantly distribute to the freshwater compartment. Distribution to other environmental compartments is considered to be negligible.

5.3. Toxicological properties

With regards to mammalian toxicological endpoints, the hypothesis/assumption that the biological effect properties of the Alkylamphoacetate analogues are similar has been verified (

Table 6 and Appendix V).

Acute tox (oral, dermal and/or inhalation)

For all alkylamphoacetate analogues the acute oral toxicity was tested in accordance with OECD test guideline 401 for the mono- and diacetate, and the LD50 was found to be at least 5000 mg/kg bw. For the monoacetate of the C8-C18 alkylamphoacetate also an LD50 >5000 mg/kg bw was determined. Based on the data for the mono- and diacetate of C12-C14 alkylamphoacetate and the monoacetate of C8-C18 alkylamphoacetate the LD50 of the diacetate of C8-C18 is also expected to be >5000 mg/kg bw. For the monoacetate of C12 alkylamphoacetate an LD50 of 3422 mg/kg bw was found.

For the analogue alkylampho(mono)acetates C8-C18, the acute dermal toxicity (OECD 402) was determined to be above 2612 mg/kg in a limit test. As alkylamphoacetates C12-C14 and alkylamphoacetates C12 have also mainly C12 and C14 mono- and diacetates similar to the tested substance, it is reasonable and scientifically justified to read-across the data to these substances, resulting in an acute dermal LD50 of >2612 mg/kg for all members of the alkylamphoacetates category.

No acute inhalation toxicity studies are available for any of the analogues. Testing for acute inhalation toxicity is not considered necessary as the exposure of humans via inhalation is not likely due to low vapour pressure of the alkylamphoacetate substances.

Corrosion/irritation (skin, eye)

Alkylamphoacetates C8-C18 (monoacetate and surrogate with unknown mono- to diacetate ratio)and alkylampho(mono)acetates C12 were tested for skin irritation/corrosion (OECD 404). Based on the results of the available studies, these 2 substances do not need to be classified as irritating to skin. As alkylamphoacetates C12-C14 has also mainly C12 and C14 mono- and diacetates similar to the tested substances, it is considered that alkylamphoacetates C12-C14 does not need to be classified as irritating to skin either.

With regard to eye irritation, all substances have been tested (according to or equivalent to OECD 405), in various concentrations in water. Aqueous solutions of the substance alkylamphoacetates C8-C18 (mono- and diacetate and surrogate with unknown ratio) were shown to be irritating to eyes (reversible effects in 3 studies; at a concentration of ≥ 38.9%), or corrosive to eyes (in 2 studies, irreversible similar grading of effects in 1 animal out of 3 in one study conducted with a diacetate form, or 1 animal out of 4 tested in a study conducted with a monoacetate form; at a concentration of ≥ 31%). Alkylampho(mono)acetates C8-C18 has also been studied as a 50% aqueous solution and in this study the observed effects do not warrant classification. Based on a worst-case approach, the substance Amphoacetates C8-18 is classified as causing irreversible effects on the eye (Category 1; H318).

In one study (OECD 405), a solution of 50% alkylampho(mono)acetates C12 showed slight irritation to the eyes, below classification criteria. In another study (OECD 405), an aqueous solution of 50% alkylampho(mono)acetates C12 was shown to be irritating to eyes. Based on a worst-case approach, the substance Alkylamphoacetates C12 is classified as irritating to eyes (Category 2; H319). In two studies at concentrations at ≤15% alkylampho(mono)acetates C12, ocular effects were below threshold criteria to warrant classification in accordance with EU CLP.

Alkylampho(mono)acetates C12-C14 has been tested for eye irritation at a concentration of approximately 16% in water, in this study observed effects do not warrant classification. The composition of this substance is more closely related to the composition of the substance alkylampho(mono)acetates C12; as it contains 69-78% of the C12 alkyl derivatives and lacks the shorter and longer alkyl chain derivatives and unsaturated C18 alkyl chain derivatives. For these reasons, it is therefore considered scientifically justified and appropriate that the classification of irritating to eyes (Category 2; H319) is read across to alkylampho(mono)acetates C12-C14.

Based on the data, >16% might be considered as a specific concentration limit for EU CLP classification of alkylamphoacetates C12 and alkylamphoacetates C12-C14 as irritating to eyes. When the eye irritation studies in rabbits in which the substances are tested at similar concentration (~50%) are compared, the average intensity of the ocular lesions are considered relatively similar. Only in 2 of the studies performed with alkylamphoacetates C8-C18 (mono- and diacetate form), the effects were irreversible which cannot be explained by differences in pH of the used solutions and also not by differences in surfactant, impurity and/or sodium chloride contents.

Skin sensitization

All analogues are surfactants, therefore results of a local lymph node assay performed with an analogue should be considered with care as it can be expected to result in a false positive outcome (OECD 336, Annex VI, Roberts et al., 2016, Ball et al., 2011). Indeed, in a LLNA performed with alkylampho(mono)acetates C12 and according to OECD guideline 429, the test item was found to induce proliferation of lymph node lymphocytes at the concentration of 50% (v/v) with an SI of >3. However, since the substance is a surfactant and showed clear irritating effects (described in scientific literature as confounding factors for false positives (OECD 336, Annex VI, Roberts et al., 2016, Ball et al., 2011), the result is considered inconclusive.

A guinea pig maximization test (GPMT), which is the preferred test for surfactants, was performed according to OECD guideline 406 with an alkylampho(mono)acetate C8-C18 (100% mono amphoacetate form). The lowest irritating concentration was chosen at the induction phase and the maximal non-irritating concentration was used at challenge. After epidermal induction performed on test day 8, slight to well-defined erythematous reactions were observed in all test animals treated with the test article at 75 % in bi-distilled water. After challenge, none of the animals of the test group were observed with positive skin reactions after treatment with the maximum non-irritant concentration of the test article of 1 % in water. Based on these results, alkylampho(mono)acetates C8-C18 was considered to be not sensitising.

Alkylampho(mono)acetate C12 (≥ 95% monoacetate form), was also tested in a GPMT in accordance with a test method equivalent to the OECD 406 guideline. Since 5 of the 20 animals (25%) were observed to have a positive response during challenge, the substance is not classified as a skin sensitiser in accordance with the CLP Regulation. It is of note that the choice for the intradermal induction exposure was based on the minimal irritating exposure in the range finding test, while it should have been the highest exposure to cause mild-to-moderate skin irritation. Therefore it cannot be excluded that the outcome is an underestimation of the actual skin sensitisation potential of the substance.

In order to substantiate the findings of the GPMT with alkylampho(mono)acetate C12, *in silico* (QSAR) predictions on the skin sensitizing potential for 4 representative C12-alkyl derivatives (mono-amphoacetate 1 and 2, and di-amphoacetate 1 and 2) and 3 potential minor constituents (by-products) present in alkylamphoacetate C12 were performed with the DEREK NEXUS

program (v 5.0.2). DEREK NEXUS is a knowledge-based system that contains more than 80 alerts specific to skin sensitization and it is recommended for KE 1 predictions in OECD TG 497. The rules are based on the presence of specific sub-structures, or chemical classes related to potential mechanisms for skin sensitization. Eight representative mono- and diacetate structures with the outer end alkyl chains, i.e. 4 representative C8-alkyl derivatives and 4 representative C18-alkyl derivatives, were investigated with DEREK NEXUS (see Appendix III: Chemical structures evaluated by DEREK NEXUS (version 5.0.2) for structures investigated). For all of these structures DEREK NEXUS did not find any sub-structures in its database that triggered an alert for sensitisation potential.

In the conclusions of a safety assessment of Cocoamphoacetates published in the Journal of the American College of Toxicology in 1990)., cocoampho(mono)acetate and cocoamphodiacetate were reported to be, at concentrations of 10% and 5% respectively, neither a skin irritant nor skin sensitizer based on a human repeated insult patch test (HRIPT) with 141 subjects. Although these results were published in a peer-reviewed journal, which can be regarded to be scientific expert judgement, the data are not found reliable due to the fact that no information was given on the exact study outline, identity and purity of the test substance and that no results were included in the report. Cocoamphoacetates were later re-assessed by the cosmetic ingredient review (CIR) expert panel in in 2005/2006. The Panel reviewed newly available studies and confirmed the safety of Cocoamphoacetate and Cocoamphodiacetate at concentrations as high as 18 and 12% respectively.

In order to conclude on the skin sensitising potential of the Alkylamphoacetates, the following aspects are considered to be crucial:

- For two analogues (alkylampho(mono)acetates C8-18 and alkylampho(mono)acetates C12), it has been shown that a GPMT study results in a negative outcome.
- DEREK NEXUS did not find any substructures in its database that triggered an alert for skin sensitisation potential for the chemical structures present in the amphoacetates, indicating both mono- and diacetates do not have functional groups in them that are known to induce skin sensitization.
- In spite of wide spread use of the alkylamphoacetates, no reports on cases of skin sensitization in the public domain or in company-owned data can be found.
- All major constituents of alkylamphoacetates C8-C18, alkylamphoacetates C12-14 and alkylamphoacetates C12 are ionized at all realistic pH levels; based on this dermal absorption is expected to be negligible or very limited (WHO, 2006) and thus sensitization events are unlikely to occur (Basketter, 2008).

Based on this information, it is considered scientifically valid to read across the data on skin sensitization potential to all other alkylamphoacetate analogues within the category.

Genotoxicity

The substances alkylampho(di)acetates C8-C18, alkylampho(di)acetates C12-C14 and alkylampho(mono)acetates C12 tested negative (with and without metabolic activation) in the *Salmonella typhimurium* reverse mutation assay conducted in accordance with OECD 471. Alkylampho(di)acetates C12-14 and alkylampho(mono)acetates C12 tested also negative (with and without metabolic activation) in the *Escherichia coli* reverse mutation assay (OECD 471).

Only alkylamphoacetates C8-C18 (monoacetate) was tested in an *in vitro* mouse lymphoma assay (OECD 476) and was shown to be negative (with and without metabolic activation).

Alkylamphoacetates C12-C14 and alkylamphoacetates C12 are mainly comprised of C12 and C14 mono- and diacetates similar to the alkylamphoacetate C8-C18 tested substance. However amphoacetates C8-C18 cover a wider distribution of chain lengths than C12-C14 or C12. Fatty alkyl chains are not electrophilic functional groups and do not exert any potential for DNA- or protein-binding; but their length may impact molecular weight and log Kow, which in turn may affect passive permeation through lipid bilayers and into mammalian cell nuclei. While cell membrane permeability is a direct function of log Kow and an indirect function of molecular size/weight, a far higher dependence on the octanol:water partition coefficient than on the latter is usually observed (Rowland, 2011). All analogues contain C12 and/or C14 alkyl chains. As C8-18 has C8 and C10 as only minor constituents, while C16 and C18 are major constituents next to C12 and C14, C8-18 can be considered a worst case for access to a cell. Conversely, diacetate forms are both bigger and more hydrophilic molecules than their monoacetate counterparts, thus they can be assumed to have lower capacity to reach the nuclei of mammalian cells than smaller and more lipophilic monoacetates. The source substance contains mainly monoacetate structures and in similar or higher ratios than the target substances. It is thus concluded that the data on substances with longer alkyl chains and a higher or similar monoacetate to diacetate ratio [i.e. alkylampho(mono)acetates C8-C18] can be used to predict the effects of alkylamphoacetates with shorter chain lengths and lower or similar monoacetate to diacetate ratios [i.e. alkylampho(di)acetates C8-C18, alkylamphoacetates C12-C14 (mono- and diacetate forms) and alkylamphoacetates C12 (mono- and diacetate forms)]. In addition, for each of the four possible C12 alkyl amphoacetate constituents (mono- or diacetate forms 1 and 2), OECD QSAR Toolbox v4.3 predicts a negative outcome of the *in vitro* mutation study in mammalian cells (Kavanagh, 2021). I*n silico* predictions using DEREK Nexus version 5.0.2 and VEGA QSAR have also been carried out. A DEREK report (Barentsen, 2017a, Barentsen, 2017b) concluded that the representative constituents, mono- and diacetate structures with the outer end alkyl chains, i.e. C8 and C18 and the minor constituent of C8-18 amphoacetate with oleic acid the monoacetate (see Appendix II for structures investigated) , do not show a potential for either mutagenicity or carcinogenicity. The VEGA QSAR software was run with two representatives of C12 Amphoacetates (C12 Alkyl amphoacetate Form 1 Monoacetate and C12 Alkyl amphoacetate Form 2 Diacetate), the VEGA models confirm absence of genotoxic activity for all tested constituents (Kavanagh, 2021).

Moreover, retrospective comparisons have shown a comparable (Kirkland, 2005; Matthews, 2006) if not better (Zeiger, 1998) performance of the Ames test in comparison to *in vitro* mutagenicity assays in mammalian cells for the prediction of rodent carcinogens. The added value of the *in vitro* mammalian gene mutation assay in the absence of positive findings with an Ames test and an *in vitro* assay in mammalian cells to identify numerical and/or structural chromosomal aberrations has been questioned "*because the bacterial gene mutation test detects all relevant modes of action specifically leading to gene mutations. Moreover, most of the substances positive in mammalian gene mutation tests also induce clastogenic effects*" (Pfuhler, 2005). The combination of the Ames test with an *in vitro* chromosomal aberration assay or the *in vitro* micronucleus test are sufficiently sensitive to predict *in vivo* genotoxins, with the *in vitro* mammalian gene mutation assay only increasing the sensitivity of the test battery from 78 % to 79 % (Kirkland, 2011). In Chapter R7.a (Endpoint-specific guidance) (ECHA, 2017b), it is acknowledged that other regulatory frameworks do not require *in vitro* mammalian gene mutation assays to confirm the absence of mutagenic potential. A reason why this approach has not been adopted for EU REACH is not provided in this guidance document.

The applicant acknowledges the information requirement for *in vitro* mammalian gene mutation for regulatory purposes, but believes that the negative outcomes in the two *in vitro* genotoxicity assays confirm that read-across from the source substance Amphoacetate C8-C18

to the target substances Amphoacetate C12 and C12-C14, together with the supporting information from several QSARs on genotoxic potential, fulfils the information requirements for *in vitro* mammalian gene mutation is sufficiently justified.

The substances alkylamphoacetates C8-C18 (monoacetate form), alkylamphoacetates C12-C14 (diacetate form) and alkylamphoacetates C12 (monoacetate form)exhibited no clastogenic effects (with and without metabolic activation) when tested in the *in vitro* chromosome aberration assay (OECD 473). In the studies with alkylamphoacetates C8-C18 and C12-C14, a dose-dependent increase in the number of polyploid cells was noted with and without the use of a metabolic activation system.

In order to determine if the positive responses seen *in vitro* for Amphoacetates C8-C18 and C12-14 were indicative for *in vivo* genotoxicity, a mouse bone marrow cytogenetic assay (OECD 475) was performed with C8-18 alkylampho(mono)acetates. Male mice (5/group) were exposed orally (gavage) to 500, 1000 or 2000 mg/kg bw/day and bone marrow was sampled 12-18 (all doses, vehicle control group and positive control group (treated with cyclophosphamide) or 36-44 (highest dose only) hours after dosing. No mortality occurred, no clinical signs were noted in any of the mice. The number of cells with chromosome aberrations found in the vehicle control animals was within the laboratory historical control data range. The positive control animals treated with cyclophosphamide induced a statistically significant increase in the number of cells with chromosome aberrations, indicating that the test conditions were adequate. C8-18 Alkylampho(mono)acetates did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations, at both sampling times. Based on these results it is concluded that C8-18 alkylampho(mono)acetates does not disturb mitotic processes and cell cycle progression and does not induce numerical chromosome aberrations *in vivo*.

Since C8-C18 contains longer alkyl chains and a higher monoacetate to diacetate ratio than ampho(di)acetates C12-C14 (this argument was explained more profoundly on the previous page for gene mutation test in mammalian cells), it is considered appropriate to read-across the available genotoxicity data to cover this substance and conclude that Ampho(di)acetates C12-C14 is negative for disturbing mitotic processes and cell cycle progression and inducing numerical chromosome aberrations in vivo.

Toxicokinetics

An assessment of the toxicokinetic behaviour of alkylamphoacetates C8-C18, alkylamphoacetates C12-14 and alkylamphoacetates C12 to the extent that can be derived from the relevant available information has been performed in accordance with ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7c (May 2008). Oral, dermal and inhalation absorption rates of 100%, 10% and 100% were estimated for each of the routes, respectively. Slight variations observed in the liver weights and/or clinical chemistry in the 28-day repeated dose toxicity study (OECD 407) with alkylampho(mono)acetates C8-C18 and the 90-day repeated dose (OECD 408) studies with alkylampho(di)acetates C12-C14 and alkylampho(di)acetates C8-C18, provided evidence of absorption by the oral route. The dermal absorption rate of 10% is supported by experimental data on a structurally related amphoteric surfactant, dodecylamidopropylbetaine (CAS# 4292-10-8) showing a dermal absorption of less than 3.5% in Wistar rats (HERA 2005). All major constituents of alkylamphoacetates C8-C18, alkylamphoacetates C12-14 and alkylamphoacetates C12 are ionized at all physiological pH levels due to their amphoteric nature, which influences the ability to cross hydrophobic membrane barriers such as skin (WHO, 2006); based on this, 10 % dermal absorption can be considered a highly conservative assumption.

Alkyl amphoacetates consist of hydrophilic constituents, with predicted Log Kow ranges of -3.58 to +1.33 (monoacetates), and -6.15 to -0.75 (diacetates), respectively (EPIsuite Kowwin v1.67). The molecular weight range of monoacetates is 310-394 g/mol, while for diacetates it is 390-474 g/mol. As discussed in the genotoxicity section, the log Kow increases with the molecular weight. It can be safely assumed that amphoacetates with a higher alkyl chain length have a similar or higher bioavailability than that of the shorter alkyl chain lengths, thus alkyl amphoacetates C8-C18 can be considered a worst-case. The values also indicate that diacetates, which are both bigger and more hydrophilic molecules, can be assumed to have lower bioavailability compared to the smaller and more lipophilic monoacetates.

The alkylamphoacetates may be distributed throughout the body based on the relatively low molecular weight. It may be expected that the amphoacetates undergo metabolic transformation and/or conjugation prior to elimination, although no empirical data is available to substantiate this.

Repeated dose toxicity

Two sub-acute repeated dose toxicity studies combined with screening for reproduction and developmental effects were performed according to OECD 422 with two representative alkylamphoacetates C8-C18. One study was conducted with C8-C18 alkylampho(mono)acetates whilst the second study was conducted with C8-18 alkylampho(di)acetates. The rationale to perform the test with both forms was to investigate whether the structural and compositional difference in chemistry exhibited an impact on systemic and reproductive/developmental. Treatment (oral, gavage) of test animals with C8-18 alkylampho(mono)acetates was associated with a few minor non-adverse changes at the highest dose group i.e. slight salivation in both sexes, lower food consumption in females in the last week of gestation and during lactation, and lower activated partial thromboplastin time in males. Serum levels of T4 in males were not affected by treatment (not measured in females or pups), and no changes in thyroid weight or histopathology were observed. No treatment-related or toxicologically relevant changes were noted in the other parameters investigated in this study. Based on the absence of adverse effects up to 1000 mg/kg bw/day, a parental No Observed Adverse Effect Level (NOAEL) C8-18 alkylampho(mono)acetates of 1000 mg/kg bw/day was established. For C8-18 alkylampho(di)acetates, there was a high mortality in the females (4/10) and one premature death in the males at 1000 mg/kg bw/day. These deaths were concluded to be related to gavage errors (test item administration-related regurgitation) and thus secondary to the test item (possibly triggered by physical/chemical properties of the test-item solution in combination with the route of administration). Serum levels of T4 in males were not affected by treatment (not measured in females or pups). No changes in thyroid weight were observed, follicular cell hypertrophy of the thyroid gland was found in males at the 1000 mg/kg bw/day dose group. Similar findings were observed at the 300 mg/kg bw/day dose group but at a slightly lower severity. These findings were considered to be non-adverse based on its low severity (up to mild) and absence of any additional degenerative, inflammatory or proliferative findings and changes in T4 hormone levels. Due to the high mortality caused by the gavage-related incidents, the high dose group (1000 mg/kg bw/day) could not be assessed and the parental No Observed Adverse Effect Level (NOAEL) of 300 mg/kg bw/day was established for C8-18 alkylampho(di)acetates.

To follow-up the results seen with C8-18 alkylampho(di)acetates, a sub-chronic 90-day toxicity study (oral, gavage) was performed according to OECD guideline 408. In the sub-chronic study, the dosing volume (1.895 ml/kg/day) was decreased compared to the 28 day sub-acute study (5 ml/kg/d) in order to minimize risk of regurgitation. In this study no mortality was seen

at all doses (100, 300 and 1000 mg/kg bw/d), which suggests the mortalities which occurred in the high dose group in the 28-day sub-acute study were indeed likely secondary to the test item. Lower TSH values in all test item-treated male groups and lower T4 values in high-dose males were observed, achieving a level of statistical significance when compared to controls. The values remained however within the Historical Control Data Range. In the absence of a dose response relationship, effects on thyroid weight or macroscopic/microscopic correlates; these values were considered to be of no toxicological significance. No toxicologically significant changes were noted in any of the parameters investigated in this study. No adverse effects were seen on reproduction parameters (estrous cycle length, spermatogenesis, weight, appearance and histopathology of reproduction organs). Based on these results, the no observed adverse effect level (NOAEL) for sub-chronic exposure was found to be 1000 mg/kg bw/day.

Furthermore, a historical 28-day study is available for alkylampho(mono)acetates C8-C18. Following repeated oral (gavage) administration to rats for 28 days, the NOAEL was found to be 92.5 mg/kg bw/day (active substance basis equivalent to 250 mg/kg bw/d test item), based on a dose-dependent effect on liver weight (without histopathological changes). At 92.5, 185 and 370 mg/kg bw/day, increase in absolute and relative liver weights was noted (+10, +21 and +21% (absolute), +4, +9 and +12% (relative to body weight) compared to controls, respectively). These effects were not seen in males and there were no other toxicologically relevant findings in both sexes up to and including 370 mg/kg bw/day (active substance basis equivalent to 1000 mg/kg bw/d active substance basis). As the parameters included in this study are limited and doses were not analytically confirmed, this study is used as supporting evidence only.

To get a better understanding of the effect of alkyl chain length (distribution) on toxicity, a sub-chronic 90-day toxicity study (oral, gavage) has been performed according to OECD guideline 408 with C12-C14 alkylampho(di)acetates. The clinical signs seen were non-adverse and comparable with those found in the 90-day study on amphoacetates C8-18 (diacetate form). Non-adverse squamous cell hyperplasia in the stomach (with hyperkeratosis) was observed in females at 300 and 1000 mg/kg bw/day, while non-adverse goblet cell hyperplasia (without cellular atypia , inflammatory or degenerative changes) of the rectum was observed in a few high-dose males, these tissues fully recovered and were consistent with a local reaction to irritation (presumptively by the test material), these effects were not seen in the 90-day study on ampho(di)acetates C8-18 (although in this study histopathological examination of stomach and rectum was only conducted in the control an high dose groups) but similar effects were seen in dams in a OECD 414 study with alkylampho(mono)acetates C12. Haematological findings comprised decreased red blood cell count and red blood cell distribution width and increased mean corpuscular volume and mean corpuscular haemoglobin in males at 1000 mg/kg bw/day, based on the absence of a histopathological correlation and/or full recovery, these changes were considered to be non-adverse; the 90-day study on amphoacetates C8-18 (diacetate form) also reports haematological changes in males (increases in platelet numbers and prothrombin time) however in this study they were considered non-test item related. Non-adverse (reversible) clinical chemistry findings (increase in triglyceride concentration) in males were observed at 1000 mg/kg bw/day, in the 90-day study on amphoacetates C8-18 (diacetate form), and non-adverse increases were observed in alkaline phosphatase cholesterol (HDL and LDL). Hormone analysis showed no effect on T3 and TSH levels in males and females, an increased T4 levels was observed in high-dose males, which recovered at the end of the recovery period. Increased T4 levels were also seen in females at 100 and 1000 mg/kg bw/day, but these changes remained within historical control range, were reversible and not accompanied by changes in thyroid weight or histopathology; changes in T4 levels were thus considered non-adverse. Finally higher kidney and liver weight was noted in females at 1000

mg/kg bw/day; increases in liver weight were also observed in the 28-day study that is available for alkylampho(mono)acetates C8-C18. No test material-related changes were noted in any of the remaining parameters investigated in this study (i.e., mortality, body weight, food consumption, ophthalmoscopy, coagulation and macroscopic pathology). Based on these results, the no observed adverse effect level (NOAEL) for sub-chronic exposure was found to be 1000 mg/kg bw/day.

As amphoacetates C12 is mainly comprised of C12 mono- ,and the diacetate of the alkylamphoacetates C8-C18 was less or just as toxic as the monoacetate of the alkylamphoacetates C8-C18, the alkylamphomonoacetate C12 can be considered similar to the tested C12-C14 alkylampho(di)acetate substance, and it is considered appropriate and justified to read-across the 90-day oral NOAEL of 1000 mg/kg bw/day to this substance. In order to substantiate the read across hypothesis for the alkylamphoacetates, predictive toxicity assessments of C8-monoacetates and diacetates and of C18-monoacetates and diacetates were performed using the *in silico* model DEREK NEXUS. In this assessment version 5.0.2 of DEREK NEXUS was used. The exact chemical structures assessed are included in Appendix III: Chemical structures evaluated by DEREK NEXUS (version 5.0.2). DEREK analysis predicts no toxicity for any endpoint present in the DEREK database that is relevant for humans for “C8- monoacetate1”, “C8-diacetate1” and “C8-diacetate2”, or for “C18-monoacetate1”, “C18-diacetate1” and “C18-diacetate2”. Also for the minor constituent of alkylamphoacetates C8-C18 with oleic acid, “C18unsat monoacetate2” no toxicity was predicted. Since the structures assessed have the same functional groups present in all analogues, it can be concluded that none of the analogues exhibits any structural alerts for adverse toxic effects. This *in silico* (QSAR) assessment is in line with the general outcome of repeated dose toxicity and reproductive/developmental screening on C8-C18 alkylamphoacetates and serves as important supporting information to justify the category and read-across approach employed.

Reproductive/developmental toxicity

As discussed above, two screening level studies for reproduction and developmental effects were performed in accordance with OECD 422 using two representative forms of alkylamphoacetates C8-C18 (high diacetate form and predominantly monoacetate form). In both studies, no reproductive toxicity effects were observed up to the highest dose level tested (1000 mg/kg bw/day). No treatment-related changes were noted in the reproductive parameters examined in both studies (i.e. mating and fertility indices, precoital time, number of implantation sites, oestrous cycle, spermatogenic profiling, and histopathological examination of reproductive organs). For the study with alkylampho(di)acetates C8-C18, the reproduction parameters were assessed for all groups, but the developmental effects could not be determined in the high dose group due to excessive mortalities in the dams caused by secondary effects via regurgitation.

In neither of the OECD 422 studies was any adverse developmental toxicity effect observed up to the highest dose level tested (1000 mg/kg bw/day or 300 mg/kg bw/day). No treatment-related changes were noted in the developmental parameters investigated in the studies (i.e. gestation, viability and lactation indices, duration of gestation, parturition, sex ratio, maternal care and early postnatal pup development consisting of mortality, clinical signs, body weight, anogenital distance (PND 1), areola/nipple retention (PND 13 males), and macroscopy).

Furthermore, no effects on body weight, macroscopy or histopathology were seen in the sub-chronic 90-day study on male and female reproductive organs performed with alkylampho(di)acetates C8-C18. Stage dependent qualitative evaluation of spermatogenesis in the testes was performed. The testes revealed normal progression of the spermatogenic cycle

and the expected cell associations and proportions in the various stages of spermatogenesis were present.

A summary of thyroid-related effects is given in Appendix IV. Given the fact that all mean hormone levels measured in exposed animals were within Historical Control Data Range (HCDR) and the large natural variability in thyroid hormone/TSH measurements (Li *et al.* 2019; Beekhuijzen *et al.* 2019), and effects were contradictory for T4 in different studies, these differences are likely caused by chance, rather than the result of a toxicological effect of the test item. Thyroid weight and histopathology should be evaluated in conjunction with changes in serum thyroid hormones (both T3 and T4) and TSH to allow correct interpretation of changes (Li *et al.* 2019), especially since the thyroid gland of rodents is much more sensitive than that of humans to loss of colloid and induction of hypertrophy and hyperplasia from a TSH increase. Following this argumentation, the fact that no effects on thyroid weight or histopathology in combination with an effect in hormone levels were observed in any of the OECD414 or OECD408 studies indicates that there were no adverse effects on the thyroid system.

Taken together, there are no indications that alkylamphoacetates C8-C18 have an adverse effect on reproduction.

Development toxicity was tested for all alkylamphoacetate analogues in accordance with OECD test guideline 414, details are provided in table 6 and appendix V.

6. DATA MATRIX

The key data for the analogues are presented in Table 4-6. All available studies, both key and supporting, are included in the tables in Appendix III, IV and V. The use of read across to meet a particular endpoint requirement is indicated in the tables by 'RA'. QSAR calculated values for physico-chemical data are presented for some endpoints next to the measured values to indicate that the difference between measured and calculated is in the same range of the variation between substances.

If for a particular endpoint no reliable data were available, this is indicated by not determined "n.d." in the tables. If filling of an endpoint is not relevant for one of the analogues related to its tonnage band, this is indicated with not applicable ("N.A.").

Endpoints with a waiving statement not relevant for read-across, such as flash point, stability in organic solvents, dissociation constant and viscosity, have not been included.

charles river

Table 4 Data Matrix, Physico-chemical Properties for the Alkylamphoacetates

REACH	Endpoint	Amphoacetates C8-C18	Amphoacetates C12-C14	Amphoacetates C12
7.1	State of the substance at ambient conditions	Pasty orange solid (containing approximately 6% residual water)	Light yellow crystals (containing approximately 2% residual water)	Light yellow powder with lumps (containing approximately 4% residual water)
7.2	Melting/freezing point [ºC]	The substance has no melting temperature, substance decomposes starting at 160°C	The substance has no melting temperature, substance decomposes starting at 150°C	40 (see comment in text)
7.3	Boiling point [ºC]	The substance has no boiling temperature, decomposition starts at 160 °C	The substance has no boiling temperature, decomposition starts at 150 °C	The substance has no boiling temperature, decomposition starts at 75 °C
7.4	Relative density at 20 ºC	**RA from C12-C14:** 1.33	1.33	**RA from C12-C14:** 1.33
7.5	Vapour pressure at 20 ºC [Pa]	$1.4*10^{-7}$ (monoacetate); $< 8.4*10^{-7}$ (diacetate)	$1.8*10^{-8}$ - $1.3*10^{-6}$ (monoacetate); $2.6*10^{-6}$ (diacetate)	$1.5*10^{-7}$
7.6	Surface tension [mN/m]	34 (concentration: 0.5 g/L) Surface active	35.4 (concentration: 1 g/L) Surface active	31.9 (concentration: 1 g/L) Surface active
7.7	Water solubility at 20 ºC	> 1000 g/L (bulk)	Between 206.4 g/L and 1032 g/L (bulk)	> 1000 g/L (bulk)
	Critical Micelle Concentration [mg solids/L]	160 (monoacetate); 158 (diacetate)	239 (monoacetate); 262 (di-acetate)	718
7.8	Partition coefficient n-octanol/water [log Pow]	-1	-1	-1
	n-octanol solubility (visually determined) [g/L]	< 0.082	< 1.1	< 1.08
7.9	Flash point [ºC]	Aqueous solutions have no flashpoint A 50% aqueous solution has a boiling temperature of approximately 105 ºC	Aqueous solutions have no flashpoint	Aqueous solutions have no flashpoint. A 39% aqueous solution has a boiling temperature of approximately 94 ºC

REACH	Endpoint	Amphoacetates C8-C18	Amphoacetates C12-C14	Amphoacetates C12
7.10	Flammability (in contact with water and pyrophoric properties)	The substance does not ignite spontaneously in contact with water and has no pyrophoric properties (based on the molecular structure of the constituents of the substance)	The substance does not ignite spontaneously in contact with water and has no pyrophoric properties (based on the molecular structure of the constituents of the substance)	The substance does not ignite spontaneously in contact with water and has no pyrophoric properties (based on the molecular structure of the constituents of the substance)
7.11	Explosive properties (based on the molecular structure of the constituents of the substance)	Negative	Negative	Negative
7.12	Self-ignition temperature	**RA from C12-C14:** not self-ignitable	Not self-ignitable	**RA from C12-C14:** not self-ignitable
7.13	Oxidising properties (based on the molecular structure of the constituents of the substance)	Negative	Negative	Negative
7.14	Granulometry	Waived: The substance is marketed and used in aqueous solutions (non-granular form)	Waived: The substance is marketed and used in aqueous solutions (non-granular form)	Waived: The substance is marketed and used in aqueous solutions (non-granular form)
7.15	Stability in organic solvents	Waived: Not a critical property	Waived: Not a critical property	Waived: Not a critical property
7.16	Dissociation constant	Waived: Test technically not feasible	Waived: Test technically not feasible	Waived: Test technically not feasible
7.17	Viscosity	Waived: Substance is a solid	Waived: Substance is a solid	Waived: Substance is a solid

Table 5 Data Matrix, Environmental Fate/Toxicity for the Alkylamphoacetates

REACH	Endpoint	Amphoacetates C8-C18	Amphoacetates C12-C14	Amphoacetates C12
9.2.1.1	Ready biodegradability[12]	Readily biodegradable under both aerobic (OECD 301 A, D, E and F) and anaerobic conditions (OECD 311)	**RA:** Readily biodegradable under aerobic conditions	Readily biodegradable under aerobic conditions (OECD 301B)
9.2.2.1	Hydrolysis as function of pH	Waived: the substance is readily biodegradable (study is scientifically not necessary)	Waived: the substance is readily biodegradable (study is scientifically not necessary)	Waived: the substance is readily biodegradable (study is scientifically not necessary)
9.3.1	Adsorption/desorption [log Koc]	The screening study is technically not feasible due to the surface active nature of the substance (OECD 121) 3.16 L/kg, calculated using the QSAR: $Koc = \frac{10.47 \cdot Kow^{0.52}}{1000}$ (with Koc in m^3/kg)	The screening study is technically not feasible due to the surface active nature of the substance (OECD 121) 3.16 L/kg, calculated using the QSAR: $Koc = \frac{10.47 \cdot Kow^{0.52}}{1000}$ (with Koc in m^3/kg)	The screening study is technically not feasible due to the surface active nature of the substance (OECD 121) 3.16 L/kg, calculated using the QSAR: $Koc = \frac{10.47 \cdot Kow^{0.52}}{1000}$ (with Koc in m^3/kg)
9.3.2	Bioaccumulation in aquatic species	Waived: low potential for bioaccumulation based on log Pow ≤ 3	Waived: low potential for bioaccumulation based on log Pow ≤ 3	Waived: low potential for bioaccumulation based on log Pow ≤ 3
9.1.1	Acute toxicity to *Daphnia*, 48h-EC50 [mg/L, based on solid content]	EC50 = 25.4; NOEC = 9.4 (monoacetate) (OECD 202)	EC50 = 67.3; NOEC = 45.5 (monoacetate) (OECD 202)	EC50 = 89 (monoacetate) (OECD 202)
9.1.2	Growth inhibition algae, 72h-ErC50, NOErC [mg/L, based on solid content]	72h-ErC50 = 13 72h-NOErC = 0.87 72h-ErC10 = 1.7 (monoacetate) (OECD 201)	**RA from C8-C18:** 72h-ErC50 = 13 72h-NOErC = 0.87 72h-ErC10 = 1.7 (OECD 201)	72h-ErL50 = 44 (monoacetate) (OECD 201)

[12] In accordance with the REACH Guidance and the OECD Guidelines, the 10-day window should not be applied to interpret the results of the tests. The substances consist of homologues of surfactants composed of alkyl chains of varying length. It is anticipated that a sequential biodegradation of the individual structures takes place.

REACH	Endpoint	Amphoacetates C8-C18	Amphoacetates C12-C14	Amphoacetates C12
9.1.3	Acute toxicity to fish, LC50 [mg/L, based on solid content]	96h-LC50: 4.0 (monoacetate) (OECD 203)	**RA from C8-C18:** 96h-LC50: 4.0 (OECD 203)	96h-LC50: 28 (monoacetate) (OECD 203)
9.1.4	Activated sludge respiration inhibition, EC50 [mg/L, based on solid content]	**RA from C12:** NOEC: 560 (OECD 209)	**RA from C12:** NOEC: 560 (OECD 209)	NOEC: 560 (monoacetate) (OECD 209)
9.1.5	Long-term toxicity to *Daphnia*, NOEC [mg/L, based on solid content]	$NOEC_{repro}$ = 3.7 $EC10_{repro}$ = >3.7 $NOEC_{growth\ reduction}$ = 1.6 $EC10_{growth\ reduction}$ = >3.7 (monoacetate) (OECD 211)	**RA from C8-C18:** $NOEC_{repro}$ = 3.7 $EC10_{repro}$ = >3.7 $NOEC_{growth\ reduction}$ = 1.6 $EC10_{growth\ reduction}$ = >3.7 (OECD 211)	**RA from C8-C18:** $NOEC_{repro}$ = 3.7 $EC10_{repro}$ = >3.7 $NOEC_{growth\ reduction}$ = 1.6 $EC10_{growth\ reduction}$ = >3.7 (monoacetate) (OECD 211)
9.1.6	Long-term toxicity to fish, NOEC [mg/L, based on solid content]	$NOEC_{growth}$ = 0.61 $EC10_{length}$ = 0.79 $EC10_{weight}$ = 0.80 (monoacetate) (OECD 210)	**RA from C8-C18:** $NOEC_{growth}$ = 0.61 $EC10_{length}$ = 0.79 $EC10_{weight}$ = 0.80 (OECD 210)	**RA from C8-C18:** $NOEC_{growth}$ = 0.61 $EC10_{length}$ = 0.79 $EC10_{weight}$ = 0.80 (monoacetate) (OECD 210)

Table 6 Data Matrix, Toxicological endpoints for the Alkylamphoacteates

REACH	Endpoint	Amphoacetates C8-C18	Amphoacetates C12-C14	Amphoacetates C12
8.5.1	Acute oral, LD50 [mg/kg bw]	>5000 (equivalent or similar to OECD 401; monoacetate)Diacetate: no data	>5939 (equivalent or similar to OECD 401; surrogate monoacetate) 7935 (equivalent or similar to OECD 401; diacetate)	3422 (equivalent or similar to OECD 401; monoacetate)
8.5.2	Acute inhalation, LC50 [mg/L]	waived	waived	waived
8.5.3	Acute dermal, LD50 [mg/kg bw]	> 2612 (OECD 402/EU Method B.3; monoacetate)	**RA from C8-C18:** > 2612	**RA from C8-C18:** > 2612
8.1	Skin irritation/corrosion	Not classified as irritating to skin (similar or according to OECD 404/EU Method B.4; monoacetate and surrogate of unknown mono-/diacetate ratio) Diacetate: no data	**RA:** not classified as irritating to skin	Not classified as irritating to skin (OECD 404/EU Method B.4; monoacetate)
8.2	Eye irritation	Classified as causing irreversible effects on the eye (Category 1), based on worst-case results (variable results were observed) (OECD 405/EU Method B.5; mono and diacetate form)	**RA from C12:** Classified as irritating to eyes (Category 2) [Conc. >16%]	Classified as irritating to eyes (Category 2) [Conc. >16%] (OECD 405/EU Method B.5; monoacetate)
8.3	Skin sensitisation	Not classified (OECD 406/EU Method B.6; monoacetate)	**RA from C8-C18 and/or C12:** not classified	Not classified (OECD 406; monoacetate)
8.4.1	In vitro gene mutation in bacteria (*Salmonella typhimurium* reverse mutation assay)	Not mutagenic with/without S9 (similar to OECD 471; diacetate)	Not mutagenic with/without S9 (OECD 471; diacetate)	Not mutagenic with/without S9 (OECD 471; monoacetate)
	In vitro gene mutation in bacteria (*Escherichia coli* reverse mutation assay)	**RA from C12-C14 and/or C12:** not mutagenic with/without S9	Not mutagenic with/without S9 (OECD 471; diacetate)	Not mutagenic with/without S9 (OECD 471; monoacetate)

REACH	Endpoint	Amphoacetates C8-C18	Amphoacetates C12-C14	Amphoacetates C12
8.4.2	*In vitro* cytogenicity in mammalian cells	Not clastogenic with/without S9 (OECD 473/EU Method B.10; monoacetate)	Not clastogenic with/without S9 (OECD 473; diacetate)	Not clastogenic with/without S9 (OECD 473/EU Method B.10; monoacetate)
8.4.3.	*In vitro* gene mutation in mammalian cells	Not mutagenic with/without S9 (OECD 476/EU Method B.17; monoacetate)	**RA from C8-C18:** not mutagenic with/without S9	**RA from C8-C18:** not mutagenic with/without S9
	In vivo mammalian bone marrow chromosome aberration test	Negative (OECD 475; monoacetate)	**RA from C8-C18:** Negative	N.A.
8.6.1	28-day repeated dose toxicity	NOAEL = 1000 mg/kg bw/day (OECD 422; monoacetate); NOAEL ≥ 300 mg/kg bw/day[13] (OECD 422; diacetate);	No data	No data
8.6.2	90-day repeated dose toxicity	NOAEL = 1000 mg/kg bw/day (OECD 408; diacetate)	1000 mg/kg bw/day (OECD 408; diacetate)	**RA from C12-C14:** 1000 mg/kg bw/day (OECD 408)
8.7.1	Screening reproductive/developmental toxicity	$NOAEL_{repro/dev}$ = 1000 mg/kg bw/day (OECD 422, monoacetate); $NOAEL_{repro}$ = 1000 mg/kg bw/day; $NOAEL_{development}$ = 300 mg/kg bw/day[12] (OECD 422, diacetate)	Waived: the study does not need to be conducted because a pre-natal developmental toxicity study is available	Waived: the study does not need to be conducted because a pre-natal developmental toxicity study is available
8.7.2	Pre-natal developmental toxicity, one species	$NOAEL_{parental}$ = 1000 mg/kg bw/day; $LOAEL_{development}$ = 100 mg/kg bw/day (OECD 414, rat, diacetate)	$NOAEL_{parental}$ = 1000 mg/kg bw/day; $NOAEL_{development}$ = 1000 mg/kg bw/day (OECD 414, rat, diacetate)	$NOAEL_{parental}$ = 1000 mg/kg bw/day; $NOAEL_{development}$ = 1000 mg/kg bw/day (OECD 414, rat, monoacetate)
	Pre-natal developmental	Test proposal (OECD 414, rabbit, diacetate)	**RA from C8-C18:** test with analogue proposed	**RA from C8-C18:** test with analogue proposed

[13] Nb: High dose group excluded from study due to mortality related to regurgitation (secondary effect)

REACH	Endpoint	Amphoacetates C8-C18	Amphoacetates C12-C14	Amphoacetates C12
	toxicity, second species			
8.7.3	Extended One Generation Toxicity Study	Test proposal (OECD 443, rat, diacetate)	N.A.	**RA:** test with analogue proposed
8.8.1	Toxicokinetic assessment	For risk assessment purposes: oral absorption 100%, inhalation absorption 100% and dermal absorption 10% (expert statement)	**RA:** For risk assessment purposes: oral absorption 100%, inhalation absorption 100% and dermal absorption 10%	**RA:** For risk assessment purposes: oral absorption 100%, inhalation absorption 100% and dermal absorption 10%

7. RETENTION OF RECORDS

The final report generated by Charles River from this study will be transferred to a Charles River archive no later than the date of final report issue.

8. CLASSIFICATION AND LABELLING (C&L) AND PBT PROPERTIES

Classification and labelling

Based on the outcome of the available studies the analogues have been assessed to require the following classification and labelling according to Regulation (EC) No 1272/2008 including related amendments (e.g., the Commission Regulations (EU) No 286/2011, No 618/2012 and No 487/2013):

Table 7 Classification and labelling for the Alkylamphoacetates

Substance	Classification	Labelling
Amphoacetates C8-C18	Classified as causing irreversible effects on the eye (Category 1), based on worst-case results (variable results were observed) Aquatic Chronic 3	Pictogram: GHS05 Signal word: Danger Hazard statement: H318, H412 Precautionary Statements: P280; P305+P351+P338; P310
Amphoacetates C12-C14	Read Across from Amphoacetates C12: Classified as irritating to eyes (Category 2) Read Across from Amphoacetates C8-C18: Aquatic Chronic 3	Pictogram: GHS07 Signal word: Warning Hazard statement: H319, H412 Precautionary Statements: P280; P305+P351+P338; P337+P313 Specific concentration limits: Eye Irrit. 2: C >16%
Amphoacetates C12	Classified as irritating to eyes (Category 2) Read Across from Amphoacetates C8-C18: Aquatic Chronic 3	Pictogram: GHS07 Signal word: Warning Hazard statement: H319, H412 Precautionary Statements: P280; P305+P351+P338; P337+P313 Specific concentration limits: Eye Irrit. 2: C >16%

PBT/vPvB properties

As the available data does not allow a definitive conclusion on the PBT or vPvB properties of alkylamphoacetates, the screening criteria as mentioned in the ECHA Guidance on information requirements and chemical safety assessment Chapter R.11: PBT Assessment (Table R. 11-1) are used to decide whether the substances potentially fulfil the PBT or vPvB criteria.

As all alkylamphoacetates are concluded to be readily biodegradable and have a log Kow lower than 4.5, the substances do not fulfil the screening criteria for classification as P, vP, B or vB.

The alkylamphoacetate substances have been concluded to exhibit acute L(E)C50 values >0.1 mg/L to aquatic organisms. Therefore, the available data demonstrate that the substances do not fulfil criteria for classification as T related to eco-toxicity endpoints. Furthermore, the substances are not classified as carcinogenic, mutagenic, toxic for reproduction (CMR) or STOT-RE. The substances therefore do not fulfill the screening criteria for T related to human toxicity endpoints either.

9. REFERENCES

ECHA Guidance for the implementation of REACH, Guidance on information requirements and chemical safety assessment, Chapter R.6 (reporting format for a chemical category). May 2008.

ECHA Guidance for the implementation of REACH, Guidance on information requirements and chemical safety assessment, Chapter R.7b: Endpoint specific guidance. Version 4.0, June 2017.

ECHA Guidance for the implementation of REACH, Guidance on information requirements and chemical safety assessment, Chapter R.7c: Endpoint specific guidance. Version 3.0, June 2017.

Read-Across Assessment Framework (RAAF), ECHA-17-R-01-EN, March 2017

ECHA Advice on using read-across for UVCB substances, May 2022; https://www.echa.europa.eu/documents/10162/11395738/advice_uvcb_read-across_en.pdf

Uphues G., 1998. Chemistry of amphoteric surfactants. Fett/Lipid, 100:490-497

Behler et al., 2001. Industrial Surfactant Syntheses. In : Reactions and Synthesis in Surfactant Systems, John Texter (ed.), CRC Press, Boca Raton, FL, 2001, 44 pp. http://uqu.edu.sa/files2/tiny_mce/plugins/filemanager/files/4281709/ch1.PDF

H. Schaefer, T. E. Redelmeier (eds). In: Skin Barrier – Principles of percutaneous absorption.S. Krager AG (publ.), Basel, Switzerland, 1996

HERA (Human and Environmental Risk Assessment on ingredients of household cleaning products) (2005), Cocamidopropylbetaine, http://www.heraproject.com/files/45-HH-05-Final%20CAPB-HH-Fraunhofer-2806.pdf

M.N. Martinez, G.L. Amidon. Mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. J Clin Pharmacol 2002, 42, p. 620-643

Final Report on the Safety Assessment of Cocoamphoacetate, Cocoamphopropionate, Cocoamphodiacetate, and Cocoamphodipropionate. International Journal of Toxicology. Volume: 9 issue: 2, page(s): 121-142. Issue published: March 1, 1990

Series on Testing and Assessment, No. 336, ANNEX 6: ANALYSIS OF LLNA REFERENCE DATA TO CONCLUDE ON PREDICTIVITY OF ALTERNATIVE METHODS FOR SKIN SENSITISATION FOR LIPOPHILIC CHEMICALS

Ball, N., et al. (2011). Evaluating the sensitization potential of surfactants: integrating data from the local lymph node assay, guinea pig maximization test, and in vitro methods in a weight-of-evidence approach. Regulatory Toxicology and Pharmacology 60.3: 389-400.

Roberts, D. et al. (2016). Chemical applicability domain of the Local Lymph Node Assay (LLNA) for skin sensitisation potency. Part 3. Apparent discrepancies between LLNA and

GPMT sensitisation potential: False positives or differences in sensitivity?. Regulatory Toxicology and Pharmacology, 80, 260-267.

Annual Review of Cosmetic Ingredient Safety Assessments: 2005/20061. International Journal of Toxicology. 2008;27(1_suppl):77-142.

Basketter D, Darlenski R, Fluhr JW. Skin irritation and sensitization: mechanisms and new approaches for risk assessment. Skin Pharmacol Physiol. 2008;21(4):191-202

WHO (World Health Organisation), 2006. Dermal Absorption. Environmental Health Criteria 235. ISBN 92-4-157235. World Health Organisation, Geneva.

Rowland M, Tozer TN (edts.) (2011). Clinical Pharmacokinetics and Pharmacodynamics – Concepts and Application. Chapter 4: Membranes and Distribution. Fourth Edition. Wolters Kluwer Health Baltimore. ISBN 978-0-7817-5009-7

Lauren Kavanagh (2021). In vitro gene mutation study in mammalian cells of C12 Amphoacetate. Innospec Limited. Unpublished report.

Barentsen HM (2017a). Predictive Toxicity Assessment of C8-monoacetates and diacetates. Charles River Laboratories Test Facility Study No. 518359. Unpublished report.

Barentsen HM (2017b). Predictive Toxicity Assessment of C18-monoacetates and diacetates. Charles River Laboratories Test Facility Study No. 518360. Unpublished report.

Kirkland D, Aardema M, Henderson L, Müller L (2005). Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens I. Sensitivity, specificity and relative predictivity. Mutation Research 584: 1-256

Kirkland D, Reeve L, Gatehouse D, Vanpary P (2011). A core in vitro genotoxicity assay comprising the Ames test plus the in vitro micronucleus test is sufficient to detect rodent carcinogens and in vivo genotoxins. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 721: 27-73

Matthews Edwin J, Kruhlak NL, Cimino MC, Benz RD, Contrera JF (2006). An analysis of genetic toxicity, reproductive and developmental toxicity, and carcinogenicity data: I. Identification of carcinogens using surrogate endpoints. Regulatory Toxicology and Pharmacology 44: 83-96

Zeiger E (1998). Identification of Rodent Carcinogens and Noncarcinogens Using Genetic Toxicity Tests: Premises, Promises, and Performance. Regulatory Toxicology and Pharmacology 38: 85-95

Li, AA et al. Practical considerations for developmental thyroid toxicity assessments: What's working, what's not, and how can we do better? Reg Toxicol Pharmacol 106: 111-136 (2019)

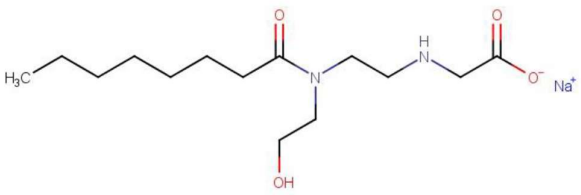
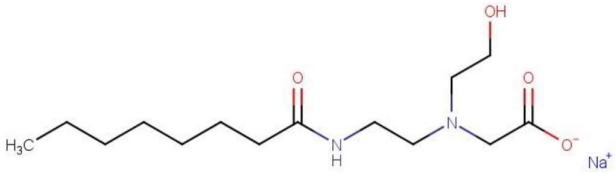
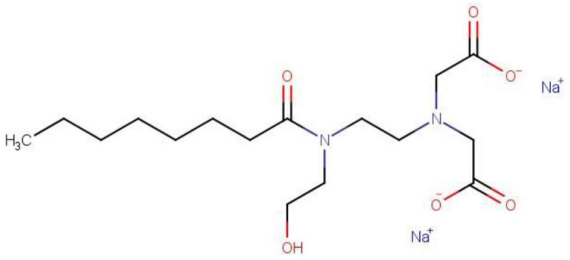
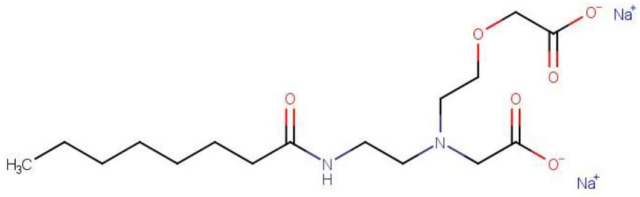
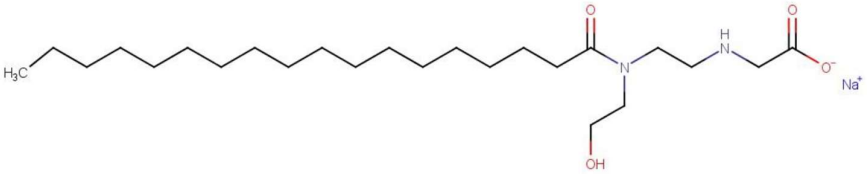
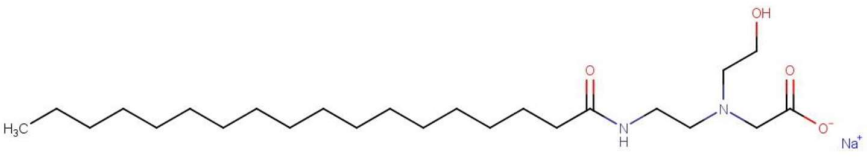
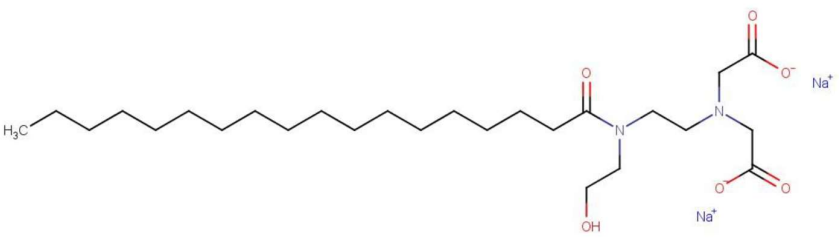
Beekhuijzen, M et al. A critical evaluation of thyroid hormone measurements in OECD test guideline studies: Is ther any added value? Reproductive Toxicology 88: 56-66 (2019)

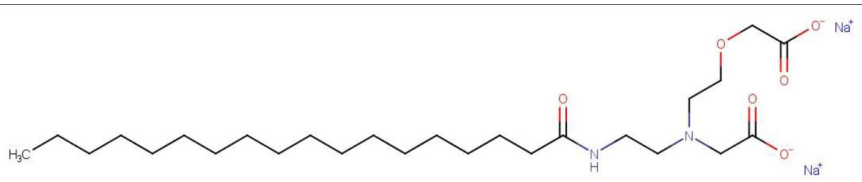
APPENDICES

Appendix I: Overview alkyl chain length distribution of the Alkylamphoacetate analogues

Alkyl chain length	Amphoacetates C8-C18	Amphoacetates C12	Amphoacetates C12-C14
C8	0-11%		
C10	0-11%		≤4%
C12	42-64%	80-99.9%	67–80%
C14	6-26%		20 –32%
C16	4-22%		≤ 4%
C18	0.1-18%		

Appendix III: Chemical structures evaluated by DEREK NEXUS (version 5.0.2)

C8-monoacetate1	
C8-monoacetate2	
C8-diacetate1	
C8-diacetate2	
C18-monoacetate1	
C18-monoacetate2	
C18-diacetate1	

C18-diacetate2	

charles river

Appendix IIIII: Overview of available ecotoxicity studies

Amphoacetates C8-18	Acute toxicity to fish	Chronic toxicity to fish	Acute toxicity to aquatic invertebrates	Chronic toxicity to aquatic invertebrates	Toxicity to aquatic plants	Activated Sludge Respiration inhibition
Mono- : 95-100% Di- : 0-5%	**96h-LC50 = 4.0 mg/L (CRL 2018)** --- 96h-LC50 = 4.2 mg/L (Bazin 1995) --- 96h-LC50 = 5.5 mg/L (Wetton 1996b) --- 96h-LC50 = 8.24 mg/L (Wehrhahn 2002)	**NOEC = 0.61 mg/L (CRL 2019)**	**48h-EC50 = 25.4 mg/L (ibacon 2017a)** --- 48h-EC50 = 8.2 mg/L (Vandendaele 2010a) --- 48h-EC50 = 6 mg/L (Vandendaele 2010b) --- 48h-EC50 = 18.5 mg/L (Wetton 1996) --- 48h-EC50 >100 mg/L (Bazin 1995)	**NOEC = 1.6 mg/L (CRL 2018)**	**72h-ErC50 = 13 mg/L (CRL 2018)** --- 72h-ErC50 = 10 mg/L (Cerbelaud 1995)	*No data*
Mono- : 80-85% Di- : 15-20%	96h-LC50 = 6.4 mg/L (Wetton 1996a) --- 96h-LC50 = 8.5 mg/L (Rudolf 2001) --- 96h-LC50 = 10 mg/L (Berger and Guhl 1998)	*No data*	48h-EC50 = 12.6 mg/L (Bazin 1994) --- 48h-EC50 = 17.9 mg/L (Wierich 2001) --- 48h-EC50 = 17.9 mg/L (Guhl 1993)	*No data*	72h-ErC50 = 28.5 mg/L (Pandard 2001) --- 72h-ErC50 = 3.7 mg/L (Safepharm 1996)	*No data*
Mono- : 50% Di- : 50%	96h-LC50 = 13.9 mg/L (Kamp 1996)	*No data*	48h-EC50 = 56.7 mg/L (ibacon 2017b)	*No data*	72h-ErC50 = 30 mg/L (Lebertz 1998)	*No data*
Undefined ratio	96h-LC50 = 23 mg/L (Burger 2002)	*No data*	48h-EC50 = 2.5 mg/L (Wetton 1992)	*No data*	*No data*	NOEC = 12.7 g/L (Weyandt 1991)

Note: bold indicate the studies used as Key studies in the IUCLID dossiers.

Amphoacetates C12	**Acute toxicity to fish**	**Chronic toxicity to fish**	**Acute toxicity to aquatic invertebrates**	**Chronic toxicity to aquatic invertebrates**	**Toxicity to aquatic plants**	**Activated Sludge Respiration inhibition**
Mono- : 95-100% Di- : 0-5%	**96h-LC50 = 28 mg/L (CRL 2018)** --- 96h-LC50 = 1.6 mg/L (Guhl 1993b)	*No data*	**48h-EC50 = 89 mg/L (Guhl 1993a)** --- 48h-EC50 > 100 mg/L (Pandard 2001)	*No data*	**72h-ErL50 = 44 mg/L (CRL 2018)** --- 72h-ErC50 = 14.8 mg/L (Bätscher 2008)	**NOEC = 560 mg/L (Notox 2012)**
Mono- : 80-85% Di- : 15-20%	*No data*		*No data*	*No data*	*No data*	*No data*
Mono- : 50% Di- : 50%	*No data*		*No data*	*No data*	*No data*	*No data*

Note: bold indicate the studies used as Key studies in the IUCLID dossiers.

Amphoacetates C12-14	**Acute toxicity to fish**	**Chronic toxicity to fish**	**Acute toxicity to aquatic invertebrates**	**Chronic toxicity to aquatic invertebrates**	**Toxicity to aquatic plants**	**Respiration inhibition**
Mono- : 95-100% Di- : 0-5%	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*
Mono- : 80-85% Di- : 15-20%	*No data*		**EC50 = 67.3 mg/L NOEC 45.5 mg/L (ibacon 2017)**	*No data*	*No data*	*No data*
Mono- : 50% Di- : 50%	*No data*		EC50 > 100 mg/L NOEC = 20.7 mg/L (ibacon, 2017)	*No data*	*No data*	*No data*

Note: bold indicate the studies used as Key studies in the IUCLID dossiers.

Appendix IVV: Overview of available thyroid-related data

	T3	T4	TSH	Thyroid weight/pathology	Notes
OECD 422 on C8-18 alkylampho(mono)acetates	n.d	unaffected	n.d	unaffected	
OECD 422 on C8-18 alkylampho(di)acetates	n.d	unaffected	n.d	**follicular cell hypertrophy of the thyroid gland was found in males**	follicular cell considered to be non-adverse based on low severity (up to mild) and absence of correlates
OECD 408 on C8-18 alkylampho(di)acetates	unaffected	**lower in males**	**lower in males**	unaffected	No dose response relationship for TSH effects. TSH and T4 within HCR
OECD 408 on C12-C14 alkylampho(di)acetates	unaffected	**higher in males and females**	unaffected	unaffected	Withing historical control range
OECD 414 on alkylampho(di)acetates C12-14	**decreased in dams**	unaffected	unaffected	unaffected	withing historical control range
OECD 414 on alkylampho(mono)acetates C12	**decreased in dams**	unaffected	unaffected	unaffected	withing historical control range

Appendix V: Overview of available toxicity studies

Amphoacetates C8-18	Acute Toxicity - Oral	Acute Toxicity- Dermal	Skin Irritation	Eye Irritation /Corrosion	Skin Sens.	Repeat-dose Toxicity	Genetic Toxicity	Developmental toxicity	Toxicity to reproduction
Mono- : 95-100% Di- : 0-5%	LD50 = 7956-9828 mg/kg bw (Gill 1977c) --- LD50 > 15 ml aqueous solution /kg bw (Middleton 1977)	*No data*	Non irritating (Harper , 1995b, 1995c and 1995d; **Haynes 1995**; Morris 1996)	*No data*	Non sensitizing (**Arcelin 1998**, Liebert 1990)	*No data*	*No data*	*No data*	*No data*
Mono- : 80-85% Di- : 15-20%	LD50 = 10413 mg/kg (Gill 1977b) --- LD50 > 15 ml aqueous solution/kg bw (Middleton 1977) --- LD50 > 15 ml/kg bw (Levenstein 1977) LD50 > 500mg/kg bw (Gloxhuber 1977) --- LD50 = 10413 mg/kg bw (Gil 1977c) --- LD50 > 5 ml/kg bw (Levenstein 1975) --- LD50 = 28 ml aqueous solution/kg bw (Shapiro 1982) --- LD50 : < 15000 mg aqueous solution/kg bw (Shapiro 1987)	**LD50 > 2612 mg/kg (Notox 2010)**	Non irritating (**Dufour 1977a**)	Not-classified (Shapiro, 1990b) --- Irritating (Shapiro 1990a) --- Eye damage(Kastner, 1987) --- Irritating; (Dufour, 1997a)	*No data*	NOAEL = 92.5 mg/kg bw/day (Potokar 1990)	Non clastogenic (OECD 473) (Notox 2010) --- Non mutagenic (OECD 476) (Notox, 2010) --- negative (OECD 475) (CRL.2018)	*No data*	**NOAEL=1000 mg/kg bw/day (both parental & reproduction) (OECD 422) (CRL, 2018)**
Mono- : 50% Di- : 50%	*No data*	*No data*	*No data*	Serious eye damage (**Bien 1995)**	*No data*	**NOAEL = 1000 mg/kg bw/day (CRL, 2016**)	non mutagenic (OECD 471) (Grotsch 1994; **Hillmann 1991**)	**maternal NOAEL = 1000 mg/kg bw/day ; developmental NOAEL = not determined (OECD 414) (CRL, 2019)**	**reproduction NOAEL = 1000 mg/kg bw/day ; Parental NOAEL = 300 mg/kg bw/day (OECD 422) (CRL, 2018)**
Undefined ratio			Non Irritating (Harper 1995a; **Dufour 1977b**)	Irritating (**Dufour 1997b)**					

Note: bold indicate the studies used as Key studies in the IUCLID dossiers.

Amphoacetates C12-14	Acute Toxicity - Oral	Acute Toxicity - Dermal	Skin Irritation	Eye Irritation /Corrosion	Skin Sens.	Repeat-dose Toxicity	Genetic Toxicity	Developmental toxicity	Toxicity to reproduction
Mono- : 95-100% Di- : 0-5%	//	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*
Mono- : 80-85% Di- : 15-20%	**LD50 > 5939 mg/kg (Levenstein 1980)**	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*	*No data*
Mono- : 50% Di- : 50%	LD50 = 7935 mg/kg bw (Sandhowe-Grote)	*No data*	*No data*	*No data*	*No data*	**NOAEL = 1000 mg/kg bw/day (CRL, 2022**)	non clastogenic (OECD 473)\ (CRL,2021) --------- Non mutagenic (OECD 471) (CRL,2021)	**maternal NOAEL ≥ 1000 mg/kg bw/day ; developmental NOAEL ≥ 1000 mg/kg bw/day (OECD 414) (CRL, 2022)**	*No data*

Note: bold indicate the studies used as Key studies in the IUCLID dossiers.

Amphoacetates C12	Acute Toxicity - Oral	Acute Toxicity - Dermal	Skin Irritation	Eye Irritation/ Corrosion	Skin Sens.	Repeat-dose Toxicity	Genetic Toxicity	Developmental toxicity	Toxicity to reproduction
Mono- : 95-100% Di- : 0-5%	LD50>2000 mg/kg bw(aqueous solution with a solid content of approximately 35%.) (Potokar 1990)	*No data*	non-iritating; (Steiling 1990a, Krächter 1992)	Non-irritating (solid content approximately 12.6%) (Esdaile 1999a and 1999b)	Non sensitizing (Steiling 199b)	*No data*	Non mutagenic (OECD 471) (Cinelli 2000, Banduhn 1990) ---------- Non clastogenic (OECD 473) (Roy, 2012)	**maternal NOAEL ≥ 1000 mg/kg bw/day ; developmental NOAEL ≥ 1000 mg/kg bw/day (OECD 414) (CRL, 2022)**	*No data*
Mono- : 80-85% Di- : 15-20%	**LD50 of 3422 mg/kg bw (Levenstein 1976)** **----------** LD50 = 6116 mg/kg bw (Levenstein 1978) ---------- LD50>2000 mg/kg bw(aqueous solution with a solid content of approximately 35%.) (Longobardi 2001)	*No data*	Miranol H2M Conc; Non-irritating (**Longobardi 2001**)	Irritating (**Longobardi 2001b,** Levenstein 1976a and 1976b)	*No data*	*No data*	*No data*	*No data*	*No data*
Mono- : 50% Di- : 50%	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
Undefined ratio	LD50 >7500 mg/kg (Middleton 1977)			Irritant (Haynes 1985)					

Note: bold indicate the studies used as Key studies in the IUCLID dossiers.

Exponent®

Expert Review of Available Repeat-Dose and Developmental and Reproductive Toxicity (DART) Studies for Amphoacetates

Exponent®

Expert Review of Available Repeat-Dose and Developmental and Reproductive Toxicity (DART) Studies for Amphoacetates

Prepared by

John M. DeSesso, Ph.D., Fellow ATS
and
Amy Lavin Williams, Ph.D., DABT
Exponent
1800 Diagonal Road, Suite 500
Alexandria, VA 22314

14 April 2023

Doc. no. 2204431.000 - 7369

Table of Contents

	Page
Table of Contents	ii
List of Tables	ii
Executive Summary	iii
1. Purpose	1
2. Study summaries	4
Dehyton® DC	4
Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (Pels Rijcken, 2018)	4
Prenatal Developmental Toxicity Study (Bressers, 2019)	8
90-Day Study (Wagenaar, 2019)	12
Miranol Ultra C32	13
Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (De Raat-Beekhuijzen, 2018)	13
Fish Early-Life Stage (FELS) Toxicity Test (Tobor-Kaplon, 2019)	17
PC-2020-926	18
Dose Range-finding Prenatal Study (Viends, 2022a)	18
Prenatal Developmental Toxicity Study (Vriends, 2022b)	20
90-Day Study (Gerding, 2022)	23
Sodium lauroamphoacetate	25
Dose Range-finding Prenatal Study (Langedijk, 2022)	25
Prenatal Developmental Toxicity Study (van Otterdijk, 2022)	27
3. Assessment	31
Parental NOAELs	31
Developmental NOAELs	32
Assessment by type of malformation	35
Assessment by malformed fetus	35
Assessment by perturbed morphogenetic process	36
Non-mammalian Data	38
Potential Role for Impurity	39
4 Conclusions	41
5 Limitations	43
6 References	44
Appendix	48

List of Tables

Page

Table 1. Reproductive and litter parameters from the combined repeat-dose/DART screening test of Dehyton® DC (Pels Rijcken, 2018) 7

Table 2. Litter parameters and fetal anomalies data from the prenatal developmental toxicity study of Dehyton® DC (Bressers, 2019) 9

Table 3. Malformation data by individual fetuses for the prenatal developmental toxicity study of Dehyton® DC (Bressers, 2019) 11

Table 4. Morbidity/mortality at 1000 mg/kg/day in the combined repeat-dose/DART screening test of Miranol Ultra C32 (De Raat-Beekhuijzen, 2018). 14

Table 5. Reproductive and litter parameters from the combined repeat-dose/DART screening test of Miranol Ultra C32 (De Raat-Beekhuijzen, 2018) 16

Table 6. Measured parameters in the fish early life-stage test of Miranol Ultra C32 (Tobor-Kaplon, 2019) 18

Table 7. Litter parameters and fetal anomalies data from the DRF prenatal developmental toxicity study of PC-2020-926 (Vriends, 2022a) 20

Table 8. Litter parameters and fetal anomalies data from the prenatal developmental toxicity study of PC-2020-926 (Vriends, 2022b) 22

Table 9. Malformation data by individual fetuses for the prenatal developmental toxicity study of PC-2020-926 (Vriends, 2022b) 23

Table 10. Litter parameters and fetal anomalies data from the DRF prenatal developmental toxicity study of sodium lauroamphoacetate (Langedijk, 2022) 27

Table 11. Litter parameters and fetal anomalies data from the prenatal developmental toxicity study of sodium lauroamphoacetate (van Otterdijk, 2022) 29

Table 12. Malformation data by individual fetuses for the prenatal developmental toxicity study of sodium lauroamphoacetate (van Otterdijk, 2022) 30

Table 13. Cardiovascular Malformations Reported in Embryofetal Definitive Prenatal Development Toxicity Studies of Amphoacetates[a] 34

Table 14. Incidence of Cardiovascular Malformations Grouped by Perturbed Morphogenetic Process in Combined Embryofetal Development Studies of Amphoacetates 38

Executive Summary

Available repeat-dose and developmental and reproductive toxicity (DART) studies of the monoacetate and diacetate forms of C8-C18 amphoacetate, C12-C14 diamphoacetate, and C12 monoamphoacetate were assessed, paying particular attention to the incidence and dose-response of developmental cardiovascular findings. Any consistencies in findings across the body of studies and the potential influence of maternal toxicity were also investigated.

A total of four amphoacetates (Dehyton® DC, Miranol Ultra C32, PC-2020-926, and sodium lauroamphoacetate; refer to appendix for detailed information on substance identification) were evaluated at doses of 0 (control), 100, 300 or 1000 mg/kg/day. The parental and developmental no observed adverse effect levels (NOAELs) in the three definitive prenatal developmental toxicity studies (for Dehyton® DC, PC-2020-926, and sodium lauroamphoacetate) were the highest dose tested (1000 mg/kg/day). The maternal NOAELs for the prenatal developmental toxicity studies of Dehyton® DC and PC-2020-926 are generally supported by results from their respective 90-day repeat-dose studies. It is noted, however, that due to perceived maternally toxic effects at the high dose in the combined 28-day repeat-dose and reproduction/developmental toxicity screening test (OECD 422) of Dehyton® DC, the high dose dams were euthanized at GD 14, which precluded examination of fetuses at term and the call of NOAELs at the next lower dose (300 mg/kg/day). The developmental NOAEL for the fourth amphoacetate (Miranol Ultra C32) was also determined to be 1000 mg/kg/day, but that assessment was based on an OECD 422, which does not include visceral examination.

There was a low incidence of cardiac and great vessel malformations in two of the three definitive prenatal developmental toxicity studies. None of the malformations was significantly increased and, within each study, the greatest number of malformations occurred in the low dose group. In order to discern if there might be a trend for cardiovascular malformations in animals exposed to the test items, the data from all three studies were combined. Whether the combined data were assessed based on the incidences of malformations, number of malformed fetuses, or underlying perturbed morphogenetic processes, there was neither statistical significance nor a dose-responsive increase.

Aminoethylethanolamine (AEEA) is a starting material in the synthesis of amphoacetates, and small amounts of residual (non-reacted) AEEA can remain in the finished products. The potential for gestational exposure to AEEA to cause congenital cardiac defects was evaluated. High doses of AEEA caused aneurysms of the aorta and alterations in the pattern of great vessels but no defects of the heart. These defects were not observed in the prenatal developmental toxicity studies for the subject amphoacetates. Additionally, the NOAEL for AEEA developmental toxicity is two orders of magnitude above the highest potential AEEA exposure that might have occurred due to amphoacetate exposure in the studies reviewed herein. Thus, AEEA is unlikely to underlie the cardiac defects observed in the prenatal development studies of the subject amphoacetates.

Taken together, in-depth analyses of the available developmental and reproductive toxicity data for the four subject amphoacetates do not support the classification of these substance for reproductive or developmental hazard. Likewise, in-depth analysis of the cardiac and great vessel systems of fetuses exposed to Dehyton® DC, PC-2020-926, and sodium lauroamphoacetate at doses as high as the limit dose does not support that these substances cause malformation of the target area. This conclusion is also supported by the absence of any treatment-related cardiac abnormalities in both the fish early-life stage toxicity test of Miranol Ultra C32 and the dose range-finding studies for PC-2020-265, and sodium lauroamphoacetate (which included visceral examinations of fetal hearts).

1. Purpose

Amphoacetates comprise amphoteric surfactants that are mild detergents. They are formed by a two-step process in which fatty acids of various chain lengths are reacted with AEEA followed by reaction with chloroacetate (Farn, 2006). The resulting products are manifold and depend on the chain lengths of the starting products. An extensive table in the Appendix presents the names, synonyms, identification numbers and physical characteristics of amphoacetates with various chain lengths discussed within this review.

Exponent scientists were requested by the REACH amphoacetates consortium member companies to review the results from the available repeat-dose and developmental and reproductive toxicity (DART) studies of the C8-C18 amphoacetates Miranol Ultra C32 (90:10 monoacetate/diacetate ratio) and Dehyton® DC (50:50 monoacetate/diacetate ratio);a C12-C14 amphoacetates PC-2020-926 (60:40 monoacetate/diacetate ratio); and a C12 monoacetate sodium lauroamphoacetate. This review focuses specifically on developmental cardiovascular findings and assesses the incidence and dose-response of these findings within and across the studies. The potential influence of any maternal effects on the reported outcomes and any consistency in findings that exist across the body of studies are also addressed.

For this assessment, Exponent relied upon the specific studies provided for review by the client's representative at Charles River Laboratories in Den Bosch in the Netherlands. These studies are as follows:

- Bressers, S. 2019. Prenatal Developmental Toxicity Study of Dehyton® DC by Oral Gavage in Rats. Charles River Laboratories Den Bosch BV. Study No. 20164358. 19 July 2019.
- De Raat-Beekhuijzen, MEW. 2018. Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Miranol Ultra C32 by Oral Gavage in Rats. Charles River Laboratories Den Bosch BV. Study No. 518373. 09 July 2018.

- Gerding, L. 2022. A 90-Day Study of PC-2020-926 by Oral Gavage in Wistar Han Rats with a 28-Day Recovery Period. Charles River Laboratories Den Bosch BV. Study No. 20297957. 27 October 2022.
- Langedijk J. 2022. Dose Range Finding Study of Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1-ethanol and sodium hydroxide by Oral Gavage in Pregnant Wistar-Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20293263. 16 February 2022.
- Pels Rijcken, WR. 2018. Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Dehyton® DC by Oral Gavage in Rats. Charles River Laboratories Den Bosch BV. Study No. 518366. 23 November 2018.
- Tobor-Kaplon, MA. 2019. Fish Early-Life Stage Toxicity Test with Miranol Ultra C32 (Flow-Through). Charles River Laboratories Den Bosch BV. Study No. 20177673. 19 August 2019.
- van Otterdijk, F. 2022. Prenatal Developmental Toxicity Study of Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1- ethanol and sodium hydroxide by Oral Gavage in Time-Mated Wistar Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20293266. 29 August 2022.
- Vriends, A. 2022a. Dose Range Finding Study of PC-2020-926 by Oral Gavage in Pregnant Wistar-Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20297966. 06 May 2022.
- Vriends, A. 2022b. Prenatal Developmental Toxicity Study of PC-2020-926 by Oral Gavage in Time-Mated Wistar Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20297978. 26 August 2022.
- Wagenaar, L. 2019. A 90-Day Study of Dehyton® DC by Oral Gavage in Wistar Rats. Charles River Laboratories Den Bosch BV. Study No. 20164357. 23 July 2019.

Exponent scientists also relied on additional information as available in the published literature, the Charles River historical control database (Charles River, 2023), and their own expertise in developmental and reproductive toxicology.

Below, the results of the individual studies are first summarized according to test article and the chronological order in which the reports were issued. Next, a comprehensive evaluation of the combined results of these studies is provided that takes into consideration embryological development processes.

2. Study summaries

Dehyton® DC

Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (Pels Rijcken, 2018)

This study was conducted in compliance with good laboratory practices (GLP) and according to the Organization for Economic Cooperation and Development (OECD) test guideline (TG) No. 422 (2016). Male and female Wistar Han rats (10/sex per group) were dosed by oral gavage with 0, 100, 300 or 1000 mg/kg/day of Dehyton® DC in water at a dosing volume of 5 mL/kg. These formulations were adjusted to account for purity of the test article (47.6%) and the top dose administered (1000 mg/kg/day) is the limit dose for this test. Males were dosed for a minimum of 28 days beginning 14 days prior to mating. Females were dosed beginning 14 days prior to mating, through mating, gestation, and 13 days of the lactation period.

Methods in Brief. Parental animals were checked twice daily for mortality/morbidity, once daily for clinical signs, and once weekly for arena observations. Females were screened for estrous cyclicity during the first 14 days prior to mating, after which mating was conducted on a 1:1 basis until mating was confirmed (designated gestational day [GD] 0). Females were allowed to litter normally. Body weights and food consumption were measured weekly in males and in females prior to mating; after mating, female body weights and food consumption were measured on GDs 0, 4, 7, 11, 14, 17, and 20 and during lactation on postnatal days (PNDs) 1, 4, 7, and 13. Functional assessments were conducted on 5 rats/sex per group in their respective last weeks of treatment. At sacrifice, blood was collected from parental animals for assessment of hematology, clinical chemistry and thyroid hormone (thyroxine; T4). Pups were checked daily for mortality/morbidity and clinical signs, and weighed on PND 1, 4, 7, and 13. The numbers of live and dead pups were recorded on PND 1, sex was assessed on PNDs 1 and 4, anogenital distance (AGD; normalized to the cube root of body weight) was measured on PND 1, and nipple retention was evaluated in males on PND 13. On PND 4, litters were culled to 8 pups each (4 of each sex), when possible. Thyroid hormone (T4) was assessed in 2 pups/litter per group from culled animals on PND 4 and in remaining pups at the end of study. At necropsy, select organs from parental

animals were weighed in all dose groups, and tissues were evaluated for histopathology in the control and high dose groups.

Results. A single female at 300 mg/kg/day died as a result of blood sampling just prior to necropsy; this death was not considered treatment related. One male and four females at 1000 mg/kg/day died or were sacrificed *in extremis* during the course of the study. Respiratory and other clinical signs were noted in these animals prior to death/sacrifice. Macroscopic findings in the lungs were also observed in these animals; additionally, in one of the females, foreign material was noted in the tracheal lumen. The laboratory concluded that these deaths were likely due to regurgitation of the test article; however, in our opinion, it cannot be excluded that these deaths may have been related to gavage error. Nonetheless, it is noted that in the later studies described below, the dosing volume for Dehyton® DC in water was reduced from 5 mL/kg to 1.796-1.895mL/kg and no other such deaths were observed in the subsequent studies. It should also be noted that, because 4 of the 10 females at 1000 mg/kg/day died or were euthanized prematurely, the remaining 6 females in this dose group were sacrificed early on GD 14. Therefore, data for the high dose females should be interpreted with some caution as animals in the control and other treatment groups were sacrificed during the postnatal lactation period rather than in gestation.

Various clinical signs, including salivation, labored breathing rales, and piloerection were noted in most animals treated at 1000 mg/kg/day and occasionally in some animals treated at 300 mg/kg/day. Significantly reduced mean body weight gain was observed in males at 1000 mg/kg/day, resulting in a 6% decreased body weight at the end of treatment compared to controls. Female body weights were unaffected by treatment and no clearly treatment-related effect on food consumption was noted for either males or females. Functional observational parameters were not affected by treatment. Significant hematologic changes were noted for both males and females at 1000 mg/kg/day compared to controls. Although a number of clinical chemistry parameters in females of the 1000 mg/kg/day dose group were significantly different from control, the changes were minimal and interpreted by the laboratory as being likely a result of the difference in physiological status (pregnancy versus lactation) rather than an effect of treatment. In females at 300 mg/kg/day, total protein and albumin were significantly lower than

control, but at the lower limit of the normal range. No toxicologically relevant clinical chemistry changes were noted for males.

Serum T4 concentrations were unaffected by treatment in males. However, an increase in the incidence of minimal to slight thyroid follicular cell hypertrophy was noted at 300 and 1000 mg/kg/day. Serum T4 data were not available for parental females; however, no increased thyroid histopathology was observed in these animals at ≤1000 mg/kg/day.

Brain and kidney weights relative to body weight were significantly increased in high dose males compared to controls. In high dose females, numerous organ weights were significantly different from control (both absolute and relative to body weight); however, because these animals were sacrificed at a different stage of physiological development, no conclusions can be drawn regarding a relation to treatment. No significant organ weight differences from control were found for females in the low and mid-dose treatment groups.

Reproductive and litter parameters are presented in Table 1. Estrous cyclicity was unaffected by treatment. No significant effect of treatment was observed on mating, fertility or gestation indices, precoital time or the mean number of implantations per female. Because females at 1000 mg/kg/day were sacrificed prior to delivery, littering and pup developmental are only available for animals in the control, low and mid-dose groups. No effects of treatment were observed on gestation duration and no indications of prolonged parturition were noted. Litter size, live birth index, and viability and lactation indices were not significantly affected by treatment at ≤300 mg/kg/day. While the number of dead pups noted at the first litter check were 0, 0 and 9 in the control, low and mid-dose groups respectively, 7 of the 9 dead pups were in a single litter. The associated dam was the only one in the dose group to have lost weight during the postnatal period.

Table 1. Reproductive and litter parameters from the combined repeat-dose/DART screening test of Dehyton® DC (Pels Rijcken, 2018)

Dose (mg/kg/day):	0	100	300	1000
Mating index (%)	100	100	100	100
Fertility index (%)	90	80	100	100
Gestation index (%)	100	100	90	
Mean precoital time (days)	2.1	2.1	2.6	2.0
Mean # implantations (± SD)	12.9 ± 1.9	12.9 ± 3.0	12.5 ± 4.2	11.3 ± 4.4
Gestation duration (days ± SD)	21.2 ± 0.4	21.3 ± 0.5	21.2 ± 0.7	
# dead pups at 1st litter check (# affected litters)	0 (0)	0 (0)	9 (3)	
Mean # live pups at 1st litter check (± SD)	12.1 ± 2.3	10.9 ± 4.1	11.6 ± 2.4	
Mean postnatal loss (± SD)	0.3 ± 0.7	0.0 ± 0.0	0.1 ± 0.3	
Post-implantation survival index (%)	94	84	90	
Live birth index(%)	100	100	92	
Viability index (%)	97	100	99	
Lactation index (%)	100	100	99	

No effects of treatment were observed on pup clinical signs, body weights, sex ratio, anogenital distance, nipple retention, or serum T4 levels in male or female pups and no macroscopic findings related to treatment were observed at necropsy.

Based on these data, the laboratory concluded that the parental no observed adverse effect level (NOAEL) was 300 mg/kg/day based on mortality and regurgitation of the formulations. While the NOAEL for parental toxicity could have been called lower due to the increased incidence of thyroid hypertrophy in males at 300 mg/kg/day, the data presented in the 90-day study (see below) indicate that the thyroid finding was spurious. The laboratory indicated the reproductive NOAEL as ≥1000 mg/kg/day based on no effects observed; however, in the absence of complete reproductive data at 1000 mg/kg/day from maternal animals that reached full term, we consider the reproductive NOAEL in this study to be ≥300 mg/kg/day. The laboratory correctly indicated the developmental NOAEL as ≥300 mg/kg/day based on no effects observed.

Prenatal Developmental Toxicity Study (Bressers, 2019)

This study was conducted in compliance with GLP and according to OECD TG No. 414 (2018). Time-mated female Wistar Han rats (22/group) were dosed by oral gavage with 0, 100, 300 or 1000 mg/kg/day of Dehyton® DC in water at a dosing volume of 1.796 mL/kg. These formulations were adjusted to account for purity of the test article (48%) and the top dose administered (1000 mg/kg/day) is the limit dose for this test. Dosing was from GD 6 though GD 20.

Methods in Brief. Dams were checked twice daily for mortality/morbidity and at least once daily for clinical signs. Body weights and food consumption were measured on GDs 2, 6, 9, 12, 15, 18, and 21. At sacrifice on GD 21, blood was collected from the dams for assessment of thyroid hormones (triiodothyronine [T3], T4, and TSH). Dams were subjected to examination of the thoracic and abdominal cavities. Uteri and thyroid glands were weighed, and the thyroid prepared for histopathologic examination. Data regarding litter indices were collected. Uteri of apparently non-pregnant rats were stained for identification of potential implantation sites. All live fetuses were sexed, weighed and examined for external anomalies; AGD (normalized to the cube root of fetal body weight) was also measured. One half of the fetuses in each litter was examined by fresh dissection for visceral anomalies; this examination included the heart and major blood vessels. The heads were examined by Wilson sectioning. The other half of the fetuses in each litter was subjected to skeletal examination using Alizarin Red S staining. Fetal data were appropriately assessed based on litter means.

Results. No treatment-related mortality or clinical signs of toxicity were observed. At 300 mg/kg/day, a single dam was euthanized *in extremis* on GD 16 due to a non-treatment related spinal injury. Also, at ≥300 mg/kg/day, rats exhibited increased salivation after dosing; this finding was considered by the laboratory to be a physiological response to the test article rather than a clinical sign of toxicity. No treatment-related effects on body weight or food consumption were observed. Slightly lower serum TSH levels were seen at ≥300 mg/kg/day. However, the differences from control were not statistically significant and individual values were within the historical control data (HCD) range; therefore, the differences were not considered to be related

to treatment. No effects on T3 or T4 levels were observed, and thyroid organ weights and histopathology were not changed from control.

Fetal litter parameters and malformation data are presented in Table 2. No treatment-related effects were observed on the number of pregnant females per group, the numbers of corpora lutea and implantation sites, early and late resorptions, pre- and post-implantation loss, the numbers of live and dead fetuses per litter, fetal sex ratio, fetal weights or fetal AGD measures.

Table 2. Litter parameters and fetal anomalies data from the prenatal developmental toxicity study of Dehyton® DC (Bressers, 2019)

Dose (mg/kg/day):	0	100	300	1000
# Females on study	22	22	22	22
# Euthanized or died on study	0	0	1	0
# Pregnant at scheduled necropsy (%)	22 (100)	22 (100)	20 (90.9)	22 (100)
Litter parameters				
Mean # corpora lutea per litter (± SD)	11.8 ± 2.34	12.4 ± 2.11	11.8 ± 2.170	12.2 ± 1.84
Mean # implantations per litter (± SD)	10.7 ± 2.98	10.7 ± 2.51	11.2 ± 1.63	10.7 ± 1.75
Mean # dead fetuses per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean # viable fetuses per litter (± SD)	10.4 ± 2.91	10.4 ± 2.63	10.7 ± 1.75	10.3 ± 2.12
Mean # early resorptions per litter (± SD)	0.3 ± 0.65	0.3 ± 0.57	0.5 ± 0.69	0.4 ± 0.67
Mean # late resorptions per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean % pre-implantation loss (± SD)	11.0 ± 18.42	13.7 ± 17.70	5.1 ± 9.87	10.8 ± 14.43
Mean % post-implantation loss (± SD)	2.7 ± 5.41	3.2 ± 6.29	4.1 ± 6.52	4.5 ± 8.27
% Males per litter	50.4	46.6	53.8	48.3
Mean male fetal weights per litter (± SD)	5.5 ± 0.25	5.4 ± 0.38	5.5 ± 0.29	5.4 ± 0.37
Mean female fetal weights per litter (± SD)	5.1 ± 0.45	5.2 ± 0.30	5.3 ± 0.26	5.2 ± 0.30
Mean male corrected AGD (± SD)	1.49 ± 0.113	1.48 ± 0.087	1.50 ± 0.096	1.47 ± 0.142
Mean female corrected AGD (± SD)	0.66 ± 0.100	0.67 ± 0.093	0.68 ± 0.091	0.67 ± 0.107

Dose (mg/kg/day):	0	100	300	1000
Fetal anomalies data				
# Fetuses (litters) examined externally	229 (22)	229 (22)	214 (20)	227 (22)
# Fetuses (litters) with external malformations	0 (0)	2 (2)	0 (0)	0 (0)
# Fetuses (litters) examined viscerally	115 (22)	117 (22)	107 (20)	114 (22)
# Fetuses (litters) with visceral malformations	0 (0)	2 (2)	1 (1)	1 (1)
# Fetuses (litters) examined skeletally	114 (22)	114 (22)	107 (20)	114 (22)
# Fetuses (litters) with skeletal malformations	3 (3)	3 (3)	1 (1)	1 (1)
Total # fetuses (litters) with anomalies	3 (3)	7 (4)	1 (1)	1 (1)

The individual fetuses with malformations are shown in Table 3. No treatment related external or skeletal malformations were observed. With regard to visceral anomalies, cardiovascular findings were noted in 0, 2, 1, and 1 fetuses in the control, low, mid, and high dose groups, respectively. The laboratory concluded that, although a dose-response could not be shown, because right-sided aortic arch occurred in the 1000 mg/kg/day dose group at an incidence above the HCD range (reported as a summary percent incidence) and other findings (transposition of the great vessels, interrupted aortic arch, retro-esophageal ductus arteriosus, and absent ductus arteriosus) were not reported in the HCD, a relation to treatment could not be excluded. We note, however, that right-sided aortic arch has been previously reported in the HCD that was provided in the study report, meaning that it has been seen in at least one control fetus in at least one past study – thus, at the same rate as in the current study. Significance of the cardiovascular findings reported in this study is discussed in greater detail in the assessment section found below.

Table 3. Malformation data by individual fetuses for the prenatal developmental toxicity study of Dehyton® DC (Bressers, 2019)

Dose (mg/kg/day):	Finding
0	Fetus A001-11 – bent limb bones (S) Fetus A005-05 – bent limb bones (S) Fetus A008-06 – vertebral anomaly with associated rib anomaly (S)
100	Fetus A029-02 – omphalocele (E) Fetus A029-07 – vertebral anomaly with associated rib anomaly (S) Fetus A039-04 – abnormal lung lobation (V), transposition of the great vessels (V) Fetus A041-07 – bent limb bones (S) Fetus A043-03 – abnormal lung lobation (V), situs inversus (V), ventricular septum defect (V), interrupted aortic arch (V), retro-esophageal ductus arteriosus (V) Fetus A043-04 – absent lower jaw (E) and cleft palate (E) *(findings confirmed at skeletal)* Fetus A043-05 – sternoschisis (S)
300	Fetus A058-03 – abnormal lung lobation (V), ventricular septal defect (V), absent ductus arteriosus (V), situs inversus (V)
1000	Fetus A068-10 – absent eyes (V), right-sided artic arch (V), ventricular septal defect (V), bent limb bones (S), skull bones fused (S), vertebral anomaly without associated rib anomaly (S)

E = external finding; S = skeletal finding; V = visceral finding

No external variations were noted. Small supernumerary liver lobes were noted as a visceral variation in the study, but the incidences were considered unrelated to treatment. At the skeletal examination, an increased incidence of 7th cervical vertebra ossification site presence was observed at 1000 mg/kg/day.

Based on these data, the laboratory concluded correctly that the NOAEL for maternal toxicity was 1000 mg/kg/day based on the absence of any observed effects. The laboratory could not come to a conclusion regarding the developmental NOAEL. As detailed further in the assessment that follows, it is our opinion that the fetal findings are not treatment-related and the NOAEL for developmental toxicity is 1000 mg/kg/day, the highest dose tested.

90-Day Study (Wagenaar, 2019)

This study was conducted in compliance with GLP and according to OECD TG No. 408 (1998); this OECD guideline has since been superseded with an updated version that includes additional assessment of various endocrine-sensitive endpoints. Wistar Han rats (10/sex per group) were dosed for 90 days by oral gavage with 0, 100, 300 or 1000 mg/kg/day of Dehyton® DC in water at dosing volumes of 1.895 mL/kg (to Day 34) and 1.796 mL/kg (beginning Day 35). These formulations were adjusted to account for purity of the test article (47.2% and 48%) and the top dose administered (1000 mg/kg/day) is the limit dose for this test.

Methods in Brief. Rats were checked twice daily for mortality/morbidity, once daily for clinical signs and once weekly for arena observations. Body weights and food consumption were measured weekly. Estrus cyclicity was assessed in female rats in weeks 11-13. Functional tests were conducted on 5 rats/sex per group in week 12. An ophthalmic examination was conducted on control and high dose rats in week 13. At sacrifice, blood was collected for assessment of hematology, clinical chemistry, and thyroid hormones (T3, T4, and TSH). Select organs were weighed and tissues collected for histopathologic examination of the control and high dose groups.

Results. No mortality and no treatment-related arena observations were observed. Salivation was noted in all animals at ≥300 mg/kg/day and incidental ploughing in all animals at 1000 mg/kg/day. The laboratory did not consider these findings to be toxicologically relevant, but rather, to be a physiological response to the taste of the test material. There were no effects of treatment on body weights and body weight gains in females at ≤1000 mg/kg/day and in males at ≤300 mg/kg/day. The terminal mean body weight for males at 1000 mg/kg/day was 88% of control; this difference was statistically significant. No significant differences from control were observed with regard to food consumption. Functional observations, including motor activity, were similar across treatment groups including control, and no effects of treatment were observed in the ophthalmic examinations or with regard to estrous cyclicity. In males, platelets were significantly increased at ≥300 mg/kg/day and alkaline phosphatase was significantly increased at 1000 mg/kg/day compared to concurrent control values, but the values were reported to be within the HCD range and considered to be not toxicologically relevant. Other statistical differences from control in

hematologic and clinical chemistry parameters were considered incidental based on their minimal degree of change and/or lack of dose-response. TSH concentrations were significantly lower than control in all groups of treated males, but without dose response. T4 was reduced in males at 1000 mg/kg/day. No changes in organ weights were considered direct effects of treatment. There were no treatment related histopathologic findings, including in the thyroid. Qualitative assessment of spermatogenesis revealed normal progression of the spermatogenic cycle.

Based on the absence of any treatment-related toxicity, the NOAEL for systemic toxicity was determined by the laboratory to be 1000 mg/kg/day, the highest dose tested. The NOAEL for females is 1000 mg/kg/day.

Miranol Ultra C32

Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (De Raat-Beekhuijzen, 2018)

This study was conducted in compliance with GLP and according to OECD TG No. 422 (2016). Male and female Wistar Han rats (10/sex per group) were dosed by oral gavage with 0, 100, 300, or 1000 mg/kg/day of Miranol Ultra C32 in water at a dosing volume of 5 mL/kg. These formulations were adjusted to account for purity of the test article (39.15%) and the top dose administered (1000 mg/kg/day) is the limit dose for this test. Males were dosed for a minimum of 29 days beginning 14 days prior to mating. Females were dosed beginning 14 days prior to mating, through mating, gestation, and 13 or 15 days of the lactation period.

Methods in Brief. Parental animals were checked twice daily for mortality/morbidity and clinical signs, and once weekly for arena observations. Females were screened for estrus cyclicity during the first 14 days prior to mating, after which mating was conducted on a 1:1 basis until mating was confirmed (designated GD 0). Females were allowed to litter normally. Body weights and food consumption were measured weekly in males and in females prior to mating; after mating, female body weights and food consumption were measured on GDs 0, 4, 7, 11, 14, 17, and 20 and during lactation on PNDs 1, 4, 7, and 13. Functional assessments were conducted on 5 rats/sex per group in their respective last weeks of treatment. At sacrifice, blood was collected from

parental animals for assessment of hematology, clinical chemistry and thyroid hormone (T4 and TSH). Pups were checked daily for mortality/morbidity and clinical signs, and weighed on PND 1, 4, 7, and 13. The numbers of live and dead pups were recorded on PND 1, sex was assessed on PNDs 1 and 4, AGD (normalized to the cube root of body weight) was measured on PND 1, and nipple retention was evaluated in males on PND 13. On PND 4, litters were culled to 8 pups each (4 of each sex), when possible. Thyroid hormone (T4) was assessed in 2 pups/litter per group from culled animals on PND 4 and in remaining pups at the end of study. At necropsy, select organs from parental animals were weighed in all dose groups, and tissues were evaluated for histopathology in the control and high dose groups.

Results. No treatment-related mortalities were observed. At 1000 mg/kg/day, 1 male and 2 females died due to gavage error; the primary findings for each of these animals are shown in Table 4. It should be noted, however, that the macroscopic and microscopic findings for Female #78 are not consistent with gavage error. No other deaths were reported on study.

Table 4. Morbidity/mortality at 1000 mg/kg/day in the combined repeat-dose/DART screening test of Miranol Ultra C32 (De Raat-Beekhuijzen, 2018).

Animal	Reported findings
Male 33 Euthanized *in extremis* on Day 17	Clinical signs: lethargy, flat posture, gasping, piloerection, squeaking, chromodacryorrhea Macroscopic findings: mucous contents in trachea; gas distension of parts of gastrointestinal tract Microscopic findings: acute inflammation of the trachea
Female 74 Found dead on GD 1	Clinical signs: gasping, dyspnea Macroscopic findings: swollen lungs with dark red foci and containing watery fluid Microscopic findings: amorphous alveolar contents and congestion of the lungs, marked bronchial mucosal erosion of the lungs, marked ulceration/erosion of the trachea
Female 78 Euthanized *in extremis* on PND 1	Clinical signs: rough fur, lunched posture, ptosis, pale appearance Macroscopic findings: gelatinous contents of the stomach, discoloration of the liver (pale), discoloration of the kidneys (greenish) Microscopic findings: marked ulceration of forestomach, moderate lymphogranulocytic inflammation of the forestomach

Salivation was noted in the animals treated at 1000 mg/kg/day and occasionally in some animals treated at 300 mg/kg/day. This finding was interpreted as a physiological response to dosing rather than a sign of systemic toxicity. Rales were noted in a few animals at 1000 mg/kg/day; however, because this finding was observed only on one or a few days, it was not considered toxicologically relevant. No statistically significant effects of treatment on male or female body weights and body weight gains were observed. However, in high dose females, body weight gain was slightly reduced at the end of gestation (~15%) and 3 females were noted as showing substantially reduced weight gain or weight loss at the end of lactation. In line with these findings, female food consumption at 1000 mg/kg/day was significantly reduced at the end of gestation (~12.5-13%) and in the last week of lactation (~20%). Male food consumption was unaffected. Functional observational and hematologic parameters were not changed by treatment. Clotting time was significantly reduced in males at 1000 mg/kg/day compared to concurrent controls, but all values were within the expected range. Alanine aminotransferase levels and bile acids were increased in males at 1000 mg/kg/day, but these differences from control were not statistically significant and the individual values were within their respective HCD ranges as reported in the study. Thus, these differences were not considered toxicologically relevant. Serum T4 concentrations were unaffected by treatment in males.

Absolute brain weight was significantly lower in high dose females (~7%) compared to controls. However, individual values at 1000 mg/kg/day were similar to the HCD mean value as reported in the study, while those of the concurrent control were above the HCD range and brain weight relative to body weight was unaffected. No treatment-related histopathology was seen in the high dose group males and females.

Reproductive and litter parameters are presented in Table 5. Estrous cyclicity was unaffected by treatment. No significant effect of treatment was observed on mating, fertility or gestation indices, precoital time or the mean number of implantations per female. No effects of treatment were observed on gestation duration and no indications of prolonged parturition were noted. Litter size was not significantly affected by treatment but was generally lower in the treated groups compared to control. Live birth and lactation indices were unaffected by treatment.

At the first litter check, 7 pups from 4 litters in the 1000 mg/kg/day dose group were found dead. One of these litters (from which 3 pups were found dead) was for a dam (No. 78) that was euthanized *in extremis* on PND 1. The number of dead pups at 1000 mg/kg/day was considered by the laboratory to be within the normal limits and unrelated to treatment. The viability index at 1000 mg/kg/day was lower than in the control group; this index was substantially affected by euthanization of the full litter (10 pups) from dam No. 78. The laboratory considered the lower viability index to be unrelated to treatment. It is noted that the viability index should have been calculated without including the litter from Dam #78, as she was euthanized, and the loss of her pups were an indication of maternal toxicity rather than a lack of maternal care or impaired pup health.

Table 5. Reproductive and litter parameters from the combined repeat-dose/DART screening test of Miranol Ultra C32 (De Raat-Beekhuijzen, 2018)

Dose (mg/kg/day):	0	100	300	1000
Mating index (%)	100	90	100	100
Fertility index (%)	80	89	90	89
Gestation index (%)	100	100	89	100
Mean precoital time (days)	2.1	2.2	2.9	2.6
Mean # implantations (± SD)	13.6 ± 1.8	13.5 ± 2.1	11.1 ± 3.9	13.1 ± 2.9
Gestation duration (days ± SD)	21.3 ± 0.5	21.5 ± 0.5	21.5 ± 0.5	21.8 ± 0.5
# dead pups at 1st litter check (# affected litters)	0 (0)	1 (1)	0 (0)	7 (4)
Mean # live pups at 1st litter check (± SD)	13.3± 2.0	11.4 ± 1.6	11.5 ± 1.1	11.0 ± 2.6
Mean postnatal loss (± SD)	0.0 ± 0.0	0.1 ± 0.4	0.3 ± 0.5	1.9 ± 3.4
Post-implantation survival index (%)	97	85	92	90
Live birth index(%)	100	99	100	93
Viability index (%)	100	99	98	83
Lactation index (%)	100	100	100	100

Clinical signs were observed in those pups that did not survive until scheduled sacrifice. Otherwise, no effects of treatment were observed on pup clinical signs, body weights, sex ratio,

anogenital distance, nipple retention, or serum T4 levels in male or female pups and no macroscopic findings related to treatment were observed at necropsy.

Based on these data, the laboratory concluded that the parental, reproductive and developmental NOAELs were all 1000 mg/kg/day, the highest dose tested. We agree with these calls.

Fish Early-Life Stage (FELS) Toxicity Test (Tobor-Kaplon, 2019)

This study was conducted in compliance with GLP and according to OECD TG No. 210 (2013). Fathead minnow (*Pimephales promelas*) were exposed to target concentrations of Miranol Ultra C32 (39.6%) of 0, 0.050, 0.13, 0.31, 0.78 and 2.0 mg solids/L in a flow-through system for 33 days. In preparing the test concentrations, a correction factor of 2.525 was applied to account for the water content of the test substance and concentrations are expressed based on solids. The test concentrations were selected based on results of a range-finding assay in which levels of embryonic mortality of 5%, 10%, and 40% were recorded at Miranol Ultra C32 test concentrations of 0.05, 0.5, and 5.0 mg solids/L, respectively; hatching was delayed by one day at the highest test concentration, and larval mortality rates of 5.3% and 75% were recorded at 0.5 and 5.0 mg solids/L, respectively. The definitive test was performed with 4 replicates of 20 eggs per group.

Methods in brief. The stages of embryonic development, hatching, survival and any abnormalities in appearance were assessed daily. At the end of the study, all surviving fish were weighed (blotted dry weights) and lengths were measured.

Results. At the target concentrations of 0.050, 0.13, 0.31, 0.78 and 2.0 mg solids/L, actual mean concentrations of 0.035, 0.090, 0.22, 0.61, and 1.6 mg solids/L were measured. The reason for the lower measured concentrations was not clear, but likely due to (a)biotic loss processes within the test system such as biodegradation and adsorption.

Parameters measured in the fish early life-stage test of Miranol Ultra C32 are shown in Table 6. Treatment had no effect on embryonic survival (i.e., the percent of embryos that hatched) or on larval survival (i.e., post-hatch mortality). However, exposure affected both larval body weight and length, with a significant effect on both parameters at the highest test concentration of 1.6 mg

solids/L. At this concentration, malformation of the caudal fin was observed in all larvae; at lower test concentrations, various abnormalities of the skeleton, eyes, swim bladder or other systems were recorded. Specific to the cardiac system, cardiac edema was reported for one larva of replicate A at 1.6 mg solids/L on Days 17 & 18 only.

Table 6. Measured parameters in the fish early life-stage test of Miranol Ultra C32 (Tobor-Kaplon, 2019)

Measured mg solids/L:	0	0.035	0.090	0.22	0.61	1.6
% Embryos hatched (Day 8)	99	100	94	100	95	95
% Post-hatch mortality (Day 33)	6	16	5	10	12	13
Mean body weight (± SD; Day 33)	74.450 ± 5.7356	73.850 ± 4.9776	76.550 ± 2.0469	69.150 ± 4.6658	74.475 ± 8.9481	46.025 ± 4.9026
% Body weight reduction (Day 33)	---	0.81	-2.8	7.1	-0.034	38*
Mean body length (± SD; Day 33)	21.21 ± 0.395	21.29 ± 0.335	21.43 ± 0.326	20.48 ± 0.268	21.26 ± 0.826	17.50 ± 0.709
% Body length reduction (Day 33)	---	-0.38	-1.0	3.5	-0.26	18*

* statistically significant

Based on these data, the laboratory considered the no observed effect concentration (NOEC) to be 0.61 mg solids/L for effects on larval growth and caudal fin malformation. The NOEC for embryonic hatching success and larval survival was 1.6 mg solids/L, the highest concentration tested. We agree with these calls.

PC-2020-926

Dose Range-finding Prenatal Study (Viends, 2022a)

In preparation for a definitive prenatal developmental toxicity study, a dose range-finding (DRF) study was conducted. The study was not GLP compliant but generally followed OECD TG No. 414 (2018) with exceptions for the number of animals per group, fetal visceral examinations limited to the heart and great vessels only, and no fetal skeletal examinations. Time-mated female Wistar Han rats (6/group) were dosed by oral gavage with 0, 300, 600 or 1000 mg/kg/day of PC-2020-926 in water at a dosing volume of 1.695 mL/kg. These formulations were adjusted to

account for water content of the test material using a correction factor of 2. Dosing was from GD 6 though GD 20.

Methods in Brief. Dams were checked twice daily for mortality/morbidity and at least once daily for clinical signs; detailed clinical observations were conducted on GD 2, 6, 15 and 21. Body weights and food consumption were measured on GDs 2, 6, 9, 12, 15, 18, and 21. On GD 21, dams were subjected to examination of the thoracic and abdominal cavities. Data regarding litter indices were collected. Uteri of non-pregnant rats were stained for identification of implantation sites. Livers were collected and weighed in the control and high dose group. All live fetuses were sexed, weighed, and examined for external anomalies. All live fetuses in each litter were also examined for visceral anomalies of the heart and great vessels. Fetal data were appropriately assessed based on litter means.

Results. No treatment-related mortality or clinical signs of toxicity were observed. Maternal body weight in the treated groups was comparable to control; mean body weight gain corrected for gravid uterine weight, however, was slightly lower at 1000 mg/kg/day compared to control. Absolute and relative liver weights at the high dose were comparable to control. Fetal litter parameters and malformation data are presented in Table 7. Compared to control, post-implantation loss was higher in the low dose group and pre-implantation loss was higher in the mid-dose group; no differences in these parameters were observed between the control and high dose group. No treatment-related effects were observed on the number of pregnant females per group, the numbers of corpora lutea and implantation sites, early and late resorptions, the numbers of live and dead fetuses per litter, fetal sex ratio, or fetal weights.

Table 7. Litter parameters and fetal anomalies data from the DRF prenatal developmental toxicity study of PC-2020-926 (Vriends, 2022a)

Dose (mg/kg/day):	0	300	600	1000
# Females on study	6	6	6	6
# Euthanized or died on study	0	0	0	0
# Pregnant at scheduled necropsy (%)	6 (100)	5 (83.3)	4 (66.7)	6 (100)
Litter parameters				
Mean # corpora lutea per litter (± SD)	12.3 ± 0.8	12.8 ± 1.3	12.5 ± 1.3	11.7 ± 2.0
Mean # implantations per litter (± SD)	11.2 ± 2.4	11.6 ± 3.3	9.5 ± 4.7	11.0 ± 1.7
Mean # dead fetuses per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean # live fetuses per litter (± SD)	10.7 ± 2.3	10.4 ± 3.0	9.3 ± 4.5	10.8 ± 1.5
Mean # early resorptions per litter (± SD)	0.5 ± 0.5	1.2 ± 2.2	0.3 ± 0.5	0.2 ± 0.4
Mean # late resorptions per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean % pre-implantation loss (± SD)	10.23 ± 14.44	10.63 ± 19.75	26.33 ± 32.74	5.46 ± 6.19
Mean % post-implantation loss (± SD)	4.23 ± 4.71	8.81 ± 15.47	1.92 ± 3.85	1.28 ± 3.14
% Males per litter	60.49	48.92	41.24	60.04
Mean male fetal weights per litter (± SD)	5.303 ± 0.215	5.203 ± 0.150	5.557 ± 0.218	5.220 ± 0.330
Mean female fetal weights per litter (± SD)	5.029 ± 0.253	4.971 ± 0.217	5.210 ± 0.080	4.917 ± 0.316

Results of the fetal external and visceral heart/great vessel examinations were reported in the individual animal data. No external or visceral malformations were observed.

Prenatal Developmental Toxicity Study (Vriends, 2022b)

This study was conducted in compliance with GLP and according to OECD TG No. 414 (2018). Time-mated female Wistar Han rats (22/group) were dosed by oral gavage with 0, 100, 300 or 1000 mg/kg/day of PC-2020-926 in water at a dosing volume of 1.695 mL/kg. These formulations were adjusted to account for water content of the test material using a correction factor of 2 and the top dose administered (1000 mg/kg/day) is the limit dose for this test. Dosing was from GD 6 though GD 20.

Methods in Brief. Dams were checked twice daily for mortality/morbidity and at least once daily for clinical signs; detailed clinical observations were conducted on GD 2, 6, 15 and 21. Body weights and food consumption were measured on GDs 2, 6, 9, 12, 15, 18, and 21. At sacrifice on

GD 21, blood was collected from the dams for assessment of thyroid hormones (T3, T4, and TSH). Dams were subjected to examination of the thoracic and abdominal cavities. Uterus and thyroid glands were weighed, and the thyroid collected for histopathologic examination. Data regarding litter indices were collected. Uteri of non-pregnant rats were stained for identification of implantation sites. All live fetuses were sexed, weighed and examined for external anomalies; AGD (normalized to the cube root of fetal body weight) was also measured. One half of the fetuses in each litter was examined by fresh dissection for visceral anomalies of the body and by Wilson's technique for visceral anomalies of the head. The other half of the fetuses in each litter was subjected to skeletal examination using Alizarin Red S staining. Fetal data were appropriately assessed based on litter means.

Results. No treatment-related mortality or clinical signs of toxicity were observed. At 300 mg/kg/day, a single dam was euthanized *in extremis* on GD 9 after exhibiting clinical signs, reduced food consumption and body weight loss; at necropsy, the intestines were found to be filled with gas. Because similar findings were not seen in other animals in this or the highest dose group, the death was considered to be unrelated to treatment. Increased salivation after dosing was observed in all dams at 1000 mg/kg/day; this finding was considered by the laboratory to be a physiological response to the test article rather than a clinical sign of toxicity. No treatment-related effects on body weight or food consumption were observed. Serum levels of T3, T4 and TSH were similar across treatment groups, and thyroid organ weights and histopathology were not changed from control.

Fetal litter parameters and malformation data are presented in Table 8. No treatment-related effects were observed on the number of pregnant females per group, the numbers of corpora lutea and implantation sites, early and late resorptions, pre- and post-implantation loss, the numbers of live and dead fetuses per litter, fetal sex ratio, fetal weights or fetal AGD measures.

Table 8. Litter parameters and fetal anomalies data from the prenatal developmental toxicity study of PC-2020-926 (Vriends, 2022b)

Dose (mg/kg/day):	0	100	300	1000
# Females on study	22	22	22	22
# Euthanized or died on study	0	0	1	0
# Pregnant at scheduled necropsy (%)	21 (95.5)	22 (100)	20 (95.2)	22 (100)
Litter parameters				
Mean # corpora lutea per litter (± SD)	12.6 ± 1.8	13.5 ± 1.7	13.6 ± 1.7	12.7 ± 3.0
Mean # implantations per litter (± SD)	12.0 ± 1.9	12.4 ± 1.7	12.9 ± 1.4	11.8 ± 2.3
Mean # dead fetuses per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean # viable fetuses per litter (± SD)	11.4 ± 2.0	11.6 ± 2.0	12.2 ± 2.0	11.3 ± 2.3
Mean # early resorptions per litter (± SD)	0.6 ± 0.9	0.7 ± 0.9	0.8 ± 1.0	0.5 ± 0.7
Mean # late resorptions per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean % pre-implantation loss (± SD)	4.88 ± 10.91	7.96 ± 7.40	4.84 ± 6.90	6.55 ± 7.65
Mean % post-implantation loss (± SD)	4.64 ± 7.23	6.03 ± 7.80	6.12 ± 7.96	3.66 ± 5.83
% Males per litter	50.82	46.36	50.00	50.89
Mean male fetal weights per litter (± SD)	5.207 ± 0.203	5.289± 0.253	5.297 ± 0.265	5.308 ± 0.348
Mean female fetal weights per litter (± SD)	4.960 ± 0.194	5.044 ± 0.205	4.998 ± 0.256	5.105 ± 0.265
Mean male corrected AGD (± SD)	1.794 ± 0.126	1.805 ± 0.076	1.782 ± 0.093	1.751 ± 0.103
Mean female corrected AGD (± SD)	0.828 ± 0.083	0.796 ± 0.068	0.822 ± 0.081	0.813 ± 0.104
Fetal anomalies data				
# Fetuses (litters) examined externally	239 (21)	256 (22)	243 (20)	249 (22)
# Fetuses (litters) with external malformations	0 (0)	1 (1)	0 (0)	0 (0)
# Fetuses (litters) examined viscerally	119 (21)	128 (22)	122 (20)	125 (22)
# Fetuses (litters) with visceral malformations	0 (0)	2 (2)	0 (0)	0 (0)
# Fetuses (litters) examined skeletally	120 (21)	128 (22)	121 (20)	124 (22)
# Fetuses (litters) with skeletal malformations	1 (1)	0 (0)	0 (0)	1 (1)
Total # fetuses (litters) with anomalies	1 (1)	2 (2)	0 (0)	1 (1)

The individual fetuses with malformations are shown in Table 9. No treatment related external, visceral, or skeletal malformations were observed. No visceral findings of the head were reported.

With regard to cardiovascular findings, we note that these were observed in two fetuses in the low dose groups only.

Table 9. Malformation data by individual fetuses for the prenatal developmental toxicity study of PC-2020-926 (Vriends, 2022b)

Dose (mg/kg/day):	Finding
0	Fetus 11-L1 – lumbar centrum, 1 or more absent (S)
100	Fetus 28-R7 – omphalocele (E), aortic arch interrupted (V), ventricular septal defect (V), trachea cartilage rings absent (V) Fetus 31-L6 – transposition of the great vessels (V), ventricular septal defect (V),
300	---
1000	Fetus 88-R10 – sternoschisis (S)

E = external finding; S = skeletal finding; V = visceral finding

No external variations were noted. Visceral variations were limited to supernumerary liver lobes, convoluted and dilated ureters and absent renal papilla, the incidences of which were unrelated to treatment. Skeletal examination revealed a diverse array of variations, none of which showed a relationship with treatment.

Based on these data, the laboratory concluded that, in the absence of any observed effects, the NOAELs for maternal toxicity and developmental toxicity were both 1000 mg/kg/day, the highest dose tested. We agree with these calls.

90-Day Study (Gerding, 2022)

This study was conducted in compliance with GLP and according to OECD TG No. 408; the exact version of the OECD guideline followed is not indicated, but based on when the study was completed, it is assumed to be the most recent (2018) version. Wistar Han rats (10/sex per group) were dosed for 90 days by oral gavage with 0, 100, 300 or 1000 mg/kg/day of PC-2020-926 in water at a dosing volume of 1.695 mL/kg. The dosing formulations were adjusted to account for water content of the test material using a correction factor of 2 and the top dose administered (1000 mg/kg/day) is the limit dose for this test. An additional 5/sex per group in the control and high dose groups were dosed as described and maintained for a 28-day recovery period post-dosing to address reversibility of any observed effects.

Methods in Brief. The rats were checked twice daily for mortality/morbidity, at least once daily for clinical signs and once weekly for arena observations. Body weights and food consumption were measured weekly. Estrus cyclicity was assessed in female rats at the end of treatment and at the end of the recovery period. Functional tests were conducted on 5 rats/sex per group in week 13. An ophthalmic examination was conducted on control and high dose rats in week 13. At sacrifice of both main study and recovery animals, blood was collected for assessment of hematology, clinical chemistry, and thyroid hormones (T3, T4, and TSH). At the end of both the main study and the recovery period, select organs were weighed (all groups) and tissues collected for histopathologic examination (control and high dose groups).

Results. No mortality was observed. Abnormal breathing sounds were noted in all treated groups, with a dose-dependent increase in incidence; deep, labored, or shallow breathing was also seen incidentally in all treated groups. Another noted clinical sign was retching in all female treated groups and in males at 100 and 1000 mg/kg/day. At ≥300 mg/kg/day, salivation and ploughing were noted, which the laboratory considered to be a physiological response to the taste of the test material and not toxicologically relevant. There were no effects of treatment on body weights, body weight gains or food consumption. With regard to functional observations, motor activity was reduced in females at 1000 mg/kg/day, but mean values were reported by the testing laboratory to be within the HCD range. No effects of treatment were observed in the ophthalmic examinations or with regard to estrous cyclicity. In males, red blood cell counts, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were significantly increased and triglycerides non-significantly in males at 1000 mg/kg/day; these values were again reported by the laboratory to be within the HCD range, and at the end of the recovery period, comparable to control. T4 concentrations were increased in females at 100 and 1000 mg/kg/day but were considered by the laboratory to be within HCD range; no effects were seen on T3 or TSH concentrations. Compared to control, absolute weights of the kidneys and liver were significantly increased in females at 1000 mg/kg/day by 18% and 20%, respectively. The weights of these organs relative to body weight were also increased 12-14%, but these differences from control disappeared during the recovery period. At necropsy, 3 of 10 males at 1000 mg/kg/day were observed with minimal hyperplasia of the goblet cells of the rectum; no such findings were noted at the end of the recovery period. We are unaware of the significance of goblet cell hyperplasia.

Squamous cell hyperplasia of the non-glandular stomach (accompanied by hyperkeratosis) was seen in 1 of 10 and 3 of 10 females at 300 and 1000 mg/kg/day, respectively; this finding was not reported in high dose animals at the end of the recovery period. At 1000 mg/kg/day, focal erosion of the non-glandular and glandular portions of the stomach were also noted in 1 of 10 females at the end of the dosing period and in 1 of 5 females at the end of the recovery period; this finding was interpreted by the laboratory to be indicative of a dosing procedure-related event rather than an effect of treatment. We disagree with this interpretation in that the findings do not seem consistent with gavage error. Thus, we cannot discount that they may possibly be treatment related.

Based on these data, the NOAEL for systemic toxicity was determined by the laboratory to be 1000 mg/kg/day, the highest dose tested. In our opinion, the NOAEL in females may be 100 mg/kg/day based on squamous cell hyperplasia with hyperkeratosis of the non-glandular stomach in 1 and 3 females at 300 and 1000 mg/kg/day, respectively. The NOAEL for males may be 300 mg/kg/day based on goblet cell hyperplasia of the rectum in 3 males at 1000 mg/kg/day. It is noted, however, that the results of this study generally support the NOAEL for maternal toxicity determined in the prenatal developmental toxicity study of PC-2020-926 (1000 mg/kg/day), as histopathologic examination is not a part of the prenatal study design, and therefore, the findings of concern from the 90-day study would not have been observed. Further, the prenatal study involves dosing for a shorter duration; thus, it is very possible that the findings from the 90-day study would not have yet developed by the end of dosing in the prenatal study.

Sodium lauroamphoacetate

Dose Range-finding Prenatal Study (Langedijk, 2022)

In preparation for a definitive prenatal developmental toxicity study, a DRF study was conducted. The study was not GLP compliant but generally followed OECD TG No. 414 (2018) with exceptions for the number of animals per group, fetal visceral examinations limited to the heart and great vessels only, and no fetal skeletal examinations. Time-mated female Wistar Han rats (6/group) were dosed by oral gavage with 0, 300, 600 or 1000 mg/kg/day of sodium lauroamphoacetate in water at a dosing volume of 2.596 mL/kg. These formulations were adjusted

to account for the water content of the test material using a correction factor of 2.83. Dosing was from GD 6 though GD 20.

Methods in Brief. Dams were checked twice daily for mortality/morbidity and at least once daily for clinical signs; detailed clinical observations were conducted on GD 2, 6, 15 and 21. Body weights and food consumption were measured on GDs 2, 6, 9, 12, 15, 18, and 21. On GD 21, dams were subjected to examination of the thoracic and abdominal cavities. Data regarding litter indices were collected. Uteri of non-pregnant rats were stained for identification of implantation sites. All live fetuses were sexed, weighed, and examined for external anomalies. All live fetuses in each litter were also examined for visceral anomalies of the heart and great vessels. Fetal data were appropriately assessed based on litter means.

Results. A single female at the mid-dose was found dead on GD 15; this death was attributed to gavage error. No treatment-related mortality was observed. Clinical signs of abnormal breathing sounds were noted for individual animals in all treated groups. Maternal body weight in the treated groups was comparable to control. Fetal litter parameters and malformation data are presented in Table 10. Compared to control, the mean number of implantations and the mean number of live fetuses was significantly increased and mean fetal weights were significantly reduced in the high dose group. The reduced fetal weight at the high dose, however, appears to be a function of the increased number of fetuses per litter (13.3 vs 9.5 in the control group) as the mean total litter weight in the high dose group was much greater than that of the control group (66.1 g compared to 50.7 g in the control group).[1] No treatment-related effects were observed on the number of pregnant females per group, the numbers of corpora lutea, early and late resorptions, the number of dead fetuses per litter, or fetal sex ratio.

[1] Calculated based on reported mean number of fetuses per litter and mean fetal weights.

Table 10. Litter parameters and fetal anomalies data from the DRF prenatal developmental toxicity study of sodium lauroamphoacetate (Langedijk, 2022)

Dose (mg/kg/day):	0	300	600	1000
# Females on study	6	6	6	6
# Euthanized or died on study	0	0	1	0
# Pregnant at scheduled necropsy (%)	5 (83.3)	6 (100)	5 (83.3)	6 (100)
Litter parameters				
Mean # corpora lutea per litter (± SD)	10.8 ± 5.5	12.8 ± 0.4	12.2 ± 2.0	14.8 ± 1.0
Mean # implantations per litter (± SD)	10.2 ± 4.8	12.7 ± 0.8	11.4 ± 1.3	13.8* ± 1.2
Mean # dead fetuses per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean # live fetuses per litter (± SD)	9.5 ± 4.8	11.3 ± 2.0	11.0 ± 1.2	13.3* ± 1.0
Mean # early resorptions per litter (± SD)	0.7 ± 0.8	1.3 ± 1.4	0.4 ± 0.5	0.3 ± 0.5
Mean # late resorptions per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.4
Mean % pre-implantation loss (± SD)	4.17 ± 7.57	1.39 ± 3.40	5.71 ± 7.82	6.69 ± 5.79
Mean % post-implantation loss (± SD)	20.40 ± 39.40	10.96 ± 11.61	3.33 ± 4.56	3.51 ± 3.88
% Males per litter	51.83	36.51	47.37	50.05
Mean male fetal weights per litter (± SD)	5.500 ± 0.350	5.617 ± 0.258	5.296 ± 0.272	5.111 ± 0.126
Mean female fetal weights per litter (± SD)	5.139 ± 0.225	5.192 ± 0.175	5.102 ± 0.172	4.815§ ± 0.126

* p≤0.05 by Kruskal-Wallis followed by Dunn test
§ p≤0.05 by ANOVA followed by Dunnet's test

Results of the fetal external and visceral heart/great vessel examinations were reported in the individual animal data. No external or visceral malformations were observed.

Prenatal Developmental Toxicity Study (van Otterdijk, 2022)

This study was conducted in compliance with GLP and according to OECD TG No. 414 (2018). Time-mated female Wistar Han rats (22/group) were dosed by oral gavage with 0, 100, 300 or 1000 mg/kg/day of the sodium lauroamphoacetate in water at a dosing volume of 2.596 mL/kg. These formulations were adjusted to account for the water content of the test material using a correction factor of 2.83 and the top dose administered (1000 mg/kg/day) is the limit dose for this test. Dosing was from GD 6 though GD 20.

Methods in Brief. Dams were checked twice daily for mortality/morbidity and at least once daily for clinical signs; detailed clinical observations were conducted on GD 2, 6, 15 and 21. Body

weights and food consumption were measured on GDs 2, 6, 9, 12, 15, 18, and 21. At sacrifice on GD 21, blood was collected from the dams for assessment of thyroid hormones (T3, T4, and TSH). Dams were subjected to examination of the thoracic and abdominal cavities. Uterus and thyroid glands were weights and the thyroid collected for histopathologic examination. Data regarding litter indices were collected. Uteri of non-pregnant rats were stained for identification of implantation sites. All live fetuses were sexed, weighed and examined for external anomalies; AGD (normalized to the cube root of fetal body weight) was also measured. One half of the fetuses in each litter was examined by fresh dissection for visceral anomalies of the body and by Wilson's technique for visceral anomalies of the head. The other half of the fetuses in each litter was subjected to skeletal examination using Alizarin Red S staining. Fetal data were appropriately assessed based on litter means.

Results. No treatment-related mortality or clinical signs of toxicity were observed. At 300 mg/kg/day, a single dam was found dead on GD 12 due to gavage error. At ≥300 mg/kg/day, rats exhibited increased salivation after dosing, which was considered by the laboratory to be a physiological response to the test article rather than a clinical sign of toxicity. Also, 2 and 4 rats at 300 and 1000 mg/kg/day, respectively, exhibited abnormal breathing sounds between GD 8 and GD 18, typically on a single day of treatment. A transient body weight loss upon initiation of treatment (GD 6-9 interval) was reported for 2 and 3 dams at 300 and 1000 mg/kg/day, respectively. Otherwise, no significant differences from control were observed for body weight or body weight gains. Compared to the control group, food consumption was significantly reduced for the dosing intervals of GD 6-9 and GD 9-12 at doses of 300 mg/kg/day (~10%) and 1000 mg/kg/day (~18-20%). Food consumption from GD 6 to GD 21, was significantly reduced at 1000 mg/kg/day by ~10% compared to control. At 1000 mg/kg/day, mean serum concentrations of total T3 were ~77% of control values, although the individual values were within the HCD range. No treatment related effects were noted on serum concentrations of T4 or TSH, and thyroid organ weights and histopathology were not changed from control. At necropsy, 12 of 22 dams at 1000 mg/kg/day exhibited irregular surface of the glandular stomach.

Fetal litter parameters and malformation data are presented in Table 11. No treatment-related effects were observed on the number of pregnant females per group, the numbers of corpora lutea

and implantation sites, early and late resorptions, pre- and post-implantation loss, the numbers of live and dead fetuses per litter, fetal sex ratio, fetal weights or fetal AGD measures.

Table 11. Litter parameters and fetal anomalies data from the prenatal developmental toxicity study of sodium lauroamphoacetate (van Otterdijk, 2022)

Dose (mg/kg/day):	0	100	300	1000
# Females on study	22	22	22	22
# Euthanized or died on study	0	0	1	0
# Pregnant at scheduled necropsy (%)	21 (95.5)	21 (95.5)	20 (95.2)	22 (100)
Litter parameters				
Mean # corpora lutea per litter (± SD)	13.0 ± 1.6	12.7 ± 2.1	12.9 ± 1.4	13.0 ± 2.1
Mean # implantations per litter (± SD)	12.2 ± 1.8	11.2 ± 2.31	12.2 ± 1.5	12.4 ± 2.4
Mean # dead fetuses per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean # viable fetuses per litter (± SD)	11.9 ± 1.6	10.9 ± 2.5	11.8 ± 1.6	12.0 ± 2.6
Mean # early resorptions per litter (± SD)	0.3 ± 0.7	0.4 ± 0.8	0.4 ± 0.5	0.4 ± 1.1
Mean # late resorptions per litter (± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean % pre-implantation loss (± SD)	5.71 ± 8.38	11.69 ± 11.69	5.29 ± 7.21	4.78 ± 9.22
Mean % post-implantation loss (± SD)	2.46 ± 4.75	3.73 ± 8.24	3.29 ± 4.15	3.39 ± 9.85
% Males per litter	48.28	46.24	51.93	48.74
Mean male fetal weights per litter (± SD)	5.275 ± 0.290	5.334 ± 0.323	5.378 ± 0.179	5.246 ± 0.272
Mean female fetal weights per litter (± SD)	4.987 ± 0.237	5.074 ± 0.260	5.065 ± 0.192	4.980 ± 0.338
Mean male corrected AGD (± SD)	1.774 ± 0.093	1.755 ± 0.113	1.730 ± 0.099	1.759 ± 0.109
Mean female corrected AGD (± SD)	0.804 ± 0.090	0.798 ± 0.089	0.778 ± 0.096	0.783 ± 0.080
Fetal anomalies data				
# Fetuses (litters) examined externally	249 (21)	228 (21)	235 (20)	263 (22)
# Fetuses (litters) w/ external malformations	0 (0)	0 (0)	0 (0)	1 (1)
# Fetuses (litters) examined viscerally	124 (21)	115 (21)	119 (20)	132 (22)
# Fetuses (litters) w/ visceral malformations	0 (0)	1 (1)	0 (0)	0 (0)
# Fetuses (litters) w/ head malformations	2 (2)	0 (0)	1 (1)	0 (0)
# Fetuses (litters) examined skeletally	125 (21)	113 (21)	116 (20)	131 (22)
# Fetuses (litters) w/ skeletal malformations	0 (0)	0 (0)	0 (0)	0 (0)
Total # fetuses (litters) with anomalies	2 (2)	1 (1)	1 (1)	1 (1)

The individual fetuses with malformations are shown in Table 12. No treatment related external, visceral, or skeletal malformations were observed. Further, no cardiovascular findings were noted.

Table 12. Malformation data by individual fetuses for the prenatal developmental toxicity study of sodium lauroamphoacetate (van Otterdijk, 2022)

Dose (mg/kg/day):	Finding
0	Fetus 05-L2 – small eye lens, right (V) Fetus 16-R7 – small eye, right (V)
100	Fetus 39-L5 – situs inversus (V)
300	Fetus 48-L9 – large eye lens, left (V)
1000	Fetus 84-L3 – subcutaneous edema (E)

E = external finding; S = skeletal finding; V = visceral finding

No external variations were noted. Visceral variations of supernumerary liver lobes, convoluted ureters, and a fluid-filled thorax were seen, but the incidences were considered unrelated to treatment. A diverse array of skeletal variations was noted across all groups without relation to treatment.

Based on these data, the laboratory concluded that, in the absence of any observed effects, the NOAELs for maternal toxicity and developmental toxicity were both 1000 mg/kg/day, the highest dose tested. We agree with these calls.

3. Assessment

A total of four commercial amphoacetate surfactant products have been evaluated in combined repeat-dose reproduction and developmental toxicity screening tests or prenatal developmental toxicity studies conducted in Wistar Han rats. In all definitive studies, mated rats received the test article by oral gavage at doses of 0 (control), 100, 300 or 1000 mg/kg/day. In the combined repeat-dose and reproduction/developmental toxicity screening tests, dosing occurred for two weeks prior to mating and throughout gestation. In the prenatal developmental toxicity studies, the treatment period occurred during presumed GDs 6-19.

In the five reproductive and developmental studies under consideration, the parental and developmental NOAELs were the highest dose tested (1000 mg/kg/day) for all test articles except Dehyton® DC. It is additionally noted that the developmental NOAEL for Miranol Ultra C32 was not based on a prenatal development toxicity study, but rather, on results from the 28-day repeat-dose reproductive and developmental toxicity screening test.

Parental NOAELs

Although Dehyton® DC was tested in a prenatal development toxicity study, it was also the subject of a 28-day combined repeat-dose and reproductive and developmental toxicity screen test wherein four of ten high-dose females died or exhibited signs of toxicity, resulting in premature euthanasia of the entire group on GD 14 due to humanitarian concerns. The premature euthanasia of pregnant dams, in turn, meant that there were no high dose (1000 mg/kg/day) fetuses to examine at term and the mid dose (300 mg/kg/day) became the *de facto* NOAEL for both maternal and developmental toxicity. The study director considered the maternal deaths to be test article related, which contributed to the determination of the mid-dose as the NOAEL. However, the data surrounding this call are complex and require further discussion.

Necropsies of the four deceased high-dose dams revealed morphologic findings in the respiratory tracts including erosions and/or ulceration of epithelium of the trachea and bronchi. There were no reports of irritation or ulceration in the esophagi of these animals. The study director ascribed

the findings to "regurgitation" and considered the findings to be test article related; however, necropsies of the remaining six high-dose dams found no lesions in the esophagus, trachea or bronchi. The absence of respiratory tract findings in any of the other female rats in this group, the absence of findings in the esophagus (which must be traversed to reach the respiratory tract in cases of regurgitation), the inability of the rat to vomit, and the lack of similar findings among the male rats suggest that the limited number of lesions may have been due to the gavage procedure. Importantly, neither the prenatal development toxicity study nor the 90-day oral gavage study of Dehyton DC in rats conducted at the same dose levels reported any lesions of the esophagus or respiratory tract. The absence of respiratory effects is of interest because of the much longer dosing periods (~50 days versus 90 days). There is, however, a difference between the dose volumes administered in the repeat-dose 28-day study (5 mL/kg) versus the other two studies 1.796 mL/kg and 1.895 mL/kg). It is likely that the reduced dosing volume reduced the likelihood of the dosing fluid escaping from the stomach. Notably, due to the differences in dosing volume, the concentration of test article at the high dose of 28-day study was 200 mg/mL whereas the concentration in the 90-day study was 557 mg/mL. In the 90-day study of PC-2020-926 (high-dose volume of 1.695 mL; concentration of test article: 590 mg/mL), the stomachs of females in the high (3/10) and mid-dose (1/10) groups exhibited epithelial hyperplasia/hyperkeratosis of the non-glandular stomach. These findings were not present at the end of the recovery period. No macroscopic changes of the viscera were reported in the prenatal development toxicity study of PC-2020-926; however, no histopathology of the stomach was conducted. Taken together, it is not possible to exclude that the lesions in the respiratory tracts of high-dose Dehyton DC treated dams were the result of the gavage procedure; consequently, these findings in the respiratory tract should not be considered as the basis for maternal toxicity. Taken in combination with the determination that 1000 mg/kg/day is the NOAEL for maternal/adult female toxicity in the other two studies, 1000 mg/kg/day might more appropriately be considered the maternal NOAEL for Dehyton® DC in the 28-day combined repeat-dose and reproductive and developmental toxicity screen test.

Developmental NOAELs

The developmental toxicity potential of the amphoacetates as a group appears to be low. With the exception of Dehyton® DC, the study directors determined the developmental toxicity NOAEL

for each of the amphoacetates to be 1000 mg/kg/day. However, due to the occurrence of several cardiovascular malformations in all Dehyton® DC treated groups in the prenatal development toxicity study, the study director did not identify a developmental NOAEL.

The collated data for this assessment were obtained from investigations of four commercial amphoacetate surfactant products with remarkably similar chemical structures (varying only in C-chain length and the proportion/ratio of monoacetate and diacetate forms). Their structural and compositional similarity allows for the findings across these compounds to be grouped, provided that the study designs are comparable.

Among the study reports supplied to us were two DRF prenatal developmental toxicity studies and three prenatal development toxicity studies conducted using Wistar Han rats. Visceral malformations of the cardiovascular system were observed in two of the three definitive studies; no cardiovascular malformations were seen in the two DRF studies, despite specific examination of the fetal hearts and great vessels in these studies . Because cardiac and other cardiovascular malformations can only be detected in prenatal development toxicity studies, the following assessment is based on visceral data for the three amphoacetates (Dehyton® DC, PC-2020-926, and sodium lauroamphoacetate) that were tested in definitive prenatal development toxicity studies. Low incidences of cardiac and great vessel malformations were observed in treated groups of two of the three studies. Consequently, it was deemed important to determine whether these compounds as a group might alter cardiovascular development. Since the test articles share similar chemical structures and all definitive studies were conducted using the same dose levels (0 [control], 100, 300, or 1000 mg/kg/day), it is instructive to display the cardiovascular malformations, as presented in Table 13 below.

Table 13. Cardiovascular Malformations Reported in Embryofetal Definitive Prenatal Development Toxicity Studies of Amphoacetates[a]

Dose (mg/kg/d)	Test Article	VSD	Trans of Great Vessels	Inter-rupted Aortic Arch	Right Sided Aortic Arch	Ductus Arteriosus Absent	Ductus Arteriosus Retro-Esophageal	# Affected Fetuses	Total # Fetuses Examined Viscerally
0	D	--	--	--	--	--	--	--	115
	P	--	--	--	--	--	--	--	119
	L	--	--	--	--	--	--	--	124
100	D	1	1	1			1	2	117
	P	2	1	1	--	--	--	2	128
	L	--	--	--	--	--	--	--	115
300	D	1				1	--	1	107
	P	--	--	--	--	--	--	--	122
	L	--	--	--	--	--	--	--	119
1000	D	1	--	--	1	--	--	1	114
	P	--	--	--	--	--	--	--	125
	L	--	--	--	--	--	--	--	132

D = Dehyton DC; P = PC-2020-926; L = sodium lauroamphoacetate; VSD = Ventricular Septal Defect

[a] The heart and great vessels were evaluated in the DRF studies for PC-2020-926 and sodium lauroamphoacetate and no malformations were found; however, because these studies were conducted with only 6 dams per group and used different doses, they were not included in this table.

Further support for the absence of compound-related cardiovascular defects for PC-20-926 and sodium lauroamphoacetate is available from the preliminary dose range finding studies (DRFs) for these chemicals. In both cases, the test agents were administered at doses of 0, 300, 600 or 1000 mg/kg bw/day via oral gavage from GD 6 – GD 19 to groups of 6 mated rats. Test agents did not increase the resorption rates or decrease the mean number of pups per litter. Mean fetal weights were not adversely affected. Fetuses were subjected to gross examination and a modified visceral examination that included the great vessels and the heart. No visceral malformations were reported in either DRF study.

Assessment by type of malformation

The overall incidence of malformations reported across the studies, both within individual studies and in all studies in total, were low. However, most malformations identified during the visceral examinations were related to the cardiovascular system (heart and great vessels). Among the three definitive prenatal development toxicity studies, the majority of the cardiovascular findings (8 of 12) were in the low dose groups and the incidences were not dose responsive. Thus, the individual malformations data do not support there being an effect due to treatment with the amphoacetates.

Further, it does not appear that these findings are indicative of a low dose effect. The condition when adverse effects occur at low doses, but not at higher doses, has been termed a "non-monotonic" dose response relationship (Vandenberg et al, 2012). Much of the data to support this concept comes from cell-based systems and involves hormones or endocrine disrupting chemicals (Vandenberg, 2012); however, data in whole animals is scant (Rhomberg and Goodman, 2012). In the case of developmental data, what may seem to be a non-monotonic effect (i.e., malformations observed in low dose animals but not in the higher dose groups) occasionally occurs because the higher doses either kill the offspring or cause severe toxicity in the pregnant dam such that she either resorbs her litter or dies. In either case, there would be fewer (or no) exposed near-term fetuses to examine in the higher dose groups, and those that remain represent a less sensitive population. In the current data set, however, there were no significant incidences of severe maternal toxicity or total litter losses at the higher doses.

Assessment by malformed fetus

The embryologic development of the heart and the aortic arch system that gives rise to the great vessels is complex. Perturbations of the morphogenetic processes involved in development of this region underlie multiple malformations that often occur together. Merely counting the number of malformations often overstates the significance of the problem. Among the three prenatal development toxicity studies considered in the present report, there were a total of 12 cardiovascular findings that occurred in a total of 6 fetuses. The distribution of malformed fetuses included 4 fetuses in the combined low dose groups and one fetus in each of the combined mid- and high-dose groups. Again, there was no dose-response when considered on a malformed fetus basis.

Note that cardiovascular malformations can be detected only during the visceral examinations that are conducted as part of a prenatal development toxicity study. As a result, roughly one half of the fetuses were examined viscerally. The grand totals of fetuses that underwent visceral examinations in the combined dose groups of the three prenatal development toxicity studies were:

0 mg/kg bw/day:	358 fetuses
100 mg/kg bw/day:	360 fetuses
300 mg/kg bw/day:	348 fetuses
1000 mg/kg bw/day:	371 fetuses

Inspection of the table reveals that a total of 12 cardiovascular malformations were reported in the treated groups; however, the malformations occurred in a total of 6 fetuses. Notably, 8 of the cardiovascular malformations (in 4 fetuses) occurred in the low dose group; two cardiovascular malformations in a single fetus were reported in each of the mid- and high dose groups. Thus, there is no dose-response when the data are considered on either a malformation basis or on the basis of malformed fetuses.

Assessment by perturbed morphogenetic process

Despite there being no clear dose-response for the observed cardiovascular findings, it is important to understand if there is a link between amphoacetate exposure and congenital heart defects. First, it should be recognized that congenital heart malformations are rarely reported in rats, perhaps due to the small size of their hearts. This means that a finding could be missed if one relies on only a single study. Among these three studies, there were low incidences of cardiac and great vessel malformations in two of the three studies.

Second, the anatomy of the heart and its embryology are complex. Because different individual cardiac defects can be caused by perturbation of a single morphogenetic process, it is possible to combine the findings of defects that result from the same morphogenetic process. As an analogy, consider for example the effect an earthquake might have on a town. The major road passes multiple buildings (e.g., the church, the school, the town hall, etc.). Because the road is destroyed, the entrances to each of these buildings may also be ruined. In a damage report, the entrances to

each of the affected buildings may be all reported separately; however, the underlying event that caused the damage (the earthquake-induced damage to the major road) is the same in all cases. The changes at each building are not independent events. In another town, the set of affected buildings due to the earthquake-induced damaged major road may be different (e.g., the department store, the firehouse, the police station), but the underlying problem is the same. By looking only at individual buildings, one can miss the bigger issue, which is that all findings were caused by the damaged major road. The situation is similar with regard to teratogen-induced malformations. The rationale for grouping defects by the embryologic process that might be perturbed is based on the well-accepted tenet that teratogens interact with embryos via specific mechanisms to cause malformation (Wilson, 1959; 1973). With regard to cardiac embryology, a set of morphogenetic processes has been proposed to underlie most of the major anatomic features of the heart and great vessels (Clark, 1986). Using an approach that allows for the grouping of various cardiac defects by a common perturbed morphogenetic process has been used to assess the potential teratogenicity of other substances (e.g., Watson et al, 2006). Additionally, it must be recognized that a given teratogen may cause malformations by means of multiple mechanisms (DeSesso and Goeringer, 1990).

In an attempt to increase the likelihood of discerning a potential class effect of amphoacetate exposure, we combined the results of all three definitive prenatal development toxicity studies and grouped the reported cardiac defects according to the underlying morphogenetic process that would have been perturbed. Thus, the reported malformations can be sorted as follows.

> Cellular migration and targeted growth. Cardiac neural crest cells and cells from the pharynx migrate into the heart and great vessels to form a population of cells that grow into the lumen of the truncus arteriosus, where they underlie successful development of the aorticopulmonary septum and the membranous portion of the interventricular septum. Perturbation of these processes can result in transposition of the great vessels and membranous ventricular septal defect. Thus, the incidences of these two malformations can be combined for analysis.
>
> Hemodynamics and cellular death. Remodeling of the aortic arch system depends upon differential blood flow strength and patterns (hemodynamics) and removal of unnecessary

potions of the vessels (controlled cellular death). Perturbations of these processes can result in aberrant great vessel patterns, including interrupted aortic arch, right sided aortic arch, and absence/misplacement of the ductus arteriosus. Thus, the incidence of these malformations can be pooled for analysis.

The merged malformation data categorized by morphogenetic process are displayed in Table 14 below. Most incidences of perturbed morphogenetic processes occurred in the low dose group, with only single occurrences in each of the mid- and high dose groups. Thus, there is no indication of a dose-response for either of the morphogenetic processes that underlie the cardiovascular malformations reported in the prenatal development toxicity studies.

Table 14. Incidence of Cardiovascular Malformations Grouped by Perturbed Morphogenetic Process in Combined Embryofetal Development Studies of Amphoacetates

Dose (mg/kg/day)	Cellular Migration & Targeted Growth	Hemodynamics and Targeted Cellular Death
0	0	0
100	5	3
300	1	1
1000	1	1

Taken together, the combined data do not support a causal relationship between the amphoacetates tested in the prenatal development toxicity studies and malformations of the heart and great vessels.

Non-mammalian Data

The results of the Fish Early-life-Stage (FELS) Toxicity Test using Miranol Ultra C32 (Tobor-Kaplon, 2019) are consistent with the mammalian data in that no gross alterations in cardiac development were reported. However, it must be noted fish hearts differ substantially from mammalian hearts in that fish hearts have only 2 chambers, undergo limited cardiac looping, and have a single circuit circulatory system making cardiovascular development much simpler than in mammals (Tang et al, 2018; Barresi and Gilbert, 2020). Further, the observations in the FELS

test were limited to gross changes in body form and/or concurrent aberrant behavioral during development rather than detailed observation of cardiac development. Observations of the heart included verification of beating; however, the rate of cardiac rhythmicity was not measured and estimates of stroke volume and cardiac output were not made. The latter data, although not typically collected in a FELS test, would have provided more critical indications of normal development (Burggren and Blank, 2009). Nevertheless, the absence of significant cardiac findings at any dose and the overall survival of fish throughout the study provide no reason to suggest that the cardiovascular system is a specific target organ for toxic effects from exposure to Miranol Ultra C32 and, by extrapolation, to the other amphoacetates reviewed herein.

There were, however, post-hatching malformations of the caudal (tail) fin observed among developing larvae in the high exposure (1.6 g/L) group. In the lower exposure groups, some larvae developed non-dose dependent, minor abnormalities that were considered not treatment related. None of these findings were associated with cardiac development.

Potential Role for Impurity

One of the ingredients used in the synthesis of amphoacetates is AEEA and residual amounts may be found in the finished products as an impurity (Foti et al., 2001). To test whether AEEA could cause adverse effects on reproduction and development, Schneider et al. (2012) performed a repeated dose and reproductive and developmental toxicity screening test (OECD 421) with AEEA in rats by oral gavage doses at doses of 0, 50, 250, or 1000 mg/kg/day. The results of that initial experiment and a follow-on experiment in the same study conducted at 0, 0.2, 1, 5, or 50 mg/kg/day found malformations of the great vessels at doses of ≥50 mg/kg/day. The NOAEL for these findings was thus 5 mg/kg/day. These malformations consisted of high aortic arch, aberrant course of the carotid arteries, and aneurysms in the walls of the aorta. Moore et al. (2012) confirmed the great vessel findings and determined that prenatal exposure was sufficient to cause the great vessel anomalies. Importantly, the findings produced by AEEA were all in the great vessels (although considerably different from the defects reported with the amphoacetates) and did not include the cardiac malformations (VSD) reported in the amphoacetate studies under consideration here.

Foti et al. (2001) measured the amounts of AEEA in a variety of preparations of all four amphoacetates used in cosmetics, the AEEA amounts were small and ranged between 4.9-15.3 ppm in the test samples.[2] AEEA analyses were conducted for all amphoacetates discussed in this report and the highest level (14 ppm) was measured in the C8-C18 amphoacetates (Appendix Table). Based on the maximum estimated concentration of residual AEEA (15.3 ppm, Foti et al, 2001), the highest dose of amphoacetates administered in the studies discussed herein (1000 mg/kg/day) would result in a potential AEEA exposure that is 2 orders of magnitude below the NOAEL of 5 mg/kg/day determined by Schneider et al. (2012).[3] It can be thus concluded that residual AEEA in the amphoacetates preparations did not cause the cardiac defects observed in the studies under consideration in this report.

[2] One measurement of Miranol HM Special (1130 ± 30 ppm) was much higher than all others and was stated to have been likely due to faulty purification of the sample.

[3] At 15.3 ppm (15.3 µg/1000 mg), an amphoacetates dose of 1000 mg/kg/day would translate to 15.3 µg/kg/day of AEEA, which is 327x below the AEEA NOAEL of 5 mg/kg/day or 5000 µg/kg/day.

4 Conclusions

The developmental and reproductive toxicity (DART) properties of four commercial amphoacetate surfactant products (Dehyton® DC, Miranol Ultra C32, PC-2020-265, and sodium lauroamphoacetate) were evaluated at doses of 0 (control), 100, 300 or 1000 mg/kg/day. Because there were few adverse fetal cardiovascular findings, the available data from all of the amphoacetates were combined to maximize the ability to discern the presence of adverse reproductive or developmental effects. The parental and developmental NOAELs in the three prenatal developmental toxicity studies (for Dehyton® DC, PC-2020-265, and sodium lauroamphoacetate,) were the highest dose tested (1000 mg/kg/day). The maternal NOAELs for the prenatal developmental toxicity studies of Dehyton® DC and PC-2020-926 are generally supported by results from their respective 90-day repeat-dose studies. It is noted, however, that for Dehyton® DC due to perceived maternally toxic effects at the high dose in the combined 28-day repeat-dose and reproduction/developmental toxicity screening test (OECD 422), the high dose dams were euthanized at GD 14, which precluded examination of fetuses at term and necessitated the call of NOAELs at the next lower dose (300 mg/kg/day). The developmental NOAEL for the fourth amphoacetate (Miranol Ultra C32) was also determined to be 1000 mg/kg/day, but that assessment was based on an OECD 422, which does not include visceral examination.

A low incidence of cardiac / great vessel malformations occurred in each of the three prenatal developmental toxicity studies. None of the malformations was significantly increased and, within each study, the greatest number of malformations occurred in the low dose group. Increased maternal toxicity and/or resorptions/post-implantation loss did not occur at higher doses; thus, there is no evidence to support this being a low-dose effect. In order to discern if there might be a trend for production of cardiovascular malformations, the data for all three definitive prenatal developmental toxicity studies were combined. Whether the combined data were assessed based on the incidences of malformations, number of malformed fetuses, or underlying perturbed morphogenetic processes, there was neither statistical significance nor a dose responsive increase.

Residual amounts of a starting material used to synthesize amphoacetates (AEEA) was also evaluated as a potential causative agent. While AEEA has been reported to cause aneurysms of the great vessels and alterations in the pattern of distribution of the vessels, it did not cause heart defects or any of types of the vessel defects observed in the subject amphoacetate studies. Additionally, the NOAEL for AEEA developmental toxicity is two orders of magnitude above the highest potential AEEA exposure that might occur due to amphoacetate exposure in the studies reviewed herein. Thus, AEEA is not likely to be a factor in any of the defects observed in the subject studies.

Taken together, in-depth analyses of the available developmental and reproductive data for the four subject amphoacetates do not support the classification of these substances as reproductive or developmental hazard. Likewise, in-depth analysis of the cardiac and great vessel systems of fetuses exposed to Dehyton® DC, PC-2020-265, and sodium lauroamphoacetate at doses as high as the limit dose does not support that these substances cause malformation of the target area. This conclusion is also supported by the absence of any treatment-related cardiac abnormalities in both the FELS toxicity test of Miranol Ultra C32 and the dose range-finding studies for PC-2020-265, and sodium lauroamphoacetate (which included visceral examinations of fetal hearts).

5 Limitations

The purpose of this analysis is limited to a review the results from the available DART studies of amphoacetates. This assessment is based on review of the individual study reports, and the authors' combined expertise in developmental and reproductive toxicology. The opinions presented herein are made to a reasonable degree of scientific certainty. Exponent reserves the right to supplement this report and to expand or modify the conclusions and findings based on the review of additional materials as they become available through additional work, or through the review of additional work performed by others. The scope of services performed during this investigation may not adequately address the needs of other users of this report, and any re-use of this report or its findings, conclusions or recommendations as presented herein are at the sole risk of the user.

6 References

Barresi MJ, SF Gilbert. 2020. Developmental Biology, 12th Edition, Sinauer Associates, New York.

Bressers, S. 2019. Prenatal Developmental Toxicity Study of Dehyton® DC by Oral Gavage in Rats. Charles River Laboratories Den Bosch BV. Study No. 20164358. 19 July 2019.

Burggren, W, T Blank. 2009. Physiological study of larval fishes: challenges and opportunities. Scientia Marina, 73(S1), 99–110.

Charles River. 2023. Reproductive Toxicology Historical Control Data for Rats at the Charles River Laboratories. https://www.criver.com/products-services/safety-assessment/toxicology-services/developmental-and-reproductive-toxicology-dart/historical-control-data?region=3601 viewed 12 March 2023.

Clark, EB. 1986. Mechanisms in the pathogenesis of congenital cardiac malformations. In: *Genetics of Cardiovascular Disease*, Pierpont ME, Moller JH, eds. Martinus Nijhoff Publishing Boston, pp. 3–11.

De Raat-Beekhuijzen, MEW. 2018. Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Miranol Ultra C32 by Oral Gavage in Rats. Charles River Laboratories Den Bosch BV. Study No. 518373. 09 July 2018.

DeSesso, JM, GC Goeringer. 1990. The nature of the embryo-protective interaction of propyl gallate with hydroxyurea, Reprod Toxicol **4**: 145–152.

Farn J. 2006. Chemistry and Technology of Surfactants. Oxford: Blackwell Pub. pp. 173–174.

Foti C, D Bonamonte, G. Mascolo, G. Tiravanti, L Rigano, G Angelini. 2001. Aminoethylethanolamine: A new allergen in cosmetics? Contact Dermatitis 45:129-33. doi: 10.1034/j.1600-0536.2001.045003129.

Gerding, L. 2022. A 90-Day Study of PC-2020-926 by Oral Gavage in Wistar Han Rats with a 28-Day Recovery Period. Charles River Laboratories Den Bosch BV. Study No. 20297957. 27 October 2022.

Langedijk J. 2022. Dose Range Finding Study of Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1-ethanol and sodium hydroxide by Oral Gavage in Pregnant Wistar-Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20293263. 16 February 2022.

Moore NP, B. Tornesi, BL Yano, FD Nitschke, EW Carney. 2012. Developmental sensitivity to the induction of great vessel malformations by N-(2-aminoethyl)ethanolamine. Birth Defects Res B Dev Reprod Toxicol. 95:116-22. doi: 10.1002/bdrb.21010.

Organization for Economic Cooperation and Development (OECD). 1998. OECD Guideline for Testing of Chemicals. No. 408. Repeated dose 90-day oral toxicity study in rodents. Adopted 21 September 1998.

Organization for Economic Cooperation and Development (OECD). 2013. OECD Guideline for Testing of Chemicals. No. 210. Fish, early-life stage toxicity test. Adopted 26 July 2013.

Organization for Economic Cooperation and Development (OECD). 2016. OECD Guideline for Testing of Chemicals. No. 422. Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test. Adopted 29 July 2016.

Organization for Economic Cooperation and Development (OECD). 2018. OECD Guideline for Testing of Chemicals. No. 414. Prenatal developmental toxicity study. Adopted 25 June 2018.

Organization for Economic Cooperation and Development (OECD). 2018. OECD Guideline for Testing of Chemicals. No. 408. Repeated dose 90-day oral toxicity study in rodents. Adopted 25 June 2018.

Pels Rijcken, WR. 2018. Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Dehyton® DC by Oral Gavage in Rats. Charles River Laboratories Den Bosch BV. Study No. 518366. 23 November 2018.

Rhomberg LR, JE Goodman. 2012. Low-dose effects and nonmonotonic dose–responses of endocrine disrupting chemicals: Has the case been made? Regul Toxicol Pharmacol 64:130-133.

Schneider S, S Treumann, NP Moore. 2012. Malformations of the great vessels in the neonatal rat induced by N-(2-aminoethyl)ethanolamine. Birth Defects Res B Dev Reprod Toxicol. 95:95-106. doi: 10.1002/bdrb.21006.

Tang JLY, Y Guo, WT Stockdale, K Rana, AC Killen, MTM Mommersteeg, Y Yamamoto. 2018. The developmental origin of heart size and shape differences in *Astyanax mexicanus* populations. Develop Biol 441:271-284.

Tobor-Kaplon, MA. 2019. Fish Early-Life Stage Toxicity Test with Miranol Ultra C32 (Flow-Through). Charles River Laboratories Den Bosch BV. Study No. 20177673. 19 August 2019.

Vandenberg LN, T Colborn, TB Hayes, JJ Heindel, DR Jacobs, Jr., D-H Lee, T Shioda, AM Soto, F. vom Saal, W. Welshons RT Zoeller, JP Myers. 2012. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocrine Reviews 33:378–455.

van Otterdijk, F. 2022. Prenatal Developmental Toxicity Study of Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1- ethanol and sodium hydroxide by Oral Gavage in Time-Mated Wistar Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20293266. 29 August 2022.

Vriends, A. 2022a. Dose Range Finding Study of PC-2020-926 by Oral Gavage in Pregnant Wistar-Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20297966. 06 May 2022.

Vriends, A. 2022b. Prenatal Developmental Toxicity Study of PC-2020-926 by Oral Gavage in Time-Mated Wistar Han Rats. Charles River Laboratories Den Bosch BV. Study No. 20297978. 26 August 2022.

Wagenaar, L. 2019. A 90-Day Study of Dehyton® DC by Oral Gavage in Wistar Rats. Charles River Laboratories Den Bosch BV. Study No. 20164357. 23 July 2019.

Watson, R, CF Jacobson, A Lavin Williams, WB Howard, JM DeSesso. 2006. Trichloroethylene-contaminated drinking water and congenital heart defects: A critical analysis of the literature. Reprod Toxicol **21:** 117-147.

Wilson, JG. 1959. Experimental studies on congenital malformations. J Chron Dis **10**: 111–30.

Wilson, JG. 1973. Environment and Birth Defects. New York: Academic Press.

Appendix

Table. Selected Amphoacetates – Identification and Characteristics.

Identification: Amphoacetates C8-C18		
	Type of substance:	UVCB Monoacetate form (contains appr. 95% monoacetates and 5% diacetates) Diacetate form (contains appr. 40% monoacetates and 60% diacetates)
	IUPAC name:	Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C7-C17 odd-numbered, C17-unsatd. alkyl) derivs. and sodium hydroxide and chloroacetic acid
	Synonyms:	C8-18 Amphoacetates Sodium Cocoamphoacetate Dehyton® DC (Diacetate form) Miranol Ultra C32 (Monoacetate form)
	CAS Number:	-
	Alternative CAS numbers	68650-39-5; 68334-21-4; 68390-66-9; 61791-32-0; 90387-76-1; 68608-65-1
	EC/List Number:	931-291-0
	Molecular Weight (for the CSA):	446 g/mol
	Compositional information (as manufactured, w/w)	
	Water	47-64%
	Total solids:	36-53%
	Total alkylamphoacetate derivatives	27-43%
	NaCl	0-15%
	Sodium glycolate	0-6%
	Alkyl amidoamine	0-3%
	Sodium chloroacetate	0-600 ppm

<table>
<tr><th colspan="3">Identification: Amphoacetates C8-C18</th></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-6 ppm</td></tr>
<tr><td></td><td>Compositional information (solvent free condition, w/w)</td><td></td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>65-86%[4]</td></tr>
<tr><td></td><td>Alkyl chain distribution, Cn</td><td>
<table>
<tr><th>Cn</th><th>Mono#</th><th>Di#</th><th>Total</th></tr>
<tr><td>C8</td><td>0-11%</td><td>0-2%</td><td>0-11%</td></tr>
<tr><td>C10</td><td>0.1-10%</td><td>0-2%</td><td>0-11%</td></tr>
<tr><td>C12</td><td>16-56%</td><td>0-36%</td><td>42-64%</td></tr>
<tr><td>C14</td><td>5-20%</td><td>0-15%</td><td>6-26%</td></tr>
<tr><td>C16</td><td>1-22%</td><td>0-8%</td><td>4-22%</td></tr>
<tr><td>C18</td><td>0.1-16%</td><td>0-7%</td><td>0.1-18%</td></tr>
<tr><td>C18:1 and/or C18:2 [5]</td><td>0-9%</td><td>0-12%</td><td>0-20%</td></tr>
</table>
</td></tr>
<tr><td></td><td>NaCl</td><td>0-26%</td></tr>
<tr><td></td><td>Sodium glycolate</td><td>0-12%[6]</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-6%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-1500ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-14ppm</td></tr>
</table>

Identification: Amphoacetates C12-C14		
	Type of substance:	UVCB Diacetate form only (contains appr. 40 to 45% monoacetate and 55 to 60 % diacetates)
	IUPAC name:	Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C11-C13 odd-numbered alkyl) derivs. and sodium hydroxide and chloroacetic acid
	Synonyms:	Acetic acid, 2-chloro-, reaction products with 2-C11-13-alkyl-4,5-dihydro-1H-imidazole-1-ethanol and sodium hydroxide

[4] The lower range figure for the surfactant fraction is due to the greater difficulty in drying the C8-18 substance and residual water

[5] Number of unsaturations per C18 alkyl chain: 0.001 - 0.15

[6] Analysed as glycolic acid and converted to sodium glycolate as this is the form more likely present in the UVCB substance. Compositional information in registration dossiers may be given as glycolic acid (due to the analytical method) and/or can be also converted to the sodium salt.

<table>
<tr><th colspan="3">Identification: Amphoacetates C12-C14</th></tr>
<tr><td></td><td></td><td>C12-14 Amphoacetates
PC-2020-926
Rewoteric AM2L</td></tr>
<tr><td></td><td>CAS Number:</td><td>1689515-39-6</td></tr>
<tr><td></td><td>Alternative CAS numbers</td><td>66161-62-4; 68608-66-2</td></tr>
<tr><td></td><td>EC/List Number:</td><td>938-645-3</td></tr>
<tr><td></td><td>Molecular Weight (for the CSA):</td><td>367 g/mol</td></tr>
<tr><td></td><td>Compositional information (as manufactured, w/w)</td><td></td></tr>
<tr><td></td><td>Water</td><td>50-51%</td></tr>
<tr><td></td><td>Total solids:</td><td>49-50%</td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>≥39%</td></tr>
<tr><td></td><td>NaCl</td><td>0-10%</td></tr>
<tr><td></td><td>Sodium glycolate</td><td>2-4%</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-2%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-65 ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-5 ppm</td></tr>
<tr><td></td><td>Compositional information (solvent free condition, w/w)</td><td></td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>≥78%</td></tr>
<tr><td></td><td>Alkyl chain distribution, Cn</td><td>
<table>
<tr><th>Cn</th><th>mono</th><th>di</th><th>total</th></tr>
<tr><td>C8</td><td>n.d.[7]</td><td>n.d.</td><td>n.d.</td></tr>
<tr><td>C10</td><td>≤2%</td><td>≤2%</td><td>≤4%</td></tr>
<tr><td>C12</td><td>26-37%</td><td>36-49%</td><td>67-80%</td></tr>
<tr><td>C14</td><td>7-16%</td><td>10-20%</td><td>20-32%</td></tr>
<tr><td>C16</td><td>≤2%</td><td>≤2%</td><td>≤4%</td></tr>
<tr><td>C18</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></tr>
<tr><td>C18:1 and/or C18:2</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></tr>
</table>
</td></tr>
<tr><td></td><td>NaCl</td><td>0-20%</td></tr>
<tr><td></td><td>Sodium glycolate[8]</td><td>6 -11%</td></tr>
</table>

[7] n.d – Not determined.

[8] Analysed as glycolic acid and converted to sodium glycolate as this is the form more likely present in the UVCB substance. Compositional information in registration dossiers may be given as glycolic acid (due to the analytical method) and/or can be also converted to the sodium salt.

Identification: Amphoacetates C12-C14		
	Alkyl amidoamine	0-6%
	Sodium chloroacetate	0-130ppm
	2-(2-aminoethylamino)ethanol	0-14ppm

Identification: Amphoacetates C12		
	Type of substance:	UVCB Monoacetate form only (contains appr. 75 to 100% monoacetate and 0 to 25% diacetates)
	IUPAC name:	Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C11 alkyl) derivs. and sodium hydroxide and chloroacetic acid
	Synonyms:	Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1Himidazole- 1-ethanol and sodium hydroxide Sodium Lauroamphoacetate C12 Amphoacetates EMPIGEN® CDL60/P
	CAS Number:	68608-66-2
	EC Number:	271-794-6
	Molecular Weight (for the CSA):	367 g/mol
	Compositional information (as manufactured, w/w)	
	Water	60-70%
	Total solids:	30-40%
	Total alkylamphoacetate derivatives	23-31%
	NaCl	5-8%
	Sodium glycolate	0.5-4%
	Alkyl amidoamine	0-0.3%
	Sodium chloroacetate	0-5000 ppm
	2-(2-aminoethylamino)ethanol	0-4 ppm

<table>
<tr><th colspan="3">Identification: Amphoacetates C12</th></tr>
<tr><td></td><td>Compositional information (solvent free condition, w/w)</td><td></td></tr>
<tr><td></td><td>Total alkylamphoacetate derivatives</td><td>76-80%</td></tr>
<tr><td></td><td>Alkyl chain distribution, Cn</td><td><table><tr><th>Cn</th><th>mono</th><th>di</th><th>total</th></tr><tr><td>C12</td><td>61-93%</td><td>0.1-21%</td><td>80-99.9%</td></tr><tr><td>Unknown</td><td>-</td><td>-</td><td>0.1-20%</td></tr></table></td></tr>
<tr><td></td><td>NaCl</td><td>16-20%</td></tr>
<tr><td></td><td>Sodium glycolate</td><td>4-8%</td></tr>
<tr><td></td><td>Alkyl amidoamine</td><td>0-0.5%</td></tr>
<tr><td></td><td>Sodium chloroacetate</td><td>0-9000ppm</td></tr>
<tr><td></td><td>2-(2-aminoethylamino)ethanol</td><td>0-10ppm</td></tr>
</table>

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 30, 2023

SUBJECT: Amphopropionates

The following REACH dossiers may provide useful information on the amphopropionate ingredients included in the report on fatty amphocarboxylates.

Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C11-17 and C17 unsatd. alkyl) derivs. and sodium hydroxide and 2-propenoic acid (EC No. 946-533-0)

N-(2-hydroxyethyl)-N-[2-[(1-oxooctyl)amino]ethyl]-ß-alanine (CAS No. 64265-45-8; EC No. 264-761-2)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 18, 2023

SUBJECT: Amphoacetates

Lubrizol Advanced Materials, Inc. 2022. Composition: Schercoteric™ MS-2 50 Imidazolinium Amphoteric (Disodium Cocoamphodiacetate).

Anonymous. 2023. Process flow diagram for Disodium Cocoamphodiacetate.

Lubrizol Advanced Materials, Inc. 2021. Range formula Sulfochem B-NBBSB Surfactant Blend (contains 3-7% Disodium Lauroamphodiacetate).

Lubrizol Advanced Materials, Inc.
9911 Brecksville Road
Cleveland, Ohio 44141-3247
216.447.5000

Schercoteric™ MS-2 50 Imidazolinium Amphoteric

INCI Name: *Disodium Cocoamphodiacetate*

COMPOSITION

Ingredient/INCI name	CAS #	EC #	Function	%
Disodium Cocoamphodiacetate	68650-39-5	272-043-5	Key Ingredient	>33
Water	7732-18-5	231-791-2	Diluent	<55
Sodium Chloride	7647-14-5	231-598-3	Impurity	<12

Lubrizol Advanced Materials, Inc.
9911 Brecksville Road
Cleveland, Ohio 44141-3247
216.447.5000

Sulfochem™ B-NBBSB Surfactant Blend

Range formula

Chemical Name	*CAS #*	*wt %*	*Function*
Water	7732-18-5	58 - 61	Diluent
PEG-80 Sorbitan Laurate	9005-64-5	10 - 18	Key ingredient
Cocamidopropyl Betaine	61789-40-0	8 - 16	Key ingredient
Sodium Trideceth Sulfate	25446-78-0	7 - 12	Key ingredient
Glycerin	56-81-5	4 - 8	Emollient
Disodium Lauroamphodiacetate	68608-66-2	3 - 7	Key ingredient
PEG-150 Distearate	9005-08-7	2 - 6	Viscosity modifier
Sodium Laureth-13 Carboxylate	33939-64-9	1 – 4	Key ingredient
Sodium Benzoate	532-32-1	0.35 – 0.50	Preservative

Process Flow Diagram
Disodium cocoamphodiacetate