OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

WASHINGTON, D.C. 20460

April 22, 2024

MEMORANDUM

SUBJECT: IN-11550 and IN-11782; 1-Propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts; and Cocamidopropyl betaine. Human Health Risk Assessment and Ecological Effects Assessment to Support Inert Ingredient Approval for use in Pesticide Formulations

PC Code: 811661 and 888402
Decision No.: 570243 and 592244
Petition No.: IN-11550 and IN-11782

CAS Reg. No.: 499781-63-4 and 61789-40-0
Registration No.: N/A
Regulatory Action: Addition to inert ingredient list

DEIRDRE SUNDERLAND Digitally signed by DEIRDRE SUNDERLAND Date: 2024.04.22 12:57:21 -04'00'

FROM: Deirdre Sunderland, MHS, Biologist
Chemistry, Inert, and Toxicology Assessment Branch (CITAB)
Registration Division (7505T)

THROUGH: Evisabel Craig, PhD, DABT, Inert Ingredients Team Leader
CITAB
Registration Division (7505T)

TO: Kerry Leifer, Branch Chief
CITAB
Registration Division (7505T)

Contents

1. EXECUTIVE SUMMARY

In 2021, Spring Regulatory Sciences on behalf of Oxiteno USA, LLC, submitted petition IN-11550 to the Environmental Protection Agency (herein referred to as EPA or the Agency). This petition requests an exemption from the requirement of a tolerance for residues of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts (CAS Reg. No. 499781-63-4) when used as an inert ingredient (adjuvant or surfactant) in pesticide formulations under 40 CFR 180.910. A limitation of 25% w/w in pesticide formulations was later proposed by the company.

In 2023, SciReg, Inc., on behalf of Bi-PA NV, submitted petition IN-11782 which requested an exemption from the requirement of a tolerance for residues of 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts (CAS Reg. No. 61789-40-0), also known as cocamidopropyl betaine, when used as an inert ingredient (surfactant) in pesticide formulations under 40 CFR 180.920 at levels up to 10% w/w in pesticide formulations. Based on their similarities, these two chemicals are appropriate for read across; therefore, they have been assessed together. The purpose of this document is to assess the risk to human health and the environment from these compounds when used as inert ingredients in pesticide products.

No chemical specific acute toxicity data is available for 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts; however, data on cocamidopropyl betaine and other surrogate chemicals, show moderate acute toxicity via the oral and dermal route. Based on their physico-chemical properties, acute toxicity via the inhalation route of exposure is expected to be low. In the rat, the oral LD_{50} ranges from 1,500 to 2,600 mg/kg, the dermal LD_{50} is >620 mg/kg.

These chemicals are expected to be moderate skin irritants and severe eye irritants. Several dermal sensitization studies were conducted with cocamidopropyl betaine. Some studies provided negative results for skin sensitization while other suggested skin sensitization potential. These differing results may be due to the varying presence of two manufacturing by-products that are both known skin sensitizers. With proper manufacturing controls, these by-products may be decreased.

The repeated-dose toxicity studies showed no concern for systemic effects. Local irritation was seen in the forestomach of dams in subchronic studies and in one developmental toxicity study following gavage administration. This forestomach irritation likely resulted in the decreased maternal body weight gain and food consumption and the associated developmental effects observed at the highest dose tested (i.e., post-implantation loss and decreased mean fetal body weight). Due to the bolus administration of the compound (which may increase the irritation potential of a chemical), the lack of a forestomach in humans, and the developmental effects occurring at very high doses only, the effects observed are not considered relevant for human health risk assessment.

No oral chronic or carcinogenicity studies are available for 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts or cocamidopropyl betaine. However, there are no structural alerts for carcinogenicity and there is low concern for genotoxicity or mutagenicity based on negative results in mammalian and bacterial genotoxicity tests.

Neurotoxicity and immunotoxicity toxicity studies are not available for review; however, no evidence of neurotoxicity was observed in the neurotoxicity screening performed in the 90-day oral rat study and no evidence of immunotoxicity was seen in the available studies.

Based on the low toxicity and no toxicological endpoint of concern selected, a qualitative assessment is appropriate for all pathways of human exposure (dietary, residential, and occupational) for these two chemicals. As part of its qualitative assessment, the Agency did not use safety factors for assessing risk, and no additional safety factor is needed for assessing risk to infants and children.

Various ecotoxicity studies on freshwater fish, aquatic invertebrates, and freshwater algae showed that cocamidopropyl betaine is moderately toxic to aquatic species. Although there is moderate aquatic toxicity, both chemicals will have a limitation in formulation and are readily biodegradable; therefore, concern for environmental effects are low.

Taking into consideration all available information, EPA concludes that there is a reasonable certainty that no harm to any population subgroup, including infants and children, will result from aggregate exposure to 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts or 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts when considering sources of pesticide exposure for which there is reliable information. Therefore, the use of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts as inert ingredients in pesticide formulations under 40 CFR 180.910 (maximum of 25% w/w), and 40 CFR 180.920 (maximum of 10% w/w), respectively, can be considered assessed as safe under section 408 of the FFDCA.

2. BACKGROUND

In 2021, EPA received a petition (IN-11550) from Spring Regulatory Sciences on behalf of Oxiteno USA, LLC (EPA Company number 89654). This petition requested an exemption from the requirement of a tolerance for residues of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts (CAS Reg. No. 499781-63-4) when used as an inert ingredient (adjuvant or surfactant) in pesticide formulations applied to growing crops and raw agricultural commodities pre- and post-harvest under 40 CFR 180.910. After further discussion with the company, the request was amended to include a limitation of 25% w/w in pesticide formulations to account for the potential for ecotoxicity seen in ecotoxicity studies conducted with a 30% active cocamidopropyl betaine solution.

In 2023, SciReg, Inc., on behalf of Bi-PA NV (EPA Company number 92083), submitted petition IN-11782 which requested an exemption from the requirement of a tolerance for residues of 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts (CAS Reg. No. 61789-40-0), also known as cocamidopropyl betaine, when used as an inert ingredient (surfactant) under 40 CFR 180.920 at levels up to 10% w/w in pesticide formulations.

These two chemicals are structurally similar and appropriate for read across as described in section 3.4; therefore, they have been reviewed together. This document provides an assessment of the risk to human health and the environment from the use of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine as inert ingredients in

pesticide formulations. Information from both submitters' petitions, as well as publicly available information, are referenced in this assessment.

3. INERT INGREDIENT PROFILE

3.1. Summary of Uses

Cocamidopropyl betaine, an amphoteric substance (i.e., can act as an acid or base by donating or accepting protons), is approved by the EPA as a nonfood inert ingredient. It can be found in a variety of products including herbicides, insecticides, fungicides, and antimicrobial products. In addition to its use in nonfood pesticide products, it is widely used in cosmetics, including bath, hair, and skincare products as well as contact lens solution and washing agents.

Cocamidopropyl betaine functions as an antistatic agent, a hair conditioning agent, a skin-conditioning agent, a surfactant-cleansing agent, a surfactant-foam booster, and a viscosity increasing agent. In 2012, cocamidopropyl betaine was used in 2,743 different cosmetic products at levels up to 11%. (MRID 52204501) Products containing cocamidopropyl betaine also include household cleaning products such as laundry detergents, dish washing liquids, and hard surface cleaners. (MRID 51393106)

1-Propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts is also an amphoteric substance. According to Oxiteno USA LLC, 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts is currently used in the cosmetic industry and "as antistatic and/or antifogging agents, coatings, corrosion inhibitors or adhesives."

3.2. Physical/Chemical Properties

Cocamidopropyl betaine is one of the alkylamidopropyl betaines. These amphoteric surfactants contain a quarternary ammonium ion, a carboxylic structure, and an amide bond. They consist of various fatty acids bound to amidopropyl betaine. They are all manufactured from oils (e.g., coconut oil, avocado oil, palm oil, sunflower seed oil, etc.) containing mixtures of C_8 to C_{18} fatty acids. 1-Propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts are condensation products of fatty acids from coconut oil and sarconsine (or 2-(methylamino)acetic acid) where the fatty acid chain varies between C_{12} - C_{18}.

These two ingredients are similar in their chemistry and makeup. In addition, they both often contain the known sensitizers 3,3-dimethylamino-propylamine (DMAPA) and fatty acid amidopropyl dimethylamine (amidoamine) as impurities. Some of the physical and chemical characteristics of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine, along with their structure and nomenclature, are found in Table 1 in Section 3.4.

3.3. Metabolism

No metabolism studies are available for the subject chemicals, but an Absorption, Distribution, Metabolism, and Excretion (ADME) study was conducted with lauramidopropyl betaine. Lauramidopropyl betaine is the main component of cocamidopropyl betaine (see section 3.4).

Lauramidopropyl betaine was administered by gavage or topically to male and female Wistar rats at doses of 10-30 mg/kg. (MRID 51393106) The ^{14}C labelled test substance was followed for up to 48 hours after dosing. Whole body autoradiography was used to study the tissue distribution of the ^{14}C.

Excreted metabolites were analyzed by thin layer chromatography (TLC). The levels of ^{14}C excreted were used to estimate intestinal and skin absorption.

As it relates to oral administration, the study found that lauramidopropyl betaine (in water) is poorly absorbed from the gastrointestinal tract following administration of 30 mg/kg or 10 mg/kg. Within 48 hours, approximately 5% of the ^{14}C dose was excreted in urine and < 2 % in expired air. While 1% remained in the body, the remainder was excreted in the feces as the unchanged parent material. Low gut absorption was confirmed by whole body autoradiography which also showed that the tissues with detectable levels of ^{14}C were those predominantly associated with urinary excretion (e.g., liver, kidney cortex, urinary bladder). Traces of parent and an unidentified polar metabolite were identified in the urine. Based on the relatively low amounts of $^{14}CO_2$ produced, the lauryl moiety does not appear to be extensively removed from the rest of the molecule (MRID 51393106).

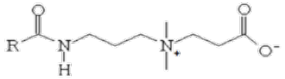
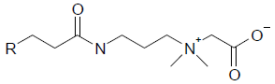
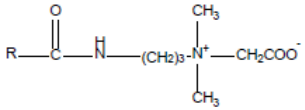
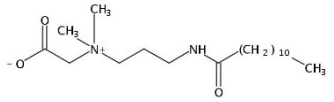
Results following dermal application were similar. Approximately 0.3mg/cm^2 of [^{14}C]LB or 0.15mg/ cm^2 of [1-^{14}C]LB in water was dermally administered and occluded. After 48 hours, approximately 3.5-6% and 2-3.5% of the test substance was absorbed in females and males, respectively. For absorbed material, urine was the major route of excretion with expired air and feces being relatively minor routes. When animals were exposed for 10 minutes, rinsed, and then a 48-hour occlusion applied, there was less than 0.2% absorption. TLC separations were not carried out for on urine from topically treated rats.

This study showed that lauramidopropyl betaine, which makes up about 50% of cocamidopropyl betaine, is poorly absorbed from the gastrointestinal tract and through the skin. Rinsing the skin reduces the absorption further. Following oral or dermal exposure, metabolism of the absorbed material occurs as indicated by the appearance of a more polar compound in the urine and by the liberation of $^{14}CO_2$.

3.4. Use of Surrogates

Although a variety of studies exist for cocamidopropyl betaine (Petition IN-11784), no repeat dose toxicity data for 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts (Petition IN-11550) were found. However, 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts is structurally similar to cocamidopropyl betaine, lauramidopropyl betaine, and coco/sunfloweramidopropyl betaine and all four compounds are members of the alkylamidopropyl betaines category of chemicals. These amphoteric surfactants contain a quarternary ammonium ion, a carboxylic structure, and an amide bond. They consist of various fatty acids bound to amidopropyl betaine. They are all manufactured from oils, usually coconut oil, containing mixtures of C_8 to C_{18} fatty acids and are generally sold as an aqueous solution containing 20 - 40 % active. (MRID 51393108)

These zwitterionic substances share structural similarities with common functional groups; however, they differ by their carbon chain length distribution and degree of saturation of the fatty acid moiety. A description of the acceptability of each surrogate is listed in Table 1. Because of the structural and functional similarities and comparable physico-chemical properties of these chemicals, a similar ecotoxicological and toxicological profile can be expected and a read across approach to the data is appropriate.

Table 1. Structure and Physico-chemical Properties of 1-Propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and Cocamidopropyl Betaine and Read Across Chemicals				
Chemical Name	1-Propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts	1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts	1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-(C8-18 and C18-unsatd. acyl) derivatives, inner salts	1-Propanaminium, N-(carboxymethyl)-N,N-dimethyl-3-[(1-oxododecyl)amino]-, inner salt
CAS Reg. No.	499781-63-4	61789-40-0	147170-44-3	4292-10-8
Molecular Formula	$C_{21}H_{44}N_2O_3$ (EPI Suite v4.11)	$C_{19}H_{38}N_2O_3$ (dominate chain length (CL)) $C_{19}H_{40}N_2O_3$ (EPI v4.11) $C_{12.8}H_{39.8}N_2O_3$-cal. Typical CL (SIAR)	Unspecified	$C_{19}H_{38}N_2O_3$ (SIAR)
Structure	 RCOOH= mixture of C_{12}-C_{18} fatty acids	 RCOOH= mixture of C_8-C_{18} fatty acids	 RCOOH= mixture of C_8-C_{18} and C_{18}-unsatd. fatty acids	
Synonyms	None found	Cocamidopropyl betaine; 1-Propanaminium, 3- amino- N-(carboxymethyl)- N,N-dimethyl-, N-coco acyl derivatives, inner salts; (N-Cocoamidopropyl)-N,N-dimethylglycin, Hydroxide, Inner Salts	Coco/sunfloweramidopropyl betaine; Babassuamidopropyl Betaine*; 1-Propanaminium, 3-amino- N-carboxymethyl) -N,N-dimethyl-, N-(C8-18 and C18-unsatd. acyl) derivs., hydroxides, inner salts	Lauramidopropyl betaine; (3 Laurylaminopropyl) dimethylaminoacetic acid, inner salt; Lauroylaminopropyldimethylaminoacetatecarboxymethyl)dimethyl-3-[(1-oxododecyl) amino]propylammonium hydroxide
Molecular Weight (g/mol)	372.60 (EPI Suite v4.11)	342.5 (Bi-PA NV) 286.4 - 426.7; 355 g/mol as typical CL distribution (SIAR)	316-426 (AG)	343.5 (PubChem, SIAR)
Boiling Point (°C) (Aqueous solition 20-40%)	>100 (Oxiteno)	104.3 (ECHA) 230°F as a 40% solution (CIR)	100 (AG)	100 - 110 (SIAR, HERA)
Water Solubility	3.321 mg/L (EPI Suite v4.11)	23.676 g/L (ECHA) >10 g/L at 20 (HERA)	10-50 g/L at 20°C (AG)	Solution: > 100 g/L at 20 °C (SIAR, HERA)

		2 g/10 ml measured (CIR)		
Vapor Pressure	1.13 x 10^{-13} Pa (EPI Suite v4.11)	Purified fractions <2x10^{-13} hPa; Pure substance (fraction of measured C8/C10): ≤ 0.0031 hPa at 20 °C (SIAR)	VP 6.63 x 10^{-3} kPa at 25 (AG)	Pure substance= 6.4 x 10^{-15} hPa (SIAR, HERA)
Partition Coefficient (Log Kow)	3.67 (EPI Suite v4.11)	-1.28-3.63 (SIAR, HERA) 2.69 (EPI Suite v4.11)	Not determined (AG)	2.69 (EPI Suite v4.11)
Relative Density (g/cm^3)	1.0249 (Oxiteno)	1.05-1.07 (SIAR, HERA)	1.046 (AG)	1.045 (SIAR, HERA)
Acceptablity of Chemical	(IN-11550) Petitioned for inert ingredient. <u>Typical Fatty acid composition</u> (registrant): C8 2-10% C10 2-10% C12 45-53% C14 13-21% C16 8-13% C18 max 5 C18:1 5-12% C18:2 max 4%	(IN-11782) Petitioned for inert ingredient. <u>Typical Fatty acid composition</u> (CIR): C8 5.6-6.0% C10 5.4-5.7% C12 53.1-53.2% C14 16.1-17.4% C16 8.1-8.3% C18 10.0-10.2% <u>Typical Fatty acid composition</u>: (SIAR) C8 ≤10% C10 ≤10% C12 47-60% C14 17-25% C16 7-14% C18 7-14%	Read Across Chemical. -R is not defined for cocamidopropyl betaine, however, it expected to have an almost identical composition to this chemical. -Difference between cocamidopropyl betaine and this chemical is the oil source from which the fatty acids are derived.* <u>Typical Fatty acid composition</u>: (AG) C8 4-8% C10 4-8% C12 44-47% C14 15-20% C16 6-9% C18 3-5% C18:1 10-12% C18:2 1-3%	Read Across Chemical. -It is the main component of cocamidopropyl betaine, making up approximately 50%. <u>Fatty Acid</u>: lauric acid, C_{12} fatty acid

*****CAS Reg. No. 147170-44-3 has been listed as "Coco/sunfloweramidopropyl betaine" and/or "Babassuamidopropyl betaine" by various reliable sources. The American Chemical Society's Chemical Abstract Service does not list common names for either of these CAS Reg. Nos so EPA is unable to verify them; therefore, we have listed both here. The only difference in the two chemicals is the source of the oil used. For coco/sunfloweramidopropyl betaine the fatty acids are derived from a blend of coconut and sunflower seed oils whereas babassuamidopropyl betaine is derived from the Babassu palm tree.

CIR= MRID 52204501; SIAR= MRID 51393108; AG= Australian Gov, https://www.industrialchemicals.gov.au/sites/default/files/LTD1578%20Public%20Report%20PDF.pdf; HERA= MRID 51393106; EPI Suite v 4.11 (see Appendix 2); ECHA= https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25362/7/1

4. HAZARD CHARACTERIZATION

4.1. Acute Toxicity Studies

Very few acute studies were conducted with 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts (CAS Reg. No. 499781-63-4); however, various acute toxicity studies have been conducted with cocamidopropyl betaine (CAS Reg. No. 61789-40-0). Based on the studies presented below, these chemicals have moderate acute toxicity via the oral and dermal routes, and they are severe eye irritants. Cocamidopropyl betaine was seen to be a moderate dermal irritant in some studies and a non-irritant in others. Although some skin sensitization effects were seen in the acute studies, these chemicals contain byproducts that are known to cause sensitization, Therefore, it is possible the effects are from chemical byproducts and with proper manufacturing controls, these irritating components can be decreased. The acute studies are presented below by route, from lowest endpoint to highest.

4.1.1. Acute Oral

No acute oral studies conducted with 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts were found. Various acute oral toxicity studies were with cocamidopropyl betaine. When the percent in formulation was clear, the LD_{50s} for the inert ingredient was calculated. The LD_{50s} from the tests summarized below ranged from 4,900 to 8,550 mg/kg of the test substance, equating to 1,500-2,600 mg/kg cocamidopropyl betaine.

In an acute oral gavage study, a solution of 30% cocamidopropyl betaine (70% water) was administered to groups of five Wistar rats at doses of 2,000, 4,000, 5,000, 6,300, 8,000, and 16,000 mg/kg. During the 14-day observation period, sluggishness, nasal hemorrhaging, diarrhea, and wetness around the hindquarters were observed. The effects increased in severity with dosage. At 4,000 mg/kg one rat died. In the 5,000 mg/kg dose group 2/5 died and at 6,300 mg/kg, 3/5 died. All rats dosed with 8,000 mg/kg or greater, died. The LD_{50} was determined to be 4,900 mg/kg or ~1,500 mg/kg cocamidopropyl betaine. (MRID 52204501, ECHA)

A solution of 30% cocamidopropyl betaine was administered by oral gavage to groups of Sprague Dawley rats (5/sex/dose) at doses 2,000, 2,710, 3,680, 5,000, or 6,780 mg/kg. The rats were then observed for 15 days. Diarrhea was seen in rats of all treatment groups. Decreased motor activity was observed in rats of all treatment groups except for the lowest dose. Dried blood around the nose and salivation were observed in male rats of the 5,000 mg/kg dose group. At necropsy, a “bloodlike viscous liquid” was found in the intestines. Mortality was observed in 2/10 at 3,680 mg/kg, 6/10 rats at 5,000 mg/kg and 8/10 rats at 6,780 mg/kg. The LD_{50} was determined to be 4,910 mg/kg or ~1,500 mg/kg cocamidopropyl betaine. (MRID 52204501, ECHA)

In an acute oral toxicity study, groups of CD rats (5/sex) were given a single oral dose of 5,000 mg/kg of a solution containing 31% cocamidopropyl betaine. The animals were then observed twice daily for the following 14 days. There were no significant treatment-related effects on survival, body weight, or necropsy findings in either sex. Clinical signs (i.e., piloerection and increased salivation) were observed in all rats shortly after dosing. Piloerection persisted throughout Day 1 and Day 2. Abnormal body carriage (hunched posture) and diarrhea were also observed on Day 2. By Day 3, recovery, as judged by external appearance and behavior, was “advanced” (piloerection alone) and complete by Day 4. The

oral LD_{50} was > 5,000 mg/kg body weight in male and female rats which equates to a LD_{50} of >1,550 mg/kg cocamidopropyl betaine. (MRIDs 52204501, 51393102, ECHA)

An acute oral toxicity study on cocamidopropyl betaine (35.61% active) was conducted with Sprague-Dawley rats (5/sex). Rats received a single, oral gavage dose of 1,800 mg/kg of the test material and were observed for 14 days. All of the female rats died on Day 2 of the study. Animals that died during the study underwent gross necropsy. Prior to death, the females exhibited salivation, diarrhea, ataxia, and/or decreased activity. Male rats exhibited similar clinical signs on Day 1 (day of dosing) and Day 2 but had recovered by Day 3. Necropsy data were not reported. The LD_{50} was determined to be greater than 1,800 mg/kg for male rats. Since all females died, a LD_{50} for females or a combined LD_{50} could not be determined. (MRIDs 52204501, 51393102, 51393108, ECHA)

Cocamidopropyl betaine (30%) was administered by oral gavage to groups of ten (5/sex/dose) Wistar rats at doses of 5,000, 6,300, 7,940, and 10,000 ml/kg. The rats were observed for 14 days. All rats had decreased motor activity, abnormal body posture, coordination disturbance, cyanosis, diarrhea, and decreased body temperature beginning approximately 20 minutes after dose administration and persisting for 24 hours. Mortality was observed in 2/10 rats at both 5,000 and 6,300 ml/kg, 6/10 at 7,940 ml/kg, and 8/10 rats in the 10,000 ml/kg dose group. Most rats died within 24 hours of treatment. Redness of the stomach and intestinal mucous membranes were observed at necropsy. The LD_{50} for the test substance was 7,970 mg/kg based on a calculation using volume per weight dosage units or 2,291 mg/kg cocamidopropyl betaine. (MRIDs 52204501, 51393108)

In another acute oral toxicity study, six groups of male and female Wistar rats (5/dose) received a single oral gavage dose of 30% cocamidopropyl betaine (70% water) at dose levels of 4,000, 8,000, 10,000, 12,500, 16,000, or 32,000 mg/kg. The rats were observed daily for two weeks after dosing. No histopathology or postmortem examinations were performed. Clinical signs included slight diarrhea and unkempt coats in animals treated with 4,000 mg/kg, and lethargy, diarrhea, nasal hemorrhage, and unkempt coats were observed in those dosed with 8,000 mg/kg or greater, with severity increasing proportionately. No rats died in the 4,000 mg/kg group. In the 8,000 mg/kg dose group 2/5 rats died and 4/5 died in the 10,000 mg/kg group. All rats died when dosed with 12,500 mg/kg or greater. The LD_{50} was determined to be 8,550 mg/kg which is equal to 2,600 mg/kg cocamidopropyl betaine. (MRIDs 52204501, 51393102, ECHA)

A solution of 30% cocamidopropyl betaine was administered by oral gavage to groups CFR Carworth mice (5/sex). The mice were dosed with 6,450 mg/kg of the test substance and observed for seven days. At 6,450 mg/kg, half of the mice died; therefore, the LD_{50} was 6,450 mg/kg which is equal to ~2,000 mg/kg cocamidopropyl betaine. (MRID 52204501, ECHA). Note: The CIR document presents the LD_{50} as 6,900 mg/kg based on a calculation using volume per weight dosage units.

4.1.2. Acute Dermal

In an acute dermal toxicity study, CD [Crl:COBS CD (SD) BR] rats (5/sex) were dermally exposed to 2,000 mg/kg of a test substance containing 31 % cocamidopropyl betaine. An area of approximately 1/10 of the body surface was treated with the test substance and then covered for 24 hours. (MRID 52204501, ECHA). Following exposure, the area was rinsed with warm water and the animals were observed daily for 14 days. There were no clinical signs of systemic reaction to treatment. At the site of application

there was slight or well-defined erythema on Day 2. Well-defined erythema persisted in three male and all female rats until Day 3. Resolution of erythema was completed by Day 6. Sloughing or hyperkeratinization affected the treated skin of 6 rats on Days 4 and 5 only. Necropsy findings were normal. The dermal LD_{50} of the test substance is >2000 mg/kg; therefore, the LD_{50} for cocamidopropyl betaine is >620 mg/kg.

4.1.3. Acute Inhalation

No acute inhalation toxicity studies are available for 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts or cocamidopropyl betaine. These chemicals are not expected to volatize due to their low vapor pressure. Similarly, the Henry's law constants calculated for all fatty acid chain lengths for alkylamidopropyl betaines indicate a very low potential for volatilization from surface waters. Subsequently, these chemicals are not expected to cause acute inhalation toxicity when used as inert ingredients in pesticide formulations.

4.1.4. Primary Dermal Irritation

Multiple dermal irritation studies and a predictive Reconstructed Human Epidermis Model study are available for this class of chemicals with varying results.

In an unpublished primary dermal irritation study, two female New Zealand White rabbits were dermally exposed to a 30% solution (70% water) of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts (CAS Reg. No. 499781-63-4) for 4 hours. At 60 minutes after patch removal, all dose sites exhibited well-defined erythema (scores = 2) and very slight edema (scores = 1). Irritation decreased over time; edema and erythema resolved by 72 hours and Day 14, respectively. Scaling was observed on 2/2 dose sites at 72 hours through Day 14 (study termination). The mean irritation score at 72 hours was 1.5; therefore, the test substance was found to be moderately irritating in this study. (MRID 51393112)

In an acute dermal irritation study, six New Zealand White rabbits were exposed to cocamidopropyl betaine for 24 hours. Two sites on each rabbit were used with one site left intact and the other abraded. Each site received 0.5 ml of a 50.7% w/v dilution of the test chemical which was then wrapped with an occlusive dressing. After 24 hours the treated sites were wiped. The Draize method was used to assess for erythema and edema. Eschar formation was observed in 3 of 6 rabbits at 72 hours post exposure in both intact and abraded skin. According to ECHA, the test chemical was corrosive to the skin of rabbits within the definition of the Federal Hazardous Substances Act and was not based on the primary irritation score of 4.54. (ECHA)

In an acute dermal irritation study, three New Zealand White rabbits were dermally exposed for 4 hours to 0.5 g of a paste containing 99.4% dried 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-(C8-18(even numbered) and C18 unsaturated acyl) derivs., hydroxides, inner salts (CAS Reg. No. 147170-44-3) moistened with saline. After removing the semi-occlusive dressing and washing the area with water, the animals were then observed for 72 hours. One hour after dosing, slight edema was observed on 2 of 3 animals and erythema was observed on all three animals. After 24 hours, erythema had lessened in 2/3 animals to score 1 and worsened in 1/3 animal to score 3. In addition, 2/3 animals showed an edema score of 1. The only notable skin reaction seen at the 48 hours was an erythema score of 2 in one animal. All symptoms were reversed by 72 hours post dosing. Thus, 1-

propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-(C8-18(even numbered) and C18 unsaturated acyl) derivs., hydroxides, inner salts is not a dermal irritant**.** (ECHA for CAS Reg. No. 147170-44-3)

Six skin irritation studies are outlined in the CIR document for cocamidopropyl betaine. Although little information is provided, concentrations ranging from 7.5-30% cocamidopropyl betaine were tested. The two studies which tested a concentration of 30% cocamidopropyl betaine, were conflicting. In one study it was a mild irritant and in the other it resulted in eschar formation. Concentrations lower than 30% were not considered irritating to the skin. (MRID 52204501)

An unpublished dermal irritation study was conducted using the Reconstructed Human Epidermis Model with 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts (CAS Reg. No. 499781-63-4) . Three disks of SkinEthic^{TM}/group were treated topically with the test substance for 42 minutes at room temperature. The relative mean viability (OD_{570}) of the test substance-treated tissues was 88.7% of the negative control mean viability. The test substance was predicted to be a non-irritant based on a mean cell viability of >50% of the control value. (MRID 51393113)

4.1.5. Primary Eye Irritation

Numerous eye irritation studies have been conducted on cocamidopropyl betaine. Table 2 below illustrates some of the studies that have been conducted. There are many more studies outlined in the ECHA dossier for these chemicals; however, they all lend to the same conclusion, cocamidopropyl betaine is an eye irritant.

Table 2. Eye Irritation Studies

Test Solution: % cocamidopropyl betaine (0.1 ml)	Test Animal	Results	Source
50.7% dilution in tap water	9 New Zealand White (NZW) rabbits	Rinsed Eyes (3 rabbits): Mean eye irritation scores (max. 110): 16.7 after 24 hours, 31.3 after 48 hours, 27.3 after 72 hours, 34.3 after 7 days, and 14.7 after 21 days Unrinsed Eyes (6 rabbits): Mean eye irritation scores (max. 110): 18.8 after 24 hours, 18.3 after 48 hours, 14.5 after 72 hours, 5.0 after 7 days, and 0.7 after 21 days *Severe irritant*	ECHA
30%	3 Albino rabbits	Mild conjunctival erythema, chemosis, and discharge from Day 1. Diffuse corneal opacity was observed by Day 3. Slight iritis by Day 4. No further details.	MRID 52204501
30%	9 NZW rabbits	Rinsed Eyes: Mean irritation score: 10.0 at 24 hours, subsided after 48 hours. Unrinsed Eyes: Mean irritation scores (scale 0-110): 32.5 after 24 hours, 31.7 after 48 hours, 41.7 after 72 hours, and 27.2 after 7 days. Corneal opacity, slight iritis, and conjunctival irritation and necrosis were seen. *Rinsed: minimal irritation; Unrinsed: severe irritant*	MRID 52204501
10%	9 NZW rabbits	Rinsed Eyes: Mean irritation score: 2.0 after 24 hours, returning to normal after 48 hours. Unrinsed Eyes: Mean eye irritation scores: 25.7 after 24 hours, 16.7 after 48 hours, 9.3 after 72 hours, and no irritation was observed on Day 7	MRID 52204501

		Unrinsed- moderately irritating; Rinsed- practically nonirritating	
5% or 10%	6 NZW rabbits	5% group- Draize score = 4.90 10% group- Draize score = 27.3 No further details. *10%- moderately irritating; 5%- not an irritant*	MRID 52204501
7.5%	6 NZW rabbits	Mild to moderate conjunctival irritation was observed in all treated animals after 24 hours. One animal had moderate corneal opacity after the second day. These alterations disappeared by the 6th day.	MRID 52204501
liquid soap formulation containing 6.5%	4 NZW rabbits	Treated eyes were rinsed. Mean corneal irritation scores (max = 80): 13.8 after 1 hour, 18.8 after 24 hours, 11.3 after 48 hours, 5 after 72 hours, and 1.3 after 7 days. Mean iridial irritation scores: 3.8 after 1 and 24 hours, decreasing to 0 after 7 days. Mean conjunctival irritation scores: 11 after 1 hour, 7.5 after 24 hours, 4 after 48 hours, 3.5 after 72 hours, and 2 after 7 days. No irritation was observed 14 days after the instillation. *Total mean irritation score of 30.0 (max = 110), considered moderately irritating.*	MRID 52204501
6.0%	6 Albino rabbits	Conjunctival irritation (mean score of 4; max = 20) was observed in all treated eyes on the first day following instillation, decreasing in severity on the second day. No corneal irritation or iritis was observed.	MRID 52204501
6.0%	3 Albino rabbits	Mild conjunctival erythema and slight discharge were observed in all treated eyes for the first two days after instillation, clearing by Day 3.	MRID 52204501
4.5%	6 Albino rabbits	There was no corneal involvement or iris congestion. <u>Rinsed Eyes</u>: Slight conjunctival irritation was observed in two of the three rinsed eyes on the first two days of observation. <u>Unrinsed Eyes</u>: Slight conjunctival erythema and chemosis were noted in all unrinsed eyes by Day 2 and subsided by Day 7.	MRID 52204501
3.0%	6 Male albino rabbits	The average ocular index was 41.6 (max = 110) 24 hours after instillation. *Considered an ocular irritant.*	MRID 52204501
3.0%	6 Albino rabbits	<u>Sample 1:</u> Scores for corneal irritation (max score = 80): 0 Day 1 and 2, 1.66 Day 3 and 4, and 4.16 on Day 7. Iritis score (scale of 0-10): 8.33 Day 1, decreased to 4.16 by Day 7. Conjunctival irritation score (scale 0-20): 15.37, decreased to 6 by Day 7. <u>Sample 2:</u> No corneal irritation observed. Iritis score (max= 10): 5 Day 1, decreased to 0 by Day 7. Conjunctival irritation score (max= 20): 14.33, decreased to 0 by Day 7.	MRID 52204501
Soap formulation containing 2.3%	9 NZW rabbits	<u>Rinsed Eyes (3)</u>: Average irritation score= 20.0 (max = 110). <u>Unrinsed Eyes (6)</u>: An average irritation score of 18.7 (max = 110) was calculated. Irritation was observed primarily in the iris and conjunctiva. *The formulation was considered moderately irritating.*	MRID 52204501
Soap formulation containing 2.3%	9 NZW rabbits	<u>Rinsed Eyes (3)</u>: Max average irritation score was 3.3. Mild conjunctival erythema and chemosis observed in all tested eyes 1-2 days following instillation.	MRID 52204501

		Unrinsed Eyes (6): Max average irritation score was 1.7 (max = 110). Slight conjunctival erythema and chemosis were observed in one rabbit two days after treatment and in the eye of another for the entire 7-day observation period. Slight discharge also was observed in the treated eye of the latter from 72 hours to 7 days following treatment. *Mildly irritating to treated, rinsed eyes. Minimally irritating to treated, unrinsed eyes.*	

In addition to the studies on cocamidopropyl betaine, a Bovine Corneal Opacity and Permeability (BCOP) test was conducted with 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts (CAS Reg. No. 499781-63-4). This study evaluated the effects of a 10-minute exposure of the test material on 3 healthy, undamaged bovine eyes/group. The IVIS (In vitro irritancy score) was calculated for each group using the mean opacity value + (15 x mean OD_{490} value). Due to an IVIS of ≥55, the test substance was classified as a severe irritant. (MRID 51393114)

4.1.6. Dermal Sensitization

Several dermal sensitization studies were conducted with cocamidopropyl betaine. Cocamidopropyl betaine is an amidopropyl betaine, these chemicals consist of various fatty acids bound to amidopropyl betaine. They also share the presence of two manufacturing by-products (3,3-dimethylaminopropylamine [DMAPA] and fatty acid amidopropyl dimethylamine [amidoamine or AA]). DMAPA and amidoamine are both known skin sensitizers. (Personal Care Products Council) As such, manufacturers keep the levels of DMAPA and amidoamine as low as possible through manufacturing controls and continual quality monitoring.

A few of the studies reviewed showed some degree of dermal sensitization. Since the concentrations of these impurities vary among samples and the study results were not consistent, it is possible the presence of these two manufacturing by-products is the reason for the effect. Cocamidopropyl betaine and other amidopropyl betaines are widely used in cosmetic products that contact the skin. Available studies are described in the CIR (MRID 52204501) and ECHA dossier are presented below.

In one study, cocamidopropyl betaine (10% active) was assessed for delayed contact hypersensitivity using a Maximization test in 20 male Pirbright white guinea pigs (5 were uses as controls). The test animals were administered 3 paired injections (6 total) at various sites: one, a 50% aqueous solution of Freund complete adjuvant; the second, a 0.5% (v/v) dilution of the cocamidopropyl betaine sample (0.05% active) in sterile isotonic saline, and finally a 0.5% (v/v) dilution of the cocamidopropyl betaine (0.05% active) sample in a 1:1 mixture of isotonic saline with Freund complete adjuvant. One week following the injections, a single occlusive 48-hour induction patch of a 60% (v/v) dilution (6% active cocamidopropyl betaine) in distilled water was applied. Two weeks after the induction, all animals received a single occlusive challenge patch of a 10% (v/v) dilution of cocamidopropyl betaine (1% active) in distilled water for 24-hours.

Temporary irritation (slight) was observed following injection of the 0.5% cocamidopropyl betaine sample in all test animals. Topical application of the 60% cocamidopropyl betaine patch resulted in slight dermal reactions. No evidence of delayed contact hypersensitivity was found; therefore, cocamidopropyl betaine was not a dermal sensitizer in this study.

In a second study, cocamidopropyl betaine (30% active) was tested for skin sensitization using a Maximization Test and a modified Draize test. Like the previous study, twenty albino guinea pigs received intradermal injections of (1) Freund complete adjuvant alone, (2) 0.1% aqueous dilution of the cocamidopropyl betaine sample (0.03% active), and (3) 0.1% aqueous dilution of the cocamidopropyl betaine sample (0.03% active) plus the Freund complete adjuvant. One week later, a topical 48-hour occlusive induction patch containing a 10% aqueous dilution of the cocamidopropyl betaine (3% active) was applied.

After three weeks of rest, single occlusive challenge patches were applied to the clipped flanks of all animals for 24 hours. A 10% aqueous dilution of the cocamidopropyl betaine sample (3% active) was applied to one flank, and water was applied to the other. Erythema, edema, and irritation were seen in 8 of the 20 test animals after the challenge application. Microscopic findings showed epidermal acanthosis, inter- and intracellular edema, and massive infiltration of the superficial layers of the dermis with lymphocytes, monocytes, and a few eosinophils with a tendency to invade the epidermis in two of the animals. Less prominent microscopic lesions of acanthosis, mild intracellular edema, and a moderate lymphomononuclear infiltrate in the superficial dermis were also found in four additional animals with slight acanthosis observed in the remaining two animals. Under conditions of this study, the chemical was a skin sensitizer.

In another study, a formulation containing cocamidopropyl betaine (0.75% active) was tested in a delayed contact hypersensitivity test. Closed patches containing the test solution were applied to the shaved area of 20 albino guinea pigs. After six hours, the patch was removed, and the area was rinsed. For the following two weeks, this procedure was repeated at the same site. Then the animals were left untreated for two weeks before the primary challenge test, where 0.01875% cocamidopropyl betaine (a 2.5% solution of the 0.75% active cocamidopropyl betaine) was applied for six hours to a clipped skin site not previously treated. Responses were graded after 24 and 48 hours. There was no evidence of sensitization following exposure to treatment.

Cocamidopropyl betaine (0.15% active) was tested for induction (0.015% for challenge) in the same laboratory as the second study mentioned above while using the same assay as that study. Slight erythema and edema were observed in 6 of the 20 animals. Slight acanthosis was observed microscopically. A few controls had moderate acanthosis with edema and vasodilation in the subjacent papillary layer of the dermis. The investigators concluded that cocamidopropyl betaine can produce a delayed-type contact sensitization.

Cocamidopropyl betaine was positive for sensitization in a local lymph node assay; however, no other information was given in the CIR document.

4.2. Repeated Dose Toxicity Studies

Various oral toxicity studies were conducted in rats and mice. With the exception of localized effects in the forestomach, no evidence of treatment related effects was seen in the repeat dose studies. These local effects are a result of the irritating properties of cocamidopropyl betaine.

Similarly, local irritation was seen in the forestomach of dams in one developmental toxicity study following gavage administration. This forestomach irritation likely resulted in the decreased maternal

body weight gain and food consumption and the associated developmental effects observed at the highest dose tested (i.e., post-implantation loss and decreased mean fetal body weight). Due to the bolus administration of the compound (which may increase the irritation potential of a chemical), the lack of a forestomach in humans, and the developmental effects occurring at very high doses only, the maternal and developmental effects observed are not considered relevant for human health risk assessment.

Note: Presented below are two subchronic oral studies on cocamidopropyl betaine. It is the opinion of the EPA that the 90-day study and 92-day study listed here are likely the same study. Although the reports are not identical, there is no conflicting information, the study parameters and results are similar, and the year of publication and the report number is identical in both references. To further support that these could be the same study, each of the various review sources (e.g., HERA, CIR, ACC, etc.) present either a 90-day study or a 92-day study, none of the reviews outline both studies. Because EPA is unable to obtain the original study/s, we have presented both here with the appropriate references.

4.2.1. 28-Day Oral

Male and female Sprague-Dawley rats (8/sex/group) were administered a 30.6% active cocamidopropyl betaine solution. (MRID 52204501) Animals were given the test material by gavage, at doses of 0, 100, 500, or 1000 mg/kg/day for 28 days. Rats were necropsied, and tissues were collected for histopathological evaluation.

Mortality was increased in the treated groups as compared to controls, but mortality did not follow a dose-response relationship. It appeared that dosing/administration errors were the likely cause for the deaths. Lesions (subacute inflammation and epithelial hyperplasia) of the nonglandular portion of the stomach (i.e., forestomach) were seen. Lesions were found in the stomachs of 1 of 5 high-dose males and in all 7 high-dose females. The loss of 3 males during the first 2 weeks of dosing prevented adequate evaluation of the response of male rats. No effects were seen in males and females of the 100 mg/kg/day dose group.

4.2.2. 28-Day Oral

The American Chemistry Council outlined a 28-day oral toxicity study with cocamidopropyl betaine. (MRID 51393102) This OECD 407 study has been further described in both the CIR and HERA documents. (MRIDs 52204501, 51393106) Sprague-Dawley rats (10/sex/dose) were gavaged with a 30% active cocamidopropyl betaine solution of 0, 250, 500, or 1000 mg/kg/day (5 days/week). For animals in the 0 and 1000 mg/kg/day dose groups, this was followed by a post-exposure recovery period of 28 days.

No treatment-related deaths or decreases in food or water consumption were observed over the course of the study. Hematological evaluations, clinical chemistry, ophthalmic examinations, and absolute and relative organ weights did not show any treatment-related effects. Head protrusion at the beginning of week 3 and salivation at the beginning of week 4 were observed in the 1000 mg/kg/day dose group.

Edema of the mucosa of the nonglandular stomach was observed during gross pathology of the 1000 mg/kg dose group. This finding of irritation in the forestomach was reversed in the recovery group rats. Microscopic examination of the 1000 mg/kg dose group found acanthosis of the gastric mucosa, inflammatory edema of the submucosa, and multiple ulcerations. The dominant findings were acanthosis and the papillomatous hyperplasia. The effects were greater in females than males. No other treatment-related effects were observed in the organs examined.

The NOAEL was 500 mg/kg/day, and the LOAEL was 1000 mg/kg/day based on the effects in the forestomach. These local effects are a result of the irritating properties of cocamidopropyl betaine together with the gavage administration and therefore, the NOAEL for systemic toxicity was 1000 mg/kg/day (highest dose tested).

4.2.3. 90-Day Oral*

According to the HERA and the OECD SIDS documents, and outlined in the ECHA dossier, a 90-day oral gavage study (OECD 408) was conducted with Sprague-Dawley rats (10/sex/dose) administered 0, 250, 500, or 1000 mg/kg/day of a 30 % cocamidopropyl betaine solution 5 days/week for 13 weeks. Gross pathology showed one male and one female rat in the 1000 mg/kg/day group with ulceration at the fundus and the cardia region of the stomach. Macroscopic and microscopic investigation revealed no evidence of toxicity in other organs.

No signs of toxicity were seen when evaluating clinical chemistry, hematology, body weight gain, clinical observations, or treatment related mortality. Histopathologic findings of forestomach gastritis with squamous hyperplasia, submucosal edema and inflammatory cell infiltration were seen in male and female rats at doses ≥ 500 mg/kg/day. Therefore, the NOAEL, based on the forestomach findings, is 250 mg/kg/day with a LOAEL of 500 mg/kg/day (MRIDs 51393108, 51393106, ECHA).

4.2.4. 92-Day Oral*

According to the CIR document and American Chemistry Council's Fatty Nitrogen Derived Amides document, groups of Crl:CF(SD)BR Sprague Dawley rats (10/sex/dose) received 0, 250, 500, or 1,000 mg/kg/day cocamidopropyl betaine (concentration not stated) in distilled water via oral gavage for 92 days. Clinical signs were recorded daily, and body weight and feed consumption were recorded once weekly. Blood and urine samples were collected, and ophthalmic examinations were performed on all groups during the final week of treatment. Surviving rats were necropsied at study termination.

Histopathology was conducted on select tissues from the rats in the control group and the 1,000 mg/kg/day group. Because treatment-related histopathological changes were observed in the stomachs of the 1,000 mg/kg/day group, stomachs of the 250 and 500 mg/kg/day groups were also examined microscopically. Forestomach gastritis was seen in six males and three females at 1000 mg/kg/day and in two males and two females at 500 mg/kg/day.

No treatment-related deaths were observed. In addition, no treatment related effects were seen in relation to clinical signs, body weights, feed consumption, ophthalmic observations, hematological parameters, clinical chemistry evaluations, urinalysis, and organ weights. Stomach ulcers at the fundic and cardiac regions in one male and one female in the high-dose group were seen during necropsy. No other treatment-related effects were observed.

The NOAEL for cocamidopropyl betaine in rats was 250 mg/kg/day and the LOAEL, based on effects in the forestomach, was 500 mg/kg/day. (MRIDs 51393102, 52204501). The NOAEL for systemic toxicity was 1000 mg/kg/day.

**It is the opinion of the EPA that the 90-day and 92-day repeat dose studies listed here are likely the same study; however, because EPA is unable to obtain the original source for either study, we have presented both with the appropriate references.*

4.2.5. Neurotoxicity

No neurotoxicity studies were available for any of the subject chemicals or appropriate surrogates. Although no specific neurotoxicity studies were conducted, there was no evidence of neurotoxicity following repeated dosing. The neurotoxicity observed following acute dosing occurred at doses not relevant for risk assessment purposes (i.e., doses >1,000 mg/kg). Therefore, 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts; and 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts are not expected to be neurotoxic to humans.

4.2.6. Reproductive toxicity

No specific reproductive toxicity studies were conducted with either of the two petitioned chemicals; however, the subchronic toxicity test/s mentioned previously in this section, show no effect on reproductive organs. Cocamidopropyl betaine was administered to male and female Sprague-Dawley rats via gavage at 0, 250, 500, or 1000 mg/kg/day for 13 weeks. There were no treatment-related effects on organ weights, nor any histopathologic changes in the testes, accessory sex organs, ovaries, uterus, and prostate. (MRIDs 51393108, 51393106, 51393102, 52204501)

4.2.7. Developmental Toxicity

Two developmental toxicity studies were reviewed. In one study conducted according to OECD Guideline 414 (MRIDs 52204520, 51393106, 51393108, ECHA), cocamidopropyl betaine was administered by oral gavage to pregnant CD rats (25/dose) at dose levels of 0, 330, 990, or 3,300 mg/kg on days 5 through 19 of gestation. At a concentration of 28.9%, the resulting cocamidopropyl betaine in the test substance was 0, 95, 286, and 950 mg/kg/day, respectively. With the exception of the high dose group, in which one additional animal was included due to the premature death of one dam, each group contained 25 animals but only twenty animals per group were evaluated for maternal toxicity.

At ≥990 mg/kg/day, effects of treatment included: 23% reduction of the net weight change (carcass weight minus day 6 body weight), reduced food consumption (up to 12 %), and, in 4 animals, ulcers and thickened mucosa.

Of the 21 dams in the 3,300 mg/kg/day group, 13 showed abdominal position and 2 showed piloerection and reduced motility. One dam died on gestation day 15. Reduced body weight (up to 17 %) and decreased body weight change were also seen. Other effects occuring at 3,300 mg/kg/day include: 67% reduction of the net weight change (carcass weight minus day 6 body weight), reduced food consumption (up to 65% each day), reduced gravid uterus weight (by 22% caused by lowered fetal

weights) and thickened mucosa with greyish discoloration in 20 animals. Two animals, and the prematurely deceased dam, were found to have ulcers.

No fetal effects were seen at 990 mg/kg/day. In the 3,300 mg/kg/day group, the number of early, late, and total resorptions was increased and the ratio of viable fetuses to implantation sites was decreased. This was attributed to a total post-implantation loss of two dams in this dose group. A statistically significant reduction in fetal weights and the number of viable fetuses compared to the control was observed in the 3300 mg/kg/day group. No external, skeletal, or soft tissue malformations and no external variations attributed to the test substance were seen.

The NOAEL for maternal toxicity was 330 mg (95 mg/kg/day cocamidopropyl betaine) based on necropsy findings in the forestomach at the LOAEL of 990 mg (286 mg/kg/day cocamidopropyl betaine). The NOAEL for fetal toxicity was 990 mg/kg/day based on the embryotoxic effects (post-implantation loss and decreased mean fetal body weight) seen at 3300 mg (950 mg/kg/day cocamidopropyl betaine).

The second developmental toxicity study was mentioned in the HERA document and outlined in the ECHA dossier. In this study, cocamidopropyl betaine (30 %) was administered via gavage to pregnant rats at doses of 0, 30, 90 or 300 mg/kg on days 6 through 17 of gestation. No treatment-related effects on the incidence of fetal malformations (external, visceral, or skeletal) or developmental variations were observed among litters in any of the treated groups. The maternal and developmental NOAEL was 300 mg/kg/day, the highest dose tested (MRID 51393106, ECHA).

4.2.8. Mutagenicity

One *in vivo* and various *in vitro* studies have been conducted to assess the mutagenic potential of cocamidopropyl betaine. Cocamidopropyl betaine was negative for mutagenicity in all studies.

4.2.8.1. In vivo

A mouse micronucleus test summarized by the American Chemistry Council (MRID 51393102) and outlined in the CIR document (MIRD 52204501) detailed the effects of cocamidopropyl betaine (concentration unknown) on groups of five male and five female OFI mice. Animals received two doses 24- hours apart of either 0.02 or 0.2 g/kg of the test substance via intraperitoneal injection (dose volume in distilled water 10 g/kg). Six hours after the second administration animals were euthanized and material and bone marrow slides were prepared. Polychromatic erythrocytes (PCEs), 1000/animal, were evaluated for the presence of micronuclei. In both dose groups, the number of micronucleated PCEs was not increased compared to controls. Cocamidopropyl betaine was not a mutagen under the conditions of this study.

4.2.8.2. In vitro

4.2.8.2.1. Bacterial reverse mutation studies

Cocamidopropyl betaine (31.0% active) was tested using *Salmonella Typhimurium (S. Typhimurium)* strains TA98, TA100, TA1535, TA1537, and TA1538, with and without metabolic activation. Concentrations of 0.004, 0.02, 0.1, 0.2, and 0.4 µL/plate were tested. Cocamidopropyl betaine was found to be toxic above 0.3 µL/plate. There were no significant increases in mutation frequency in any of the strains tested, with or without metabolic activation. (MRID 52204501)

Cocamidopropyl betaine (30% active) was tested using S. *Typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, with and without metabolic activation. Eight concentrations between 0.001 and 0.300 µL/plate were tested. Cocamidopropyl betaine did not produce an increase in mutation frequency, with or without metabolic activation. (MRID 52204501)

Cocamidopropyl betaine (28.5-30.5% active) was tested using *S. Typhimurium* strains TA98, TA1535, TA1537, and TA1538, with and without metabolic activation, at 0, 50, 150, 500, 1,500, or 5,000µg/plate. Cytotoxicity was observed starting at 150 µg/plate. Cocamidopropyl betaine was non-mutagenic in this study. (MRIDs 52204501, 51393108, 51393106)

Cocamidopropyl betaine (30%) was evaluated for mutagenicity using *S. Typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, with and without metabolic activation. The sample was tested at 1, 4, 16, 64, or 256 µg/plate without S-9 activation and at 4, 16, 64, 256, and 1,024 µg/plate with S-9 activation. Cocamidopropyl betaine did not increase the mutation frequency, with or without metabolic activation. (MRIDs 51393102, 51393108, 51393106)

Cocamidopropyl betaine was tested using *S. Typhimurium* strains TA 1538, 1535, 1537, 98, 100, and *E. coli* WP2 in the presence and absence of S9 metabolic activation. The test concentrations of cocamidopropyl betaine (dissolved in DMSO) were 1, 5, 10, 50, 100, 500, 1000, and 5000 µg/plate. The plates were incubated at 37°C for 48 hours, and then the number of revertant colonies on each plate was scored with an automated colony counter. The background bacterial lawn was checked routinely by dissected microscope. A negligible amount of revertant colonies were observed as compared to positive controls. Test chemical did not induce gene mutation in *Salmonella Typhimurium* strains TA 1538, 1535, 1537, 98, 100 and *E. coli* WP2 in the presence and absence of S9 metabolic activation system and hence it is not likely to classify as a gene mutant in vitro. (MRID 52204522, ECHA)

4.2.8.2.2. Mammalian Cell Mutation Studies

An *in vitro* mammalian chromosome aberration study was performed using Chinese hamster V79 cells in the presence and absence of S9 metabolic activation system to determine the mutagenic nature of cocamidopropyl betaine. The doses, based on the preliminary dose range-finding study, were 1.0 µg/mL without S9-mix and 10.0 µg/mL with S9-mix for 7 hours; 0.3, 1.0, and 3.0 µg/mL without S9-mix and 1.0, 3.0 and 10.0 µg/mL with S9-mix for 18 hours; and 3.0 µg/mL without S9-mix and 10.0 µg/mL with S9-mix for 28 hours. Two hours (7-hour interval) or 2.5 hours (18- and 28-hour intervals) before the end of the incubation period, colcemid was added to the cultures. Cultures were scored for structural chromosomal aberrations (breaks, fragments, deletions, exchanges, and chromosomal disintegrations). Chromosomal gaps were recorded separately.

There were no biologically relevant increases in cells with structural aberrations after treatment with the test substance at any fixation interval with or without metabolic activation. As such, the test chemical did not induce chromosome aberration in the Chinese hamster V79 cells both in the presence and absence of S9 activation system. (MRID 52204523, ECHA)

An *in vitro* mammalian chromosome aberration study was performed with cocamidopropyl betaine using CHL/IU cells in the presence and absence of S9 metabolic activation. Cocamidopropyl betaine was dissolved in saline and used at dose level of 0, 100, 125, 150, 175 or 200µg/mL (short-term treatment method) and 0, 50, 75, 100, 125 or 150 µg/mL (main test) and 0, 100, 110, 120, 130, 140 or

150 µg/mL (additional test) without S9 in the continuous treatment method. The test was performed by continuous treatment method for 24 hrs and short-term treatment method for 6 hrs. The cells were allowed to express for 18 hrs in short-term treatment method. Two hours before the end of the culture, colcemid was added to the culture solution to a final concentration of about 0.1 µg/mL.

Chromosomes were analyzed based on the classification method by the Japan Society for Environmental Mutagenesis and Mammalian Testing for structural abnormalities such as breakage and exchange of chromosome type or chromosome type, presence and absence of gap, and ploidy. Based on the observations made, the test chemical did not induce chromosome aberration in the CHL/IU cells both in the presence and absence of S9 activation system; therefore, cocamidopropyl betaine is not likely to be gene mutant in vitro. (MRID 52204523, ECHA)

Cocamidopropyl betaine was tested in a L5178Y TK+/- Mouse Lymphoma Mutagenesis assay in the presence and absence of rat liver S-9 to determine its mutagenic potential. The study was performed at various concentrations between 0.0038 to 0.050 µl/ml (10 concentrations) without S9 and 0.012 to 0.16 µL/ml (10 concentrations) with S9 metabolic activation. The cells were exposed for 4 hours and an expression time of 2 days was allowed with cell population adjustment at 24 and 48 hours. At the end of the expression period, the cells were placed in a cloning medium. None of the treated cultures that were cloned exhibited a significant increase in mutant frequency over the average mutant frequency of the solvent controls, and no dose response was observed. Thus, cocamidopropyl betaine was considered to be non-mutagenic. (MRID 52204523, ECHA)

The mutagenic potential of cocamidopropyl betaine (30.9% active) was tested in a L5l78Y TK ± mouse lymphoma assay, with and without metabolic activation. The test substance was solubilized in water and diluted for testing at concentrations of 0.001, 0.01, 0.1, 1.0, 10, and 100 µL/mL. None of the treated cultures had a significant increase in mutation frequency over the average mutant frequency of the solvent controls. (MRID 52204501)

4.2.9. Chronic/Carcinogenicity

No oral chronic or carcinogenicity studies are available for cocamidopropyl betaine or any of the acceptable surrogates. However, there are no structural alerts for carcinogenicity for cocamidopropyl betaine (see Appendix 1), no adverse effects were identified in the available subchronic toxicity studies, and there was no evidence of mutagenicity in the available *in vivo* and i*n vitro* studies. Given that cocamidopropyl betaine is an unsaturated fatty acid derived from coconut oil, coupled with the lack or mutagenicity, genotoxicity, and low toxicity as described above, 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine are unlikely to be carcinogenic.

4.2.10. Immunotoxicity

No immunotoxicity studies are available in the database. However, no evidence of immunotoxicity was observed in the submitted studies.

4.3. Toxicity Endpoint Selection

The available toxicity data for 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine show moderate acute toxicity via the oral and

dermal routes. Based on their physico-chemical properties, acute toxicity via the inhalation route of exposure is expected to be low. These chemicals are expected to be moderate skin irritants and severe eye irritants. While some skin sensitization effects were seen in the studies reviewed, it is possible that the effects are from a byproduct of the chemical and with proper manufacturing controls, these sensitizing elements can be decreased.

Except for the irritation effects on the forestomach, no adverse effects were reported in the repeat dose animal studies at any dose tested. Studies where forestomach irritation was observed used gavage administration, which may increase the irritation potential of a chemical. Unlike a human stomach, the rodent stomach contains two areas- a (non-glandular) forestomach with a mucosa of stratified squamous epithelium and a glandular stomach. Because there are inherent anatomic differences between the stomach of rodents and that of the humans, and because the doses were administered via gavage, the effect in the forestomach alone would not be the basis for endpoint selection.

While some fetal effects were found in the developmental toxicity study in rats, they were only seen in the presence of material effects. A second study showed no effect of treatment on developmental or maternal parameters. No signs of neurotoxicity were reported in any of the studies at doses that were relevant for risk assessment. Furthermore, concern for carcinogenicity is low based on negative results in mutagenicity studies, and the lack of structural alerts for carcinogenicity. Therefore, based on the low toxicity of cocamidopropyl betaine and 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts, no endpoint of concern was identified for the oral, dermal, or inhalation exposure assessments, and a quantitative risk assessment is not necessary.

4.4. Safety Factor for Infants and Children (FQPA Safety Factor)

FFDCA Section 408(b)(2)(C) provides that EPA shall apply an additional tenfold (10X) margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the database on toxicity and exposure unless EPA determines based on reliable data that a different margin of safety will be safe for infants and children. This additional margin of safety is commonly referred to as the Food Quality Protection Act (FQPA) safety factor. In applying this provision, EPA either applies the default value of 10X, or uses a different additional safety factor when reliable data available to EPA support the choice of a different factor.

Based on the low toxicity in the available studies, EPA has concluded that there are no toxicological endpoints of concern for the U.S. population, including infants and children. As part of its qualitative assessment, the Agency did not use safety factors for assessing risk, and no additional safety factor is needed for assessing risk to infants and children.

5. EXPOSURE ASSESSMENT

5.1. Dietary Exposure

Dietary exposure may occur from eating foods treated with pesticide formulations containing these inert ingredients and drinking water containing runoff from soils containing the treated crops. However, no toxicological endpoints of concern were selected, and therefore, a quantitative dietary exposure assessment was not conducted.

5.2. Residential Exposure

The term “residential exposure” is used in this document to refer to non-occupational, non-dietary exposure (e.g., for lawn and garden pest control, indoor pest control, termiticides, and flea and tick control on pets). Cocamidopropyl betaine is a nonfood use inert ingredient and can be found in various pesticide formulations including herbicides, insecticides, and antimicrobial products. This group of chemicals is also used extensively in cosmetics. Although the current and proposed uses of cocamidopropyl betaine and 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts can result in residential exposures, no toxicological endpoints were selected, and therefore, it is not necessary to conduct a quantitative assessment of residential exposures and risks.

5.3. Occupational Exposure

Similarly, although the current and proposed uses of cocamidopropyl betaine and 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts can result in occupational exposures, no toxicological endpoints were selected, and therefore, it is not necessary to conduct a quantitative assessment of occupational exposures and risks.

6. AGGREGATE RISK ASSESSMENT

The Federal Food, Drug, and Cosmetic Act (FFDCA) section 408 directs EPA to consider available information concerning exposure from the pesticide residue in food and other non-occupational exposures to determine that “there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information”.

Because no toxicological endpoints were selected, a qualitative risk assessment was conducted and subsequently, it is not necessary to aggregate dermal and inhalation residential exposures with estimated dietary exposures.

7. CUMULATIVE EXPOSURE

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider “available information” concerning the cumulative effects of a particular pesticide's residues and “other substances that have a common mechanism of toxicity.”

EPA has not found 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts or 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts to share a common mechanism of toxicity with any other substances, and these chemicals do not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance exemption, therefore, EPA has assumed that neither 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts nor 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivatives, hydroxides, inner salts have a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see EPA's website at https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/cumulative-assessment-risk-pesticides.

8. ENVIRONMENTAL FATE AND ECOTOXICITY

8.1. Environmental fate

Alkylamidopropyl betaines are typically sold in commerce as a 20-40% solution in water with a small amount of sodium chloride (~5%). Most of the tested data is done on this solution. Modeled data, on the other hand, is based on the neat chemical.

According to the OECD SIDS assessment, which used the Mackay Level I V2.11 model to predict fate, lauramidopropyl betaine and cocamidopropyl betaine will be mainly distributed to the hydrosphere (59 - 100 %), and to a lesser extent to soil and sediment (0 - 20 % each). EPI Suite v 4.11 Level III Fugacity Model, however, shows a greater affinity to soil (83%) and to a lesser extent water (16.5%). The soil sorption coefficients (KOC) calculated indicate a low to moderate potential for sorption to organic matter of soils and sediments of the alkylamidopropyl betaines. The predicted Koc and therefore, predicted adsorption to organic matter, increases with increasing chain length. A significant distribution to soil and sediment is expected only for the C_{16} and C_{18} betaines. (MRID 51393108) Using the screening HPLC-method according to OECD TG 121, experimentally obtained values for laurylamidopropyl betaine (C_{12} derivative) and tetradecylamidopropyl betaine (C_{14} derivative) showed low sorption potential. (MRID 51393108) Since these are the main two components of cocamidopropyl betaine, it too is expected to have low sorption potential.

The Henry's law constants for alkylamidopropyl betaines indicate a very low potential for volatilization from surface waters. Similarly, the chemical is not expected to volatilize due to the low vapor pressure.

Cocamidopropyl betaine was tested for biodegradable under aerobic and anaerobic conditions. Experimental results from several biodegradation studies (e.g., OECD 301A, 301B, 302B, 301D) are outlined in the ECHA dossier on cocamidopropyl betaine. For illustrative purposes, two aerobic studies and the study conducted under anaerobic conditions are described below. All of the biodegradation studies demonstrated that cocamidopropyl betaine is readily biodegradable.

Various ecotoxicity studies on freshwater fish, aquatic invertebrates, and freshwater algae showed that cocamidopropyl betaine was moderately toxic to aquatic species.

8.2. Biodegradation

To assess biodegradation, cocamidopropyl betaine (2 mg/L) was exposed to microorganisms from activated sludge collected from a municipal sewage treatment plant. The extent of biodegradation was determined by CO_2 evolution in an OECD 301D study. At the end of the 28-day incubation period, biodegradation reached 93%. Measurements taken at 5 and 15-days showed biodegradation at 44% and 82%, respectively. Based on these results, cocamidopropyl betaine is readily biodegradable. (MRID 51393102)

In a second OECD 301D study, two concentrations (2 mg/L and 5 mg/L) of a ~30% cocamidopropyl betaine solution were assayed. At the end of the 28-day incubation period, biodegradation was 86% at the 2 mg/L dose. There was insufficient residual oxygen in the test system to get an accurate read at the 5 mg/L dose. Measurements taken at 7 and 21-days showed biodegradation at 40% and 80%, respectively. Based on these results, cocamidopropyl betaine is readily biodegradable. (MRID 51393102).

The anaerobic biodegradability of cocamidopropyl betaine was also tested. The initial test substance concentration used for the 42-day study was 50 mg/L. The percent degradation of test chemical ranged from 25.1 to 60.9% by using the evolution of CO_2, CH_4 and dissolved inorganic carbon % of organic carbon of test substance. Based on the results of these studies, cocamidopropyl betaine was shown to be inherently biodegradable in nature under anaerobic conditions. (ECHA)

8.3. Ecotoxicity

8.3.1. Freshwater Fish

In an acute freshwater fish study conducted with zebrafish (*Brachydario rerio* also known as *Danio rerio*), the fish were exposed to nominal concentrations of 0, 5.66, or 8.0 mg/L of a 30% active cocamidopropyl betaine solution for 96 hours. All fish exposed to 8.0 mg/L died within the first 24 hours of exposure. The 96-hour median lethal concentration (EC_{50}) for the test chemical was 2.0 mg/L. (ECHA)

Two additional tests were outlined in the ECHA dossier for cocamidopropyl betaine. A semi-static test (OECD 203) was conducted using zebrafish, *Danio rerio* and a short-term toxicity test was conducted using carp, *Cyprinus carpio.* In the test with zebrafish, nominal concentrations of 0, 5.66, or 8.0 mg/L of a cocamidopropyl betaine solution were tested for 96-hours. All 10 fish exposed to 8.0 mg/L died within the first 24 hours of exposure. None of the animals exposed to 5.66 mg/L died. The 96-hour LC_{50} was determined to be 6.73 mg/L. In the short-term toxicity test conducted using carp, a 29.6% cocamidopropyl betaine solution was tested in concentrations of 0, 1,.0, 1.7, 3.0, 5.0 and 9 mg/L. The 96-hour LC_{50} was determined to be 1.9 mg/L.

A long-term fish toxicity test was conducted with rainbow trout, *Oncorhynchus mykiss* under OECD Guideline 204. In this study, based on mortality, the NOEC and LOEC after 28 days were 0.16 mg/L and 0.5 mg/L, respectively. (ECHA)

8.3.2. Aquatic Invertebrates

In an acute study conducted according to OECD Guideline 202, freshwater invertebrates, *Daphnia magna*, were exposed to a nominal concentration of 0, 0.5, 1, 2, 4, 8, and 16 mg/L cocamidopropyl betaine for 48 hours. There was no immobilization at the 0.5 mg/L concentration. At concentrations of 1 and 2 mg/L, 2 and 3 daphnia, respectively, were found immobile. The 4 mg/L and 8 mg/L concentrations caused immobilization in 5 and 9 daphnia, respectively. Although the report is not clear, it appears 20 daphnia were in each test group. All the animals treated with 16 mg/L were observed to be immobile. Immobilization in the control group was recorded at 1.7%. The Litchfield and Wilcoxon Method was used to determine the 50% immobilization (EC_{50}) of 6.40 mg/L. (ECHA)

In another study, following OECD Guideline 202, *Daphnia magna* were exposed to nominal concentrations of 0, 6.25, 12.5, 25, 50 and 100 mg/L cocamidopropyl betaine. There were 10 daphnids per dose. The results showed a 48-hour 50% immobilization (EC_{50}) of 21.5 mg/L. (ECHA)

8.3.3. Algae

The toxicity of cocamidopropyl betaine to marine microalgae, *Ulva lactuca*, was tested with nominal concentration of 0, 5, 10, 15, 20, 25, 30, 35, and 40 mg/L. The 50% effect on biomass was observed at a concentration 30 mg/l after 48 hours of exposure. Therefore, the 48-hour EC_{50} for freshwater algae was 30 mg/L. (ECHA)

In an OECD Guideline 201 test, cultures of *Scenedesmus subspicatus* (now known as *Desmodesmus subspicatus*) were exposed to cocamidopropyl betaine at nominal concentrations of 0.32, 1.0, 3.2, 10, 32, and 100 mg/L under static conditions for 72 hours. The 72-hour EC_{50} for biomass and growth rate were found to be 30, and 48 mg/L, respectively. (ECHA)

In a 96-hour toxicity study conducted to assess growth inhibition, cultures of *Scenedesmus subspicatus* were exposed to nominal concentrations of cocamidopropyl betaine at 0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, and 10 mg/L. A sample from each flask was taken at 24, 48, 72 and 96 hours and the density of algal cells was measured. Each experimental group was replicated three times. The 96-hour EC_{50} was 1.84 mg/L. (ECHA)

9. RISK CHARACTERIZATION

Based on the low toxicity of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine in the available studies, EPA has concluded that there are no systemic toxicological endpoints of concern for the U.S. population, including infants and children, and a qualitative assessment is appropriate for all pathways of human exposure. As part of its qualitative assessment, the Agency did not use safety factors for assessing risk, and no additional safety factor is needed for assessing risk to infants and children.

Based on the ecotoxicity and environmental fate data available, 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine may be moderately toxic to aquatic organisms, however, a percent limitation in formulation is being placed on each chemical. In addition, they are expected to readily biodegrade and therefore, there is low concern for environmental toxicity.

Taking into consideration all available information, EPA concludes that there is a reasonable certainty that no harm to any population subgroup will result from exposure to 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine when considering sources of pesticide exposure for which there is reliable information. Therefore, the use of 1-propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acyl derivatives, inner salts and cocamidopropyl betaine as an inert ingredient in food-use pesticide formulations under 40 CFR 180.910 at a maximum concentration of 25% of the finished product and 180.920 at a maximum concentration of 10% of the finished product , respectively can be considered assessed as safe.

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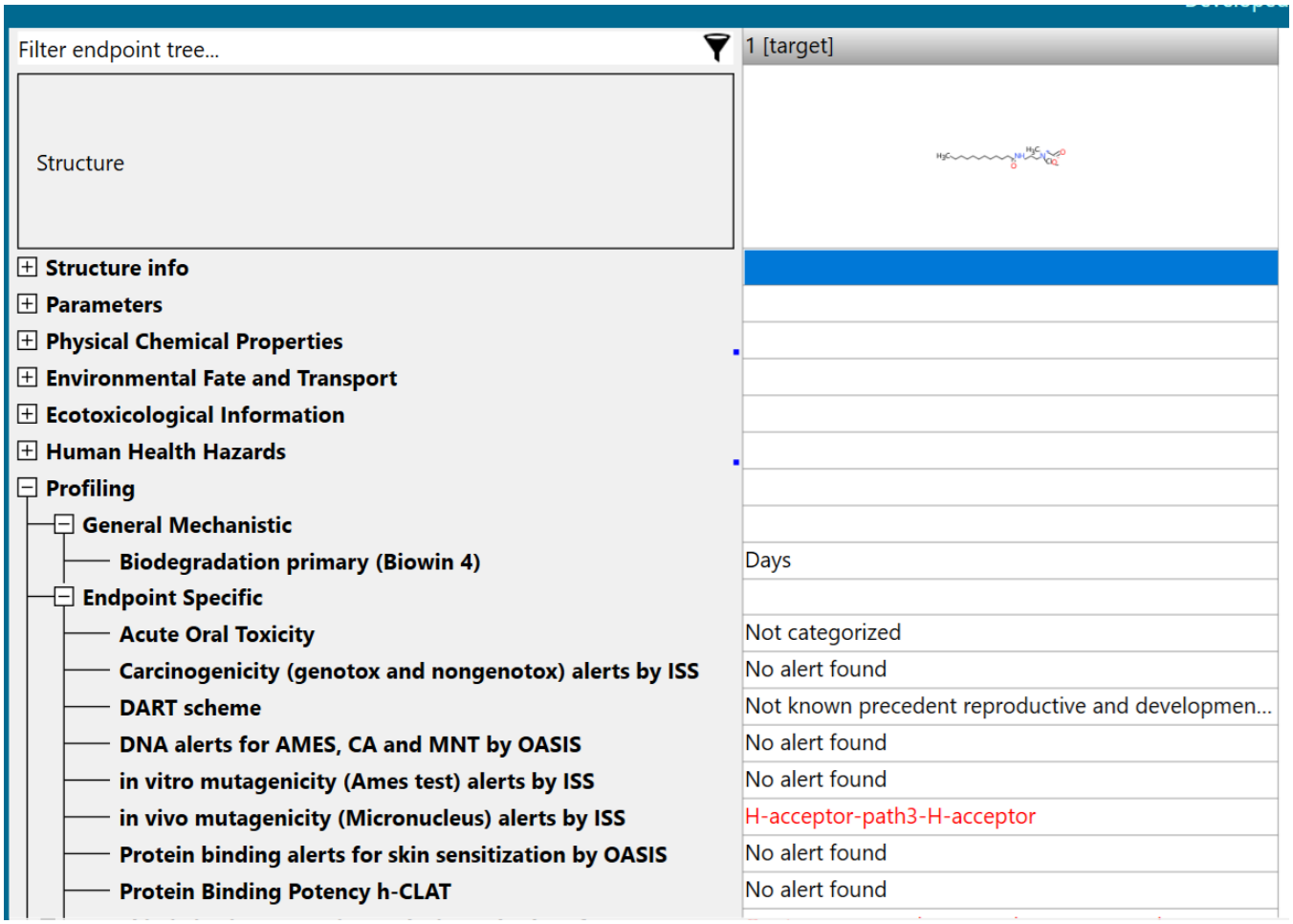
Appendix 1. Results of QSAR Analysis for cocamidopropyl betaine (monoconstituent) using the QSAR toolbox version 4.5



Filter endpoint tree...	1 [target]
Structure	
⊞ **Structure info**	
⊞ **Parameters**	
⊞ **Physical Chemical Properties**	
⊞ **Environmental Fate and Transport**	
⊞ **Ecotoxicological Information**	
⊞ **Human Health Hazards**	
⊟ **Profiling**	
⊟ **General Mechanistic**	
Biodegradation primary (Biowin 4)	Days
⊟ **Endpoint Specific**	
Acute Oral Toxicity	Not categorized
Carcinogenicity (genotox and nongenotox) alerts by ISS	No alert found
DART scheme	Not known precedent reproductive and developmen...
DNA alerts for AMES, CA and MNT by OASIS	No alert found
in vitro mutagenicity (Ames test) alerts by ISS	No alert found
in vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor
Protein binding alerts for skin sensitization by OASIS	No alert found
Protein Binding Potency h-CLAT	No alert found

Appendix 2. EPI Suite v4.11 for CAS Reg. No. 61789-40-0 and 499781-63-4

CAS Number: 61789-40-0
SMILES : CCCCCCCCCCCC(=O)NCCCN(H)(C)(C)CC(=O)(O)
CHEM : Cocamidopropyl betaine
MOL FOR: C19 H40 N2 O3
MOL WT : 344.54
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.68 estimate) = 2.69

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 650.63 (Adapted Stein & Brown method)
Melting Pt (deg C): 282.96 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 4.81E-015 (Modified Grain method)
VP (Pa, 25 deg C) : 6.41E-013 (Modified Grain method)
Subcooled liquid VP: 3.44E-012 mm Hg (25 deg C, Mod-Grain method)
: 4.59E-010 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 33.91
log Kow used: 2.69 (estimated)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2.3891 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Amides -acid

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 3.90E-022 atm-m3/mole (3.96E-017 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:

HLC: 6.430E-017 atm-m3/mole (6.516E-012 Pa-m3/mole)
VP: 4.81E-015 mm Hg (source: MPBPVP)
WS: 33.9 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 2.69 (KowWin est)
Log Kaw used: -19.797 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 22.487
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.9748
Biowin2 (Non-Linear Model) : 0.9642
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.0465 (weeks)
Biowin4 (Primary Survey Model) : 4.2107 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.6787
Biowin6 (MITI Non-Linear Model): 0.7162
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.1753
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 4.59E-010 Pa (3.44E-012 mm Hg)
Log Koa (Koawin est): 22.487
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 6.54E+003
Octanol/air (Koa) model: 7.53E+009
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 1
Mackay model : 1
Octanol/air (Koa) model: 1

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 48.4209 E-12 cm3/molecule-sec
Half-Life = 0.221 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 2.651 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):

1 (Junge-Pankow, Mackay avg)
1 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 647.5 L/kg (MCI method)
Log Koc: 2.811 (MCI method)
Koc : 33.96 L/kg (Kow method)
Log Koc: 1.531 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 1.850 (BCF = 70.79 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -0.7016 days (HL = 0.1988 days)
Log BCF Arnot-Gobas method (upper trophic) = 1.489 (BCF = 30.81)
Log BAF Arnot-Gobas method (upper trophic) = 1.489 (BAF = 30.81)
log Kow used: 2.69 (estimated)

Volatilization from Water:
Henry LC: 3.9E-022 atm-m3/mole (estimated by Bond SAR Method)
Half-Life from Model River: 2.787E+018 hours (1.161E+017 days)
Half-Life from Model Lake : 3.04E+019 hours (1.267E+018 days)

Removal In Wastewater Treatment:
Total removal: 3.77 percent
Total biodegradation: 0.11 percent
Total sludge adsorption: 3.66 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	3.59e-009	5.3	1000
Water	16.5	360	1000
Soil	83.1	720	1000
Sediment	0.403	3.24e+003	0

Persistence Time: 779 hr

CAS Number: 499781-63-4
SMILES : O=C(O)CCN(H)(C)(C)CCCNC(=O)CCCCCCCCCCCC
CHEM : 1-Propanaminium, 3-amino-N-(2-carboxyethyl)-N,N-dimethyl-, N-coco acy
l derivs., inner salts

MOL FOR: C21 H44 N2 O3
MOL WT : 372.60
---------- EPI SUMMARY (v4.11) ----------
Henry LC (atm-m3/mole) : ------
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Water Solubility (mg/L): ------
Physical Property Inputs:
Vapor Pressure (mm Hg) : ------
Melting Point (deg C) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.68 estimate) = 3.67

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 673.84 (Adapted Stein & Brown method)
Melting Pt (deg C): 293.80 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 8.51E-016 (Modified Grain method)
VP (Pa, 25 deg C) : 1.13E-013 (Modified Grain method)
Subcooled liquid VP: 8.34E-013 mm Hg (25 deg C, Mod-Grain method)
: 1.11E-010 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 3.321
log Kow used: 3.67 (estimated)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.21787 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Amides -acid

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 3.45E-023 atm-m3/mole (3.49E-018 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.256E-016 atm-m3/mole (1.273E-011 Pa-m3/mole)
VP: 8.51E-016 mm Hg (source: MPBPVP)
WS: 3.32 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used: 3.67 (KowWin est)
Log Kaw used: -20.851 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 24.521
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.9614
Biowin2 (Non-Linear Model) : 0.9476
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.9845 (weeks)
Biowin4 (Primary Survey Model) : 4.1702 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.6941
Biowin6 (MITI Non-Linear Model): 0.7261
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.1233
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 1.11E-010 Pa (8.34E-013 mm Hg)
Log Koa (Koawin est): 24.521
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 2.7E+004
Octanol/air (Koa) model: 8.15E+011
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 1
Mackay model : 1
Octanol/air (Koa) model: 1

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 54.8650 E-12 cm3/molecule-sec
Half-Life = 0.195 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 2.339 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
1 (Junge-Pankow, Mackay avg)
1 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc : 2151 L/kg (MCI method)
Log Koc: 3.333 (MCI method)
Koc : 118.3 L/kg (Kow method)
Log Koc: 2.073 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 1.850 (BCF = 70.79 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -0.4233 days (HL = 0.3773 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.081 (BCF = 120.4)
Log BAF Arnot-Gobas method (upper trophic) = 2.081 (BAF = 120.4)
log Kow used: 3.67 (estimated)

Volatilization from Water:
Henry LC: 3.45E-023 atm-m3/mole (estimated by Bond SAR Method)
Half-Life from Model River: 3.276E+019 hours (1.365E+018 days)
Half-Life from Model Lake : 3.574E+020 hours (1.489E+019 days)

Removal In Wastewater Treatment:
Total removal: 17.51 percent
Total biodegradation: 0.22 percent
Total sludge adsorption: 17.29 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.38e-009	4.68	1000
Water	15.9	360	1000
Soil	83	720	1000
Sediment	1.11	3.24e+003	0

Persistence Time: 789 hr