# Safety Assessment of PEGs Cocamine and Related Ingredients as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date to comment on this Safety Assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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 Director, Dr. Lillian J. Gill.*

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This safety assessment was prepared by Ivan J. Boyer, Ph.D., Senior Toxicologist, Christina L. Burnett, Senior Scientific Analyst/Writer, and Bart Heldreth, Ph.D., Chemist.


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## ABSTRACT

The CIR Expert Panel assessed the safety of 47 PEGs cocamine and related ingredients. These ingredients comprise mixtures of mostly tertiary amines that have alkyl groups derived from plant or animal fatty acids and an average number of polyethylene glycol groups equal to the number in the chemical name. Most of these ingredients are reported to function as surfactants or antistatic agents. The Panel reviewed the available test data and an SAR-based read-across assessment to evaluate the safety of these ingredients. The Panel concluded that 32 of these ingredients are safe in the current practices of use and concentration when formulated to be non-irritating; this conclusion supersedes the 1999 conclusion issued on six PEGs cocamine ingredients. The data were insufficient to determine the safety of the 15 other ingredients included in this safety assessment, all of which have PEG-2, -3, -4, or -5 in the ingredient names.


## INTRODUCTION

The CIR Expert Panel issued a final report on the safety assessment of PEG-2, -3, -5, -10, -15, and -20 cocamine, which was published in 1999.[1] The Panel concluded that the data were insufficient to support the safety of these ingredients for use in cosmetic products.

Genotoxicity data were available from a single non-standard bacterial mutagenicity test in which PEG-15 cocamine was negative. Repeated-dose toxicity data were available from a single study in which 10% PEG-15 cocamine was applied to the shaved skin of rats 5 days per week for 6 weeks (30 applications), and no signs of systemic toxicity were found. However, no dermal sensitization data were available for these ingredients. Thus, the CIR Expert Panel determined that the additional data needed included:

- Physical and chemical properties, including impurities (especially nitrosamines)
- Genotoxicity in a mammalian test system (if the results are positive then a dermal carcinogenesis study may be needed)
- 28-Day dermal toxicity using PEG-2 cocamine
- Dermal sensitization data on PEG-2 cocamine

Data specifically on PEG-2 cocamine were needed to demonstrate that relevant exposures to the ingredient with the lowest molecular weight in this group of would not be toxic.[1]

The CIR Science and Support Committee (SSC) contended that the gaps in genotoxicity and systemic toxicity data can be filled by applying the framework developed by Wu et al.[2] to identify and evaluate analogs for read across analyses. The framework is based on the assessment of structure activity (SAR) relationships, and enables the incorporation of information from the literature and predictive computational tools on physicochemical properties, chemical reactivity, metabolism and toxicity to identify suitable analogs and develop an overall weight-of-evidence safety assessment. The CIR SSC submitted two reports to the Panel, one in 2011[3] and another in 2012,[4] in which the framework was used to identify and evaluate structural analogs for a representative set of PEGs cocamine, and to read across from the data available for the analogs. The second CIR SSC submission was preceded by Dr. Karen Blackburn's presentation at the CIR Expert Panel Workshop in March 2012, in which she explained the framework and illustrated how the framework could be used for read-across assessment of the PEGs cocamine and related ingredients.[5]

The read-across analysis presented in these two CIR SSC submissions,[3,4] and illustrated in Dr. Blackburn's presentation to the Panel,[5] indicates that these ingredients will not exhibit genotoxicity or systemic toxicity when used as intended in cosmetics. In addition, the CIR SSC's submissions include data and computational analyses indicating that the PEGs cocamine, like the PEGs, are not dermal sensitizers.[3,4,6]

This safety assessment presents data and analyses from multiple sources, including the Council and the CIR SSC, which may enable or facilitate assessing the safety of the PEGs cocamine and related ingredients. CIR staff conducted a thorough search of the published scientific literature for information on the toxicity of all of the ingredients (original and proposed add-ons) and the analogs selected for read across in the CIR SSC's submissions. The search yielded nothing of likely relevance for the assessment of these ingredients, except for the information presented in CIR's original safety assessment of PEG-2, -3, -5, -10, -15, and -20 cocamine, and possibly some toxicity information published on polyoxyethyleamine tallow amine (the predominant surfactant in Roundup®).

In this safety assessment, selected excerpts from the original safety assessment are presented as *italicized text*.

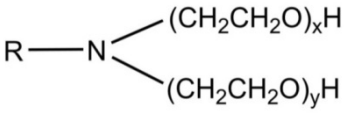
## CHEMISTRY

### Definition and Structure

***PEGs Cocamine***

*PEG-2, -3, -5, -10, -15, and -20 (CAS# 61791-14-8 [generic]) cocamine are the polyethylene glycol ethers of the primary aliphatic amine derived from coconut oil*[1]*. Other names for these compounds include polyethylene glycol (x + y) coconut amine, polyoxyethylene (x + y) coconut amine and polyoxyethylene (POE) cocamine.*

The basic structure of the PEGs cocamine (CAS# 61791-14-8, generic) is as follows:



The PEGs cocamine are a series of tertiary amines that conform to the formula shown above, where R represents the alkyl groups derived from the fatty acids of coconut oil and the x+y of the polyethylene glycol groups has an average value equal to the number in the chemical name. The structure of the smallest member of the group, PEG-2 cocamine, will have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2. The possibility of similar structural variations is notable for PEG-3, -4, and -5 cocamine.

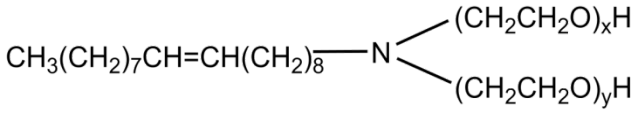
The distribution of chain lengths and degree of unsaturation of the fatty acids in coconut oil are described in Table 1. Thus, each PEGs cocamine is a mixture of compounds with the major fatty-acid derived chain lengths of C12 and C14.

The PEGs cocamine listed in the International Cosmetic Ingredient (INCI) Dictionary include PEG-2, -3, -4, -5, -8, -10, -12, -15 and -20 cocamine. All of these ingredients, except PEG-4 cocamine, were addressed in the original CIR Expert Panel assessment published in 1999.

The CIR Expert Panel concluded tentatively that the ingredient group should include other, structurally-similar ingredients listed in the INCI Dictionary, including PEGs oleamine, PEGs tallow amine, PEGs hydrogenated tallow amine, PEGs soyamine, PEG-2 rapeseedamine, PEGs stearamine, PEG-2 lauramine, and PEG-12 palmitamine. Description of the chemical structures of these other, related tertiary amines is provided below.

***PEGs Oleamine***

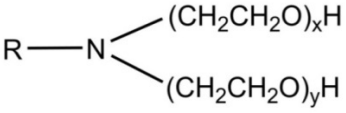
The basic structure of the PEGs oleamine (CAS# 26635-93-8, generic) is as follows:



The PEGs oleamine are a series of tertiary amines that conform to the formula shown above, where x+y has an average value equal to the number in the chemical name. The PEGs oleamine listed in the INCI Dictionary include PEG-2, -5, -6, -10, -15, -20, -25 and -30 oleamine. The structure of the smallest member of the group, PEG-2 oleamine, will have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2. The possibility of similar structural variations is notable for PEG-5 oleamine.

***PEGs Tallow Amine***

The basic structure of the PEGs tallow amine (CAS# 61791-26-2, generic) is as follows:



The PEGs tallow amine are a series of tertiary amines that conform to the formula shown above, where R represents the alkyl groups derived from the fatty acids of tallow and x+y has an average value equal to the number

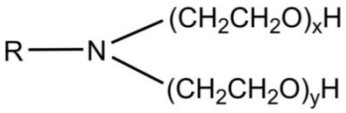
in the chemical name. The distribution of chain lengths and degree of unsaturation of the fatty acids in tallow are described in Table 2.

Therefore, each PEGs tallow amine is a mixture of compounds with the major fatty-acid derived chain lengths of C16 and C18 with a considerable fraction consisting of unsaturated alkyl groups.

The PEGs tallow amine listed in the INCI Dictionary include PEG-2, -7, -11, - 15, -20, -22, -25 and -30 tallow amine. The structure of the smallest member of the group, PEG-2 tallow amine, will have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2.

***PEGs Hydrogenated Tallow Amine***

The basic structure of the PEGs hydrogenated tallow amine (CAS# 6I79I-26-2, generic) is as follows:

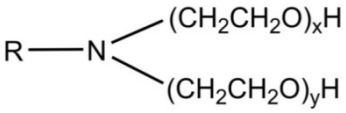



The PEGs hydrogenated tallow amine are a series of tertiary amines that conform to the formula shown above where R represents the alkyl groups derived from the fatty acids of hydrogenated tallow and x+y has an average value equal to the number in the chemical name. In hydrogenated tallow, the degree of unsaturation of the fatty acids is reduced or eliminated by hydrogenation.

The PEGs hydrogenated tallow amines listed in the INCI Dictionary include PEG-2, -5, -8, -10, -15, -20, -30, -40, and -50 hydrogenated tallow amine. The structure of the smallest member of the group, PEG-2 hydrogenated tallow amine, will have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2. The possibility of similar structural variations is notable for PEG-5 hydrogenated tallow amine. Partial hydrogenation of the tallow used to produce this ingredient may yield PEGs hydrogenated tallow amine with trans-fatty acid moieties.

***PEGs Soyamine***

The basic structure of the PEGs soyamine (CAS# 61791-24-0, generic) is as follows:

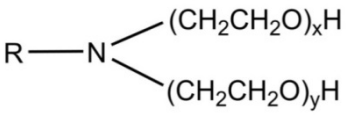



The PEGs soyamine are a series of tertiary amines that conform to the formula shown above, where R represents the alkyl groups derived from the fatty acids of soy and x+y has an average value equal to the number in the chemical name.

The PEGs soyamine listed in the INCI Dictionary include PEG-2, -5, -8, -10 and -15 soyamine. The structure of the smallest member of the group, PEG-2 soyamine, will have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2. The possibility of similar structural variations is notable for PEG-5 soyamine.

***PEG-2 Rapeseedamine***

The basic structure of PEG-2 rapeseedamine (no CAS# provided) is as follows:

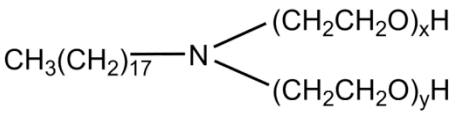



PEG-2 rapeseedamine conforms to the formula shown above, where R represents the alkyl group derived from the fatty acids of rapeseed oil and x+y has an average value of 2. The structure of PEG-2 rapeseedamine will

have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2.

***PEGs Stearamine***

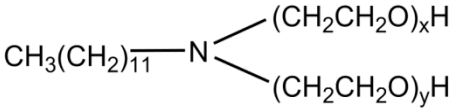
The basic structure of the PEGs stearamine (CAS# 9003-93-4, generic) is as follows:



The PEGs stearamine are a series of tertiary amines that conform to the formula shown above, where x+y has an average value equal to the number in the chemical name. The PEGs stearamine listed in the INCI Dictionary include PEG-2, -5, -10, -15 and -50 stearamine. The structure of the smallest member of the group, PEG-2 stearamine, will have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2. The possibility of similar structural variations is notable for PEG-5 stearamine.

***PEG-2 Lauramine***

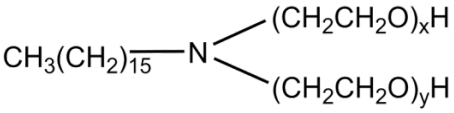
The structure of PEG-2 lauramine (no CAS# provided) is as follows:



PEG-2 lauramine conforms to the formula shown above, where the alkyl group is derived from lauric acid (C12) and x+y has an average value of 2. The structure of PEG-2 lauramine will have two hydroxyethyl groups, rather than polyethoxyl groups, if x and y both equal 1. The structure will have one hydroxyethyl group and one polyethoxyl group if x=0 and y=2.

***PEG-12 Palmitamine***

The structure of PEG-12 palmitamine (CAS# 68155-33-9, generic) is as follows:



PEG-12 palmitamine conforms to the formula shown above, where the alkyl group is derived from palmitic acid (Cl6) and x+y of the polyethylene glycol groups has an average value of 12.

**Physical and Chemical Properties**

*PEG-15 cocamine is a clear, light brown, oily liquid.*[1] *It is soluble in water, isopropyl alcohol, and benzene. The specific gravity ranges from 1.040 to 1.046. Allowable moisture and ash are 3% and 0.5% maximum, respectively.*

Specifications for some of the PEGs cocamine and related ingredients can be found in Tables 1, 2 and 3.

**Method of Manufacture**

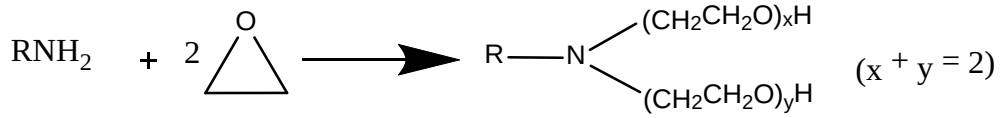
*The PEG-n cocamine polymers are manufactured by condensing coconut acid with the ingredient's corresponding number of moles (n) of ethylene.*[1]

*PEGs are formed by condensing ethylene oxide and water, with the average number of moles of ethylene oxide polymerized indicated by the number in the name.*[7]

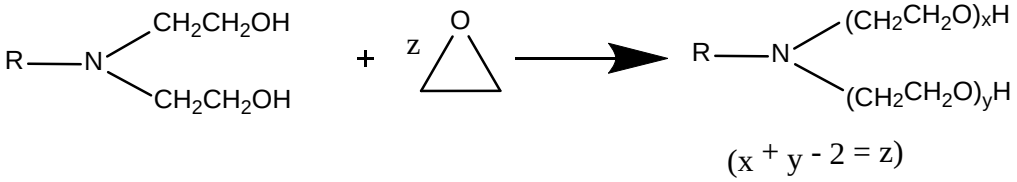
*Coconut acid is a mixture of fatty acids derived from coconut oil. Coconut oil is obtained by expression from the kernels of the seeds of Cocos nucifera. The primary constituents of coconut oil are trimyristin, trilaurin,*

*tripalmitin, tristearin, and various other triglycerides. About 90% of the oil is saturated. The expressed material has a water content of coconut oil. The fatty material is isolated after hydrolysis of coconut oil and then distilled to form coconut acid.*

The synthesis of ethoxylated fatty acids is essentially a two-step process.[5] The first step is illustrated as follows:




This reaction proceeds until all of the primary and secondary amine is consumed, yielding the smallest group members of this class, which the nomenclature calls PEG-2s. The second step, which is illustrated below, requires a catalyst.




The chain length of the polyethylene glycol groups depend on the duration of the reaction, and these groups may not be symmetrical; typically, this reaction yields a range of polyethylene glycol chain lengths.

### Impurities/Constituents

*Coconut oil is usually low in color bodies, pigments, phosphatides, gums, and other nonglyceride substances commonly found in larger quantities in other vegetable oils. It may contain free fatty acids, low concentrations of sterols, tocopherol, and squalene. The characteristic coconut flavor is due to the presence of approximately 150 ppm lactones that are present as a series of d-lactones with 6, 8, 10, 12, and 14 carbon atoms. Crude samples of coconut oil contain traces of polycyclic aromatic hydrocarbons, particularly when the copra is smoke-dried. A combination of activated charcoal treatment and steam vacuum deodorization are the common refining methods most likely to remove the hydrocarbons from the edible oils. Aflatoxin contamination of raw and dried copra have been reported. Improper drying, handling, and storage greatly increase the possibility of contamination by aflatoxins, secondary metabolites of the mold Aspergillus flavus, which grows on copra. Smoke drying of copra inhibited aflatoxin formation.*

The PEGs cocamine and related ingredients, like the PEGs, may contain traces of 1,4-dioxane, which is a by-product of ethoxylation, and ethylene oxide as impurities.[1,7,8] In addition, these ingredients are mixtures of tertiary alkyl amines that may also contain some secondary or primary amines. Thus, the formation of nitrosamines in formulation should be considered.

## USE

### Cosmetic

Tables 4 and 5 present the historic and current product-formulation use data for PEGs cocamine and related ingredients. These ingredients function primarily as surfactants and antistatic agents.[9]

The Food and Drug Administration (FDA) collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). VCRP data obtained from the FDA in 2014 and data received in response to a survey of the maximum reported use concentration by category conducted by the Council in 2014 indicate that 10 ingredients included in this report are used in cosmetic formulations. According to the 2014 VCRP survey data, PEG-2 rapeseedamine has the most reported uses in cosmetic and personal care products, with a total of 255;[10] all reported uses were in rinse-off hair-coloring preparations. PEG-2 oleamine has the second greatest number of overall uses reported, with a total of 239, all in rinse-off hair-coloring preparations.

Of the PEGs cocamine still reported to be in use in 2014, the frequency of use totaled 15 for PEG-2 cocamine and 28 PEG-15 cocamine in 1996, compared to 107 for PEG-2 cocamine and 4 for PEG-15 cocamine in

2014. The highest maximum use concentration for PEGs cocamine (length of ethoxy moieties not specified) was 20% in 1995, compared to 3% PEG-15 cocamine and 3.5% PEG-2 oleamine in 2014.[1]

No use concentrations were reported for PEG-2 rapeseedamine in the Council's 2014 use-concentration survey.[11] PEG-2 oleamine had a maximum-use concentration range of 0.1% to 3.5%, with 3.5% reported in hair dyes and colors.

Table 6 lists the 37 PEGs-cocamine ingredients not indicated to be in use, based on the 2014 VCRP data and the results of the Council's 2014 concentration of use survey.

Some of the ingredients in use are reported to be used in body and hand sprays and could possibly be inhaled. For example, PEG-15 cocamine was reported to be used in body and hand sprays at a highest maximum concentration of 3%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm compared with pump sprays.[12,13] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[14,15]

### Non-Cosmetic

The predominant surfactant in Roundup® is a polyoxyethyleneamine tallow amine (aka polyoxyethyleneamine or POEA),[16,17] which is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids.[17] The molecular size of POEA is not specified in the literature. However, its size probably fits into the range of sizes of the ingredients used in cosmetic products.

Roundup® contains 15% or more POEA, which has the same generic CAS# (61791-26-2) as several of the cosmetic ingredients addressed in this safety assessment (i.e., PEGs tallow amine and PEGs hydrogenated tallow amine).[17] POEA is listed by US EPA as a pesticide inert ingredient (http://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:2:0::NO).

A published article summarized several unpublished studies on glyphosate (i.e., the active ingredient) and the other major components of Roundup®, including POEA.[17] The results reported for POEA were:

- Acute oral (rats) and dermal (rabbits) $LD_{50}$ 1200 mg/kg and 1260 mg/kg, respectively
- Severely irritating to the skin and corrosive to the eyes in rabbits
- Subchronic oral NOAEL 500 ppm in the diet (36mg/kg/day) in rats
- Subchronic oral NOAEL could not be derived in dogs because of emesis and diarrhea at the lowest dose tested (30 mg/kg/day)
- Maternal NOAEL 15 mg/kg/day and developmental NOAEL 300 mg/kg/day (lowest dose tested) in rats by gavage on gestation days 6 through 15
- 1 mg/plate inactive in Ames tests

One study found that a single intratracheal (0.2 g/kg) or oral (1 g/kg) dose of POEA caused lung hemorrhage and lung epithelial damage.[18] In this study, POEA caused immediate respiratory effects and death in some animals within 1 hour after intratracheal exposure. Oral exposure caused diarrhea and blood-stained weeping from the nose, as well as death among the animals within 24 hours. The irritant and corrosive effects of POEA observed in such studies are consistent with the surface-active properties of surfactants in general.[17]

## TOXICOKINETICS

*PEG cocamine absorption and metabolism data were not available.[1] PEG absorption is related to whether the substance is a liquid or a solid. PEGs were readily absorbed through damaged skin. Oral and intravenous studies on the PEGs indicated that these substances were excreted, unchanged, in the urine and feces. Ingested Coconut Oil was almost entirely absorbed with no mortality.*

## TOXICOLOGICAL STUDIES

*The oral $LD_{50}$ of PEG-15 cocamine in rats was 1.2 g/kg, and for PEG-2 cocamine, the $LD_{50}$ ranged from 0.75 g/kg to 1.3 g/kg.[1] No systemic toxic effects occurred in rats following a 6-week dermal application study using 10% PEG- 15 cocamine. PEGs have low oral and dermal toxicity; generally, the greater molecular weight PEGs appear to be less toxic than the lighter PEGs in oral studies. Coconut oil and hydrogenated coconut oil are relatively nontoxic by ingestion.*

*PEG-2 cocamine was classified as a moderate cutaneous irritant, and PEG-15 cocamine was considered a mild irritant. PEGs were nonirritating to the skin of rabbits and guinea pigs, and PEG-75 was not a sensitizer, PEG-2 cocamine was considered an ocular irritant, and PEG-15 cocamine caused corneal irritation.*

*In mutagenicity studies, PEG-15 cocamine was negative. PEG-8 was negative in the Chinese hamster ovary cell mutation test and the sister chromatid exchange test. At concentrations up to 150 g/l, PEG-150 was not mutagenic in the mouse lymphoma forward mutation assay. PEG-8 was not carcinogenic when administered orally, intraperitoneally, or subcutaneously.*

*Although monoalkyl ethers of ethylene glycol are reproductive toxins and teratogenic agents, it was considered unlikely that the PEG cocamine compounds would cause reproductive or teratogenic effects based on their structural characteristics. In subchronic and chronic feeding studies, PEG-6-32 and PEG-75 did not induce reproductive effects in rats.*

*In clinical studies, PEG-8 was a mild sensitizer and irritant. Contact dermatitis and systemic toxicity in burn patients were attributed to a PEG-based topical ointment. Bar soaps containing 13% coconut oil, when tested using Draize procedures, produced minimal skin reactions.*

## IRRITATION AND SENSITIZATION

### Dermal Sensitization

No dermal sensitization studies were found or submitted for PEG-2 cocamine. However, the Council submitted data from two dermal sensitization HRIPTs in 2011.[3,19,20] In one of these HRIPTs, a hair styling formulation containing 1.0% PEG-15 cocamine was not sensitizing in 212 subjects.[19] In the other HRIPT, an adult sunscreen formulation containing 2.9% PEG-15 cocamine was not sensitizing in 201 subjects.[20]

Summary data from a photoallergy study (116 subjects) and a phototoxicity study (22 subjects) were submitted to the CIR in 2011.[3,21,22] In these studies, no photoallergic or other phototoxic effects were found in the skin after exposure to an adult sunscreen formulation containing 2.9% PEG-15 cocamine (no details of these studies were provided)

## FRAMEWORK FOR IDENTIFYING AND EVALUATING ANALOGS FOR READ ACROSS

The CIR SCC used the framework described below, and in greater detail in a paper published by Wu *et al.* (2010),[2] to evaluate and integrate data and the results of computational analyses for read-across assessments of the PEGs-cocamine ingredients.

The development of the framework was informed by the stepwise approach for analog read across proposed by the European Union (EU) Organisation for Economic Co-operation and Development (OECD) Guidance on Grouping of Chemicals (2007).[23] The steps include:

1. Identifying potential analogs
2. Gathering data on these potential analogs
3. Evaluating the adequacy of data for each potential analog
4. Constructing a matrix with available data for the target and analog(s)
5. Assessing the adequacy of the analog(s) to fill the data gap
6. Documenting the entire process

The guidance also emphasizes the importance of comparing the physicochemical properties of the analogs and the structure of interest (SOI) to be evaluated (e.g., a cosmetic ingredient), and assessing the likely toxicokinetics of the analogs and the SOI, including the possibility that divergent metabolic pathways could be important.[23,24]

Using the OECD guidance as a foundation, Wu *et al.* (2010) presented a formal, systematic, comprehensive, expert-driven framework to identify, evaluate the suitability of, and select analogs, based on similarities in chemical structure, reactivity, and metabolic and physicochemical properties, for use in read-across assessments.[2,25,26]

The framework is amenable to incorporating the results of (Q)SAR analyses to fill data gaps for specific endpoints or to inform the overall weight of evidence analysis that is integral to the exercise of the framework.[2,23,25,26]

The framework was developed to facilitate the objective and reproducible selection of analogs and enhances transparency in read-across assessments. The framework enables classifying candidate analogs in a manner that reflects the assumptions and uncertainties associated with their use in a safety assessment, based on structural, reactive, metabolic and physicochemical similarities to the SOI (i.e., the chemical with missing toxicological data),

and differences in physicochemical properties that could affect bioavailability and, consequently, the biological responses that can be expected *in vitro* or *in vivo*.

The framework includes a decision tree that depicts the series of questions that a medicinal chemist addresses about the similarities of a candidate analog and an SOI in structure, reactivity, metabolism, and physicochemical properties.[2] The result of applying the decision tree typically yields a series of "pre-ranked" analogs that are presented to the toxicologists for the read-across assessment.

The results include the classification of each candidate analog as (1) suitable, (2) suitable with interpretation, (3) suitable with a precondition or (4) not suitable:

1. Analogs categorized as "suitable" have the same functional groups, core structure and prevalence and location of reactivity-modifying double bonds as the SOI
2. Analogs categorized as "suitable with interpretation" have the most salient features relevant for reactivity and toxicological activity in common with the SOI, but have other characteristics that differ (i.e., primarily differing physicochemical properties), but these differences do not affect reactivity or do not lead to metabolic divergence that could result in different toxicological profiles
3. Analogs categorized as "suitable with precondition" typically require a hydrolytic or enzymatic reaction to yield the SOI or a close analog.
4. Not suitable for read across to the SOI

In addition, the outcome includes a qualitative characterization of (1) the strength of the evidence supporting the hypothesis of similarity between each candidate analog and the SOI, and (2) the uncertainties associated with the use of the analogs selected for read across.

An important element of the framework is the emphasis on evaluating the potential that an analog and the SOI could show toxicologically significant metabolic convergence or divergence. The search for analogs begins with analysis of key structural or substructural features and functional groups of the SOI and its likely metabolites. Metabolic pathways and major metabolites are identified based on a review of published information or on predictive software.[2]

The authors have also developed a promising battery of models to evaluate the potential of chemicals to cause developmental and reproductive toxicity (DART), including an empirically-based decision tree informed by the principles of estrogen receptor interactions combined with the CEASAR model.[25,26] This tool was designed to serve as another important element in the overall weight-of-evidence analyses conducted using the framework.

Searching for candidate analogs using the framework requires databases that support substructure and structure similarity searches and facilitate the identification of similar structures for which there are relevant toxicological data (e.g., AMBIT®, ChemIDPlus®, Scifinder®, The OECD Toolbox, and DSSTox).[2] Each candidate analog is then compared to the SOI to identify features that could affect toxicity, including:

- Common structural alerts (e.g., using DEREK® software)
- Key functional groups (e.g., ester, aldehyde, amide, or amine)
- Core structures (e.g., phenyl ring, alkyl chain, double bonds conjugated or positioned close to functional groups)
- Differences in physicochemical properties (e.g., molecular weight, $pK_a$, log P, log D and solubility estimated using ACD/Labs® property estimation software)

Evaluating the potential for the metabolism of the analog and the SOI to diverge is accomplished using combinations of metabolism databases (e.g., DiscoveryGate® or Metabolism®), scientific literature searches, substructure searches, software prediction tools (e.g., METEOR®), *in vitro* test results, and the expert judgment of a medicinal chemist.

All of the relevant toxicological data available for the SOI and analogs classified as "suitable," "suitable with interpretation" or "suitable with precondition" are then compiled and reviewed by toxicologists for consistency or concordance of toxicological responses and mechanisms and/or modes of action across multiple endpoints.[2,23]

If a candidate analog has a different toxicity profile than the other candidate analogs, then a well-documented, clear rationale for why that chemical does not fit is needed before moving forward with the read-across assessment; otherwise, more data will be needed to support a decision to move forward with an analysis more likely to have an acceptable degree of uncertainty.

Corroborating data on the SOI may be available to consider for one or more toxicological endpoints. For example, toxicity data may be missing for the SOI for one toxicological endpoint, but data for the SOI for other endpoints may serve as "anchor data" to compare with the corresponding data available for the analog(s). Confidence in the selection of analogs can also be bolstered by knowledge of the molecular mechanism(s), mode(s) of action, or adverse outcome pathway(s) of analogs that can be toxic. The number and the suitability of the analogs that can be identified to evaluate the SOI, and the quality of the study data on the analogs, are other important factors to consider when characterizing the uncertainty associated with a read-across assessment.

The outcome of the classification of the analogs and the integrated review of the analog toxicology data enables a transparent characterization of the uncertainty associated with using the analogs to conduct a read-across assessment of the SOI.[25]

**Uncertainty Rankings**

| High Uncertainty | Moderate Uncertainty | Low Uncertainty |
|---|---|---|
| Read across not recommended | Read across may be possible for some endpoints – larger margin of exposure required | Read across does not require a larger margin of exposure |

All of the data are taken together to develop an overall weight of evidence assessment, including a detailed review for consistency of the toxicology data for the analogs and the SOI, to develop a statement of confidence in the read-across assessment. Exercising the framework can identify multiple analogs of similar suitability for a SOI.

If the weight of evidence supports the use of these analogs for read across, then the most toxic ("worst-case") analog for each hazard endpoint can be identified to enable selecting the critical effect and the point of departure, such as a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL), for the rest of the safety assessment.[2]

In a series of blinded case studies of diverse SOIs, the framework performed well for the endpoints examined (genotoxicity, repeated-dose toxicity, developmental toxicity, and reproductive toxicity).[25,26] Estimates of points of departure (PODs) in the case studies were comparable to conservative PODs that had been independently derived from toxicity data by regulatory and advisory agencies.[25] Predictions of 14 blinded case studies were:

- Genotoxicity (+/-); All correct predictions
- Repeated-dose toxicity (surrogate NOAEL estimates); No underestimates
- Developmental toxicity (critical effect +/-; if +, surrogate NOAEL estimates); No underestimates
- Reproductive toxicity (critical effect +/-; if +, surrogate NOAEL estimates); No underestimates

The read-across results were protective compared to *bona fide* toxicity data on the case-study chemicals. The authors concluded that the process can be successfully applied to develop surrogate toxicity values for safety assessments.[3,4] However, Dr. Blackburn emphasized that the successful application of the approach requires substantial expertise and discipline to avoid stepping over the boundaries of the defined analogs and the suitability rating system.

In sum, the case studies showed that applying the framework can enable or facilitate the conduct of transparent, reproducible, and conservative read-across assessments.

## APPLICATION OF THE FRAMEWORK TO EVALUATE PEGS COCAMINE INGREDIENTS

### Analog Selection

Across the PEGs cocamine ingredients, there are substantial differences in physicochemical properties, potential reactivity, and possibly metabolism. Thus, the group was divided into discrete subgroups, each with its own spectrum of analogs, for the initial assessment.

In accordance with guidance from a medicinal chemist, the initial subgrouping was based primarily on the ethylene glycol chains, rather than the fatty-amine chains, because of the potential impact of the ethoxy chains on physicochemical properties, reactivity, and metabolism. The potential impact of the amine-chain lengths was not ignored, but was considered to be secondary.

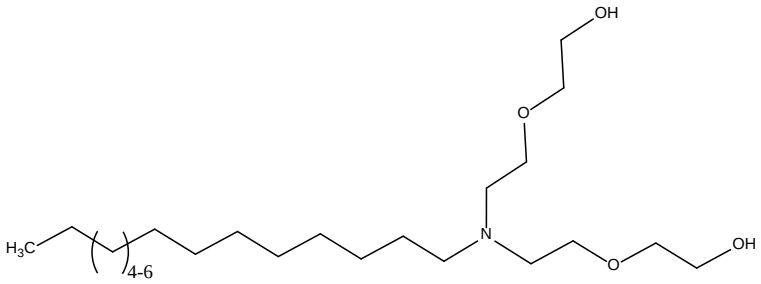
Another important criterion during this early stage of analog selection was based on evidence in the literature on ethylene glycol that polyethylene glycol chains >8 ethoxy (EO) units are not metabolized. Thus, it was

important to separate the shorter PEGs cocamine from longer PEGs cocamine at the EO = 8 break point, at least initially.

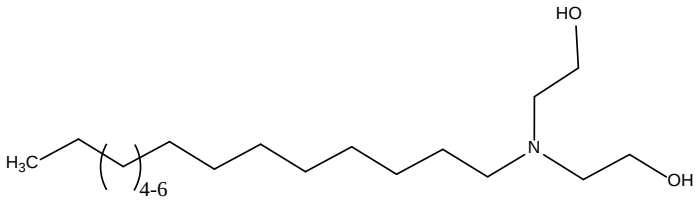
Four PEGs cocamine were selected as the structures of interest (SOIs) to cover the range of polyethylene glycol side-chain lengths for identifying analogs. The alkyl-amine chain length and degree of unsaturation were considered when evaluating the suitability of the analogs identified for each of these four PEGs cocamine. The four PEGs cocamine selected as SOIs are:

- PEG-2 cocamine (Analog Group 1)
- PEG-4 cocamine (Analog Group 2)
- PEG-10 cocamine (Analog Group 3)
- PEG-15 cocamine (Analog Group 4)

Following is an example that illustrates the preliminary selection of 3 of the 5 analogs identified for PEG-4 cocamine (Analog Group 2) as an SOI. The structure of one major component of PEG-4 cocamine is:

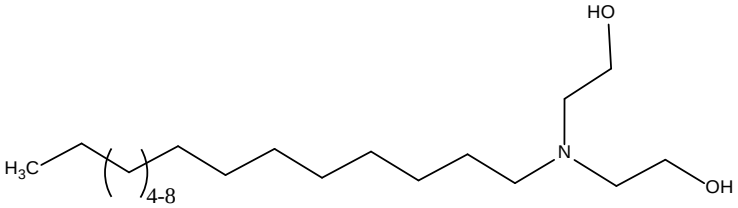



One analog identified as "suitable with interpretation" for PEG-4 cocamine is PEG-2 cocamine (CAS# 61791-31-9), which has the following structure:




Interpretation is needed because of the presence of hydroxyethyl groups, rather than the polyethoxyl groups of PEG-4 cocamine. The absence of polyethoxyl groups on the side chain is highly likely to result in a different metabolic fate and toxicity of the analog, compared with PEG-4 cocamine.

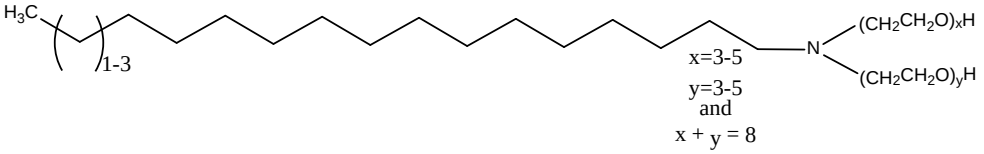
Another analog identified as "suitable for interpretation" for PEG-4 cocamine is tallow bis(2-hydroxyethyl)amine C16-C18 (CAS# 61791-44-4), which has the following structure:




Like PEG-2 cocamine, interpretation was necessary for this analog because of the presence of hydroxyethyl groups, rather than the polyethoxyl groups. However, the chain-length distributions of the tallow of the analog and the coconut oil of the PEG-4 cocamine overlap, thus the difference in the distributions is not expected to cause significant differences in the toxicity profiles of these substances.

The tallow amines have a greater degree and percentage of unsaturation of the fatty acid moieties, compared to the coconut oil fatty acids and the hydrogenated tallow amines. The tallow amines are potentially more toxic than the cocamines and the hydrogenated tallow amines because the unsaturated fatty acid moieties of the tallow amines are susceptible to epoxidation and hydroperoxidation.

A third analog, which was identified as "suitable" for PEG-4 cocamine, is PEG-8 hydrogenated tallow amine (CAS# 26635-92-7), which has the following structure:




The differences in chain-length distribution between tallow and coconut oil would not be expected to yield substantial differences in the toxicity profile of the analog, compared with the SOI.

Figures 1 through 4 show the representative structures of each of the SOIs and the corresponding analogs for each analog group. The PEG-cocamine and related ingredients of each group are shown in red in these figures. Some of the analogs illustrated in each group are PEGs cocamine or related ingredients for which toxicological data were not available for read across, including PEG-4 cocamine, Peg-10 cocamine and PEG-15 cocamine.

Many of the analogs are the larger tallow derivatives, rather than the smaller cocamine derivatives, which will generally have greater degrees of unsaturation as well as longer alkyl chain lengths. Hydrogenated tallow will be saturated, but PEGs hydrogenated tallow amines will still have larger alkyl groups than the corresponding PEGs cocamine.

## Chemical Structure

The SOIs and selected analogs were evaluated for commonality of structural alerts (e.g., Ashby alerts for genotoxicity and DEREK® alerts for several toxicity endpoints), key functional groups and core substructures, as well as for the presence of additional functional groups. This effort showed a satisfactory degree of commonality in structural features and alerts across the SOIs and analogs.

No structural alerts were found for genotoxicity when the SOIs and analogs were evaluated using the DEREK® and TIMES® prediction models.

The SOIs and analogs with ethoxylated chains consistently yielded a "rapid prototype" DEREK® alert for nephrotoxicity, which is associated in the software with the structural description of "1,2-ethyleneglycol or derivative." However, as the CIR SSC noted, the specificity of a "rapid prototype alert" is likely to be low. DEREK® does not reveal the structures of the proprietary ethylene glycol derivatives that led to the development of this rapid prototype alert.

***DEREK® Rapid Prototype Alert Notation***

*"This alert describes the nephrotoxicity of 1,2-ethyleneglycol and its derivatives. This is a rapid prototype alert derived using a proprietary data set of 731 chemicals, classified on the basis of the presence or absence of histopathologic lesions in the kidney in oral rat repeated-dose studies mostly of 28-days duration. Eleven chemicals in this data set activated this rapid prototype alert and five of these were nephrotoxic."*

The rapid prototype alerts are based on a single set of data from one source. They are intended to signal a potential toxicophore, but have not been subjected to the same level of review that is usual for the standard alerts in the DEREK® knowledge base.

The CIR Expert Panel has evaluated the available data on triethylene glycol and other PEGs with average x+y >2, including the reports of renal toxicity when PEGs have been used on severely damaged skin, as in burn patients.[8] The Panel determined that the PEGs are not metabolized to ethylene glycol, at least under normal homeostasis, and oral and dermal toxicity studies of the PEGs yielded no evidence of the type of nephrotoxicity produced by ethylene glycol and diethylene glycol. PEGs-induced nephrotoxicity has been observed only in

patients with severe burns over large surface areas of the body. The Panel concluded that there was no reason for concern for PEGs in rinse-off products, and there is a large margin of safety for leave-on products containing PEGs, after reviewing PEG-4 dermal penetration data for normal skin and skin in which the *stratum corneum* was removed.

If the ethoxyl chains are metabolized to yield acid metabolites, then it would be reasonable to anticipate that the PEGs cocamine and related ingredients could cause nephrotoxicity at high doses. However, these materials are so irritating in the digestive tract that they cannot be tested at doses sufficiently high to cause nephrotoxicity.

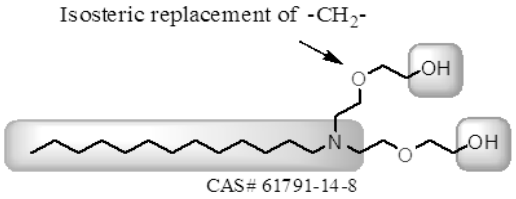
**Physicochemical Properties**

There are substantial differences in physicochemical properties across the PEGs-cocamine SOIs and their corresponding analogs. These differences would undoubtedly affect bioavailability, and in a manner dependent on the route of exposure. The longer alkyl chain-lengths derived from the fatty acids of tallow or hydrogenated tallow and longer polyethoxy chains are generally expected to reduce bioavailability, compared to the shorter alkyl-chain lengths derived from the fatty acids of coconut oil and shorter polyethoxy chains. However, longer polyethoxy chain-lengths will be associated with greater polarity, which may offset the effect of the greater molecular weight of the tallow-derived analogs to some extent.

**Chemical Reactivity**

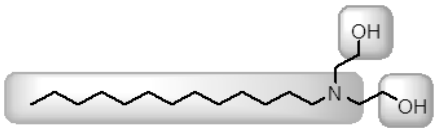
As noted above, chain-length mix skews longer with tallow than with coconut oil. In addition, the degree of unsaturation is greater in tallow than in coconut oil, but hydrogenated tallow has the lowest degree of unsaturation. Unsaturated fatty acids may form epoxides when metabolized.

Another noteworthy difference among the SOIs and analogs is that some of them have hydroxyethyl side chains (e.g., the analog tallow bis(2- hydroxyethyl)amine; CAS# 61791-44-4) and others have polyethoxyl side chains (e.g., the SOI PEG-4 cocamine; CAS# 61791-14-8), as shown below.




CAS# 61791-14-8

Representative PEG-cocamine

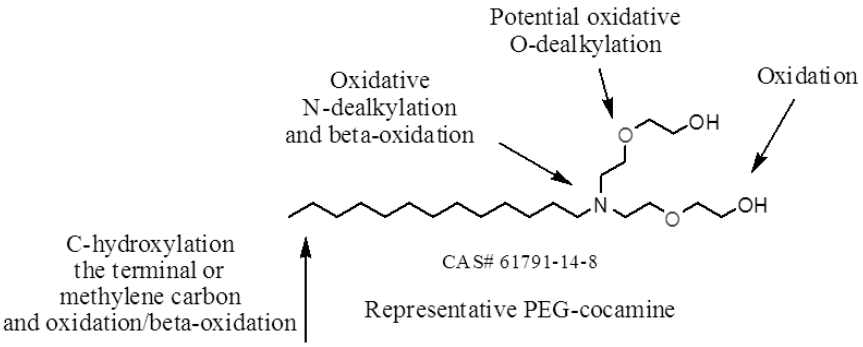



CAS# 61791-44-4

However, the ether linkage is isosteric with a $-CH_2-$ linkage. Isosteric substituents have similar molecular shapes and volumes, approximately the same distributions of electrons and, thus, would not be expected to be very different in chemical reactivity. Thus, these isosteric groups should have similar toxicology profiles if there is no metabolism (e.g., for SOIs and analogs with $x+y > 8$).

**Metabolism**

There is likely to be some metabolism of the smaller PEGs cocamine and related ingredients (i.e., those with $x+y \leq 8$). The CIR SSC and Council member companies evaluated the potential metabolic transformations of the polyethoxyl moieties of the PEGs cocamine based on data for the PEGs from peer-reviewed publications and predictions from the application of computational tools, such as METEOR®. Theoretical metabolic transformations of the PEGs cocamine are illustrated below.

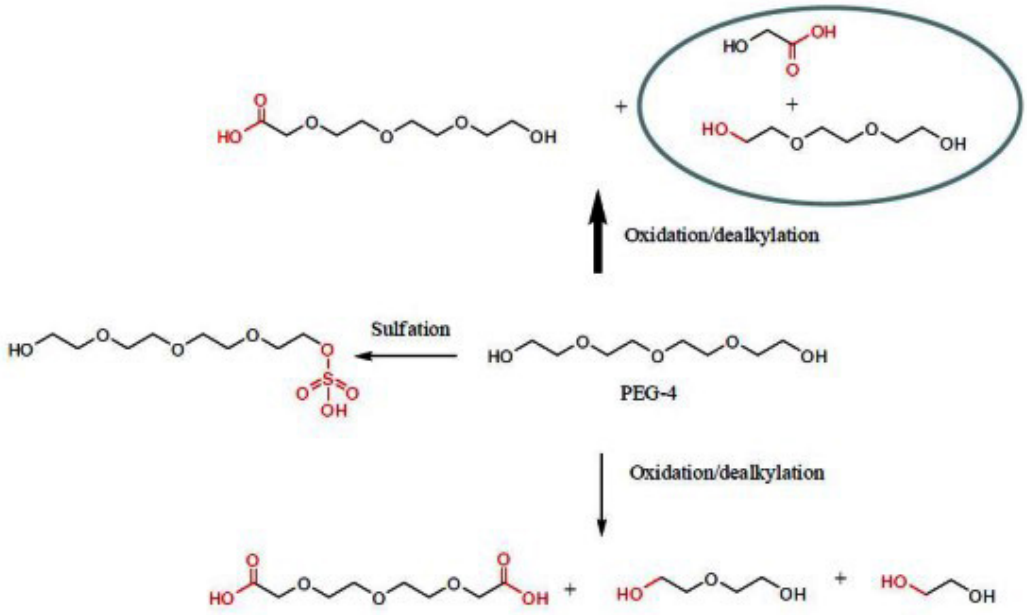



Differences in chemical structure that could affect metabolism across the analogs include the presence of hydroxyethyl groups in SOIs and analogs for which x+y=2, rather than the polyethoxyl groups in SOIs and analogs for which x+y≥4. O-dealkylation is not possible for PEG-2 cocamine and the analogs lacking polyethoxyl groups.

The potential for O-dealkylation of polyethoxyl groups of the PEGs cocamine and analogs was addressed through a search of the literature on the metabolism of PEGs.

The metabolism of the polyethoxylate groups in PEGs cocamine is anticipated to be similar to the metabolism of PEGs. PEGs are excreted mainly unchanged in the urine and feces after oral or intravenous exposure.[27,28] The extent of metabolism depends on molecular weight; there is little or no metabolism of PEGs with molecular weights >5000 Da (e.g., PEG-100).

The metabolism of PEGs involves oxidation of the terminal alcohol groups to yield carboxylic acids, which is likely mediated by alcohol dehydrogenases or possibly sulfate conjugation of the terminal alcohol groups by sulfotransferases.

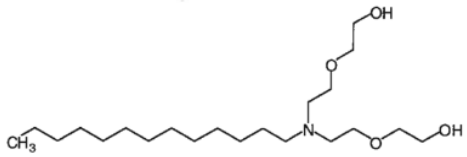



However, O-dealkylation is not a major route of metabolism. Only very small amounts of oxalic acid are formed from the O-dealkylation and alcohol oxidation of PEGs for which x+y=5 to 8 (and no detectable amounts of oxalic acid formed from PEGs for which x+y≥8). Ethylene glycol has not been shown to be formed as a metabolite of the PEGs.

An additional consideration, as noted above, is that the unsaturated fatty acids of tallow (not hydrogenated tallow) in the structure of some of the ingredients and analogs may be metabolized to form epoxide metabolites. PEGs-cocamine structures that have no unsaturated fatty-acid amine moieties do not have this potential.

None of the final metabolites of PEG-4 cocamine were predicted to be of toxicological concern using computational tools. PEG-4 cocamine was chosen in two studies as a model compound to predict metabolic transformations and toxicity.

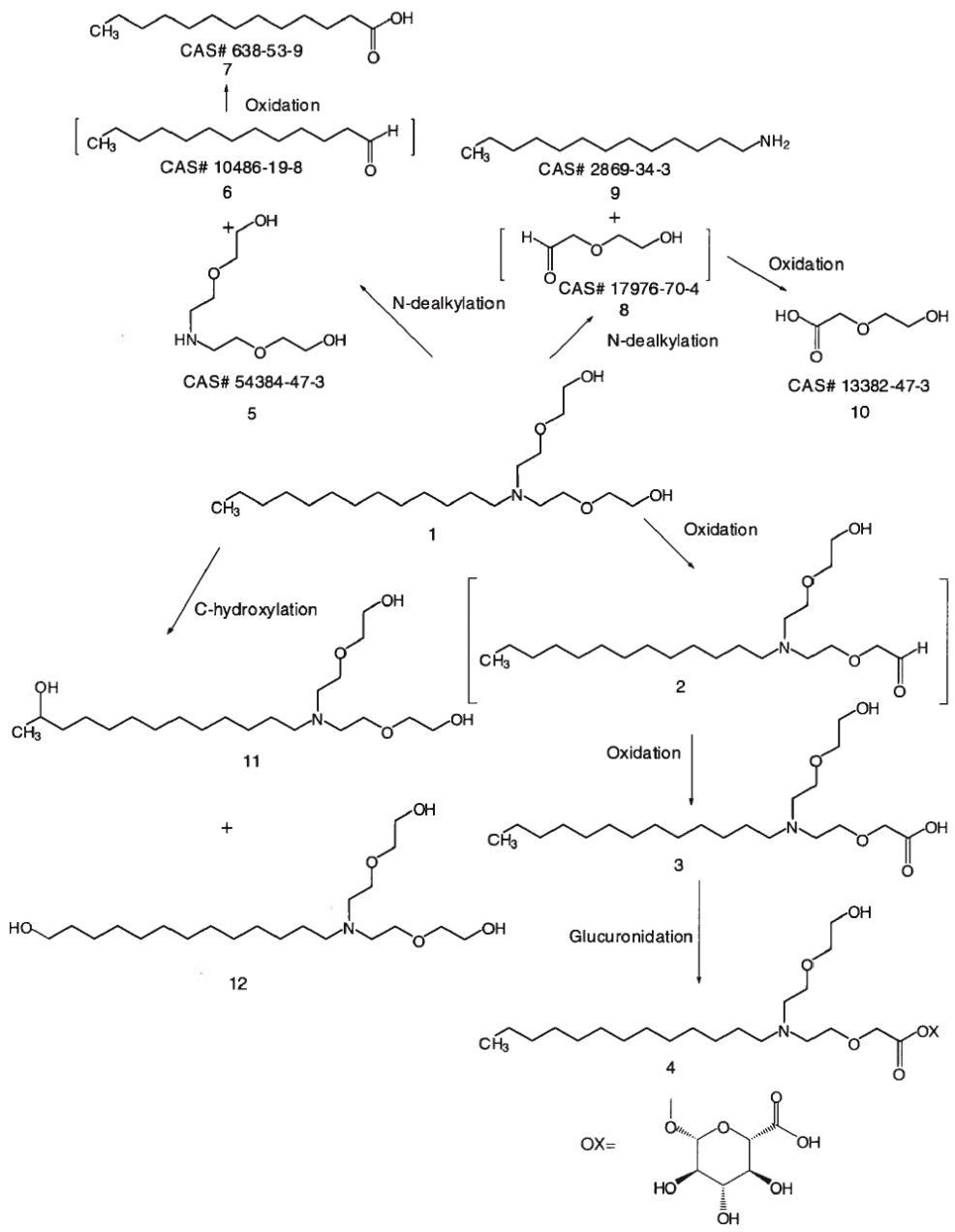
In the first of these studies, the structural features of PEG-4 cocamine were examined, and substructure searches and METEOR® were used to predict the metabolic fate of the PEG-4 cocamine having the structure depicted below.




PEG-4 cocamine may undergo oxidation, C-hydroxylation or N-dealkylation to form corresponding metabolites. The possible major metabolic fate of PEG-4 cocamine predicted from this analysis is depicted below, where compound (1) is PEG-4 cocamine.

In the second computational study, the software used included:

- Vitic (http://www.lhasalimited.org/)
- LEADSCOPE (http://www.leadscope.com/ )
- OECD Toolbox (http://www.oecd.org)
- METEOR® (http://www.Ihasalimited.org/)
- TIMES® (http://oasis-lmc.org)
- DEREK® for windows (http://oasis-lmc.org)
- MC4PC (Multicase) (http://oasis-lmc.org)
- Toxtree (http://ambit.acad.bg)
- VirtualToxLab (http://www.biograf.ch)

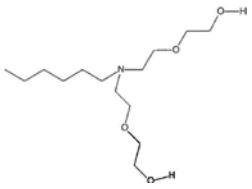



The oxidation of ethoxyl ethanol may yield the corresponding metabolite (3) through an aldehyde (2) intermediate. The ezymes that catalyze the metabolism of primary alcohols to aldehydes and then to carboxylic acid have broad substrate specificity. Subsequently, the metabolite (3) could be glucuronidated to yield metabolite (4).

The oxidative N-dealkylation of (1) may yield metabolites (5), (7) or {9), (10). The formation of metabolites (7) and (10) would proceed through the corresponding intermediate aldehydes (6) and (8). Oxidative N-dealkylation (aka deamination) involves hydrogen abstraction and oxygen addition (hydroxylation) at a carbon atom $\alpha$ to the nitrogen atom.

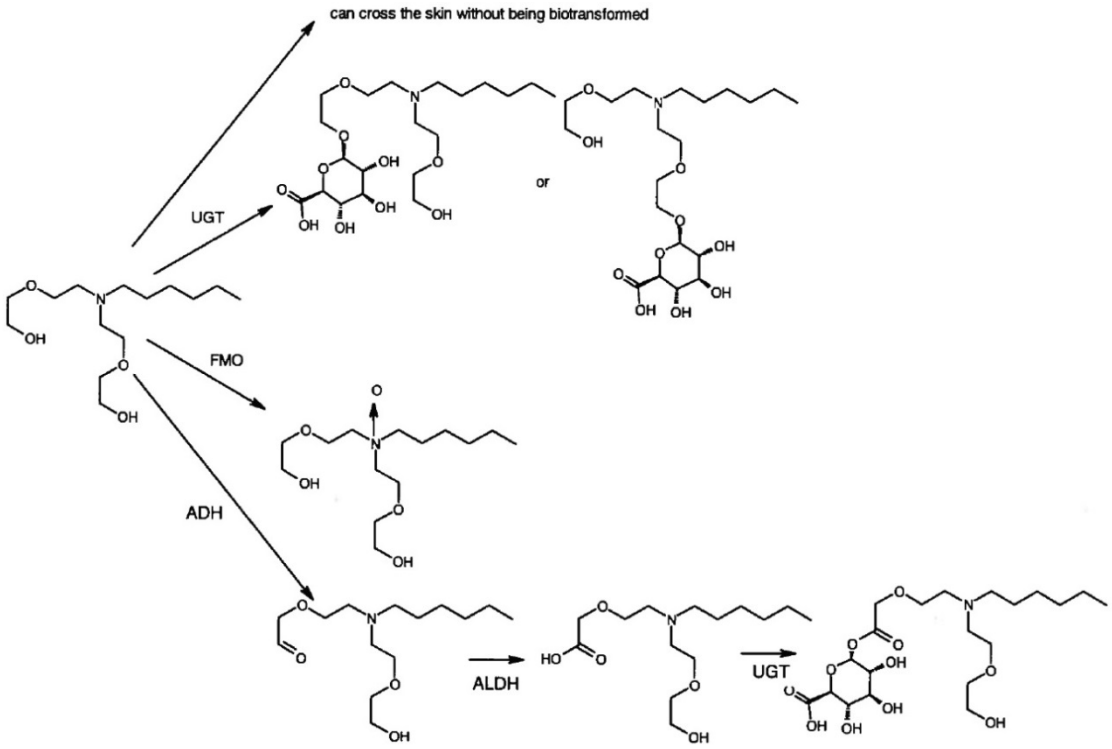
In addition, C-hydroxylation reactions of the alkyl chain to yield (11) and (12) are possible. For longer alkyl chains, hydroxylation of a methylene group may occur, as well as hydroxylation at the terminal methyl group.

The structure of PEG-4 cocamine analyzed in this study is presented below.

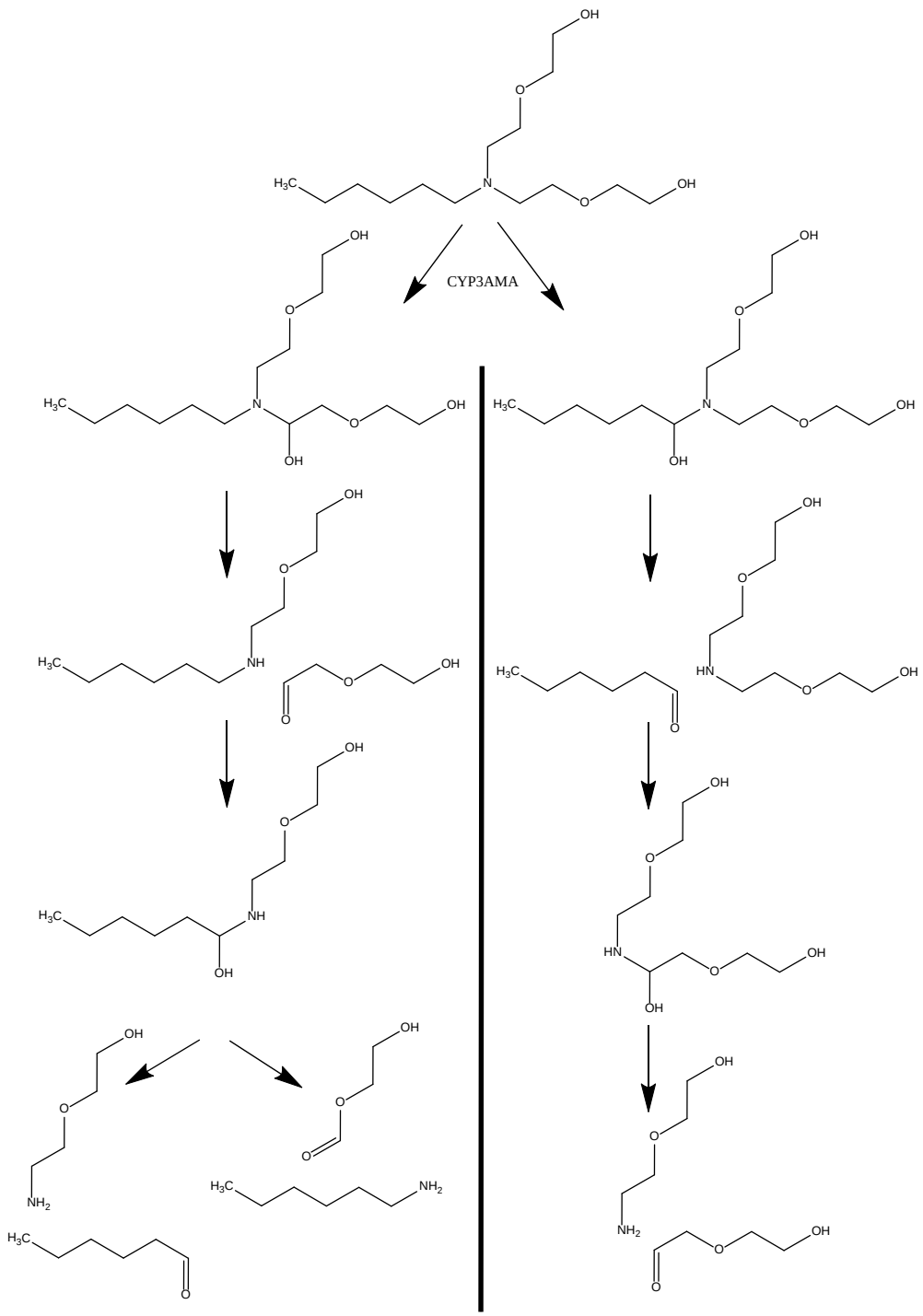


The authors noted that PEG-4 cocamine has a MW of 277 and an estimated log P of 1.961, which suggests that its rate of absorption into the skin would be similar to that of ethanolamine.[3] In the skin, PEG-4 cocamine could be metabolized or enter the systemic circulation and the liver unchanged. Plausible metabolic reactions in the skin are depicted below, where:

- UGT = Uridine diphosphate-glucuronyl transferase
- FMOs = Flavin monooxygenases
- ADH = Alcohol dehydrogenases
- ALDH = Aldehyde dehydrogenases




N or O-dealkylations are possible, as illustrated below; these are major types of metabolic reactions in the liver, although uncertain in the skin.




Hexanal, if formed via dealkylation (as shown in the figure above) can be metabolized to yield hexanoic acid, which can form a glucuronyl conjugate. Hexamine, if formed, can be oxidized to yield 1,6-hexanediol.

The authors listed the main enzymes expressed in the skin:[3]

- Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the major mRNA-expressed mRNA Phase-I metabolizing enzymes

- Flavin-containing monooxygenases (FMO) and monamine oxidase A (MAO A) are expressed only at a low level
- Cytochromes P-450 (CYP450s) are expressed at a very low level
- UDP glucoronosyl transferases (UGTs) are Phase-II metabolizing enzymes expressed in the skin, but at a lower levels than glutathione transferases (GSTs), N-acetyl transferase (NAT), and catechol-*o*-methyl transferase (COMT)

Other reactions that can occur in the skin and liver include:

- Oxidation of the terminal methyl group of the aliphatic chain
- Oxidative deamination of aliphatic amine

The second study includes a simulation of metabolic transformations in the liver using METEOR® and TIMES®. The primary biotransformations predicted where oxidation and glucuronidation of primary alcohols and dealkylation. TIMES® gives preference to the O-dealkylation. METEOR® gives preference to N-dealkylation (CYP3A3-dependent), which is consistent with the results of *in vitro* and *in vivo* experiments using N- or O-alkylated compounds.

If an ingredient is available to biotransformation enzymes, an increase in polyethoxy-chain length might increase the potential of the ingredient to interact with enzymes that catalyze O-dealkylation. CYPl and 3 families of biotransformation enzymes are expressed at low levels in the skin, but are highly expressed and functional in the liver.

On the other hand, an increase of the fatty-acid chain length would favor β-oxidation, if the compound is available to mitochondrial enzyme systems. The effect of alkyl-chain length on N-dealkylation is not known.

The authors noted that metabolism of polymers like the PEGs cocamine and related ingredients could occur at three levels on or in the skin:[3]

- In the skin microflora, if the polymer can penetrate bacteria or fungi and reach oxidative enzymes (there is no information on this topic)
- In the skin, if the molecule can penetrate the skin and contact mitochondrial enzymes (which would enable the oxidation of fatty-acid chains or the O-dealkylation of glycol groups)
- In the liver, if the polymer can reach the systemic circulation and the liver

## Analog Toxicity Data Review

Tables 7-10 summarize the toxicological data available for the analogs identified for each of the four PEGs cocamine selected as SOIs.

The data provided in Tables 7-10 address repeated-dose toxicity, genotoxicity, and DART as toxicological endpoints. Note that a rat DART screening test was identified for PEG-2 cocamine (Table 7).

Note, also, that the Appendix of this safety assessment presents Table 11 (Repeated-Dose Toxicity), Table 12 (Genotoxicity *In vitro*) and Table 13 (Genotoxicity *In vivo*), which are robust summaries of toxicity tests performed on the analog tallow bis(2-hydroxyethyl) amine C16-C18 (x+y=2). Tables 11-13 are from Appendix A of US EPA's Fatty Nitrogen Derived (FND) Amines High Production Volume (HPV) Category Chemicals Challenge,[29] which served as the source of the toxicity data for tallow bis(2-hydroxyethyl) amine presented in Tables 7-10 of this safety assessment.

### Oral Repeated-Dose Toxicity

Oral repeated-dose toxicity studies, including 28- and 90-day studies, have been conducted in rats and dogs with tallow-derived analogs that cover x+y=2 (i.e., three studies for tallow bis(2-hydroxyethyl) amine) (Tables 7 and 8) and x+y=15 to 17 (i.e., two studies, each, for PEG-15 tallow amine and POE-5/POP-12 tallow amine) (Tables 9 and 10). In addition, a 90-day rat study and 90-day dog study on the analog amines, C13-C15-alkyl, ethoxylated (x+y=2) were performed (Tables 7 and 8).

These studies showed local effects on the gastrointestinal tract, but little or no evidence of other treatment-related effects. No evidence of nephrotoxicity was observed in any of these studies. The studies are reasonably consistent in their reported NOAELs or NOELs, given the variety of dose ranges tested in these studies.

The authors noted that the potential differences in chemical reactivity, physicochemical properties, or metabolism of the analogs that were identified during analog evaluation and categorization are not evident in the outcomes of the repeated-dose oral toxicity studies.[4]

Analogs derived from tallow amine comprise the majority of the identified analogs with repeated-dose toxicity data. The higher degree of unsaturation in these analogs, compared with the PEGs cocamine, presents the potential for epoxide formation, suggesting that using these analogs for read-across analysis is a conservative approach to the safety assessment of these ingredients.

In several of the oral studies, histiocytosis (the presence of foamy macrophages) was noted in the small intestines and mesenteric lymph nodes of the test animals. The prevailing scientific opinion is that, without additional evidence of concurrent toxicity, the presence of foamy macrophages in organs such as the intestine should not be considered an adverse effect.[30-33] These lesions are attributable to the clearance of oils with high molecular weight, and are not associated with long-term effects."[31-33] Furthermore, as the authors suggested, histiocytosis in the small intestines and mesenteric lymph nodes observed in a repeated-dose oral toxicity study does not represent well the intended route of human exposure (dermal) for use of the PEGs cocamine ingredients in cosmetic products.[4]

**Percutaneous Repeated-Dose Toxicity**

Percutaneous 28-day repeated-dose toxicity studies have been conducted in rabbits with tallow bis(2-hydroxyethyl) amine (x+y= 2; one study; Tables 7 and 8) and PEG-20 tallow amine (x+y=20, two studies; Table 10). Local skin irritant effects were noted in these studies, but there was no evidence of systemic toxicity.

**Genotoxicity**

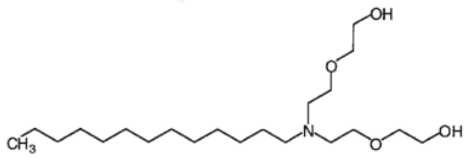
Both *in vitro* and *in vivo* genotoxicity studies have been conducted with tallow amine analogs (Tables 7-10), including:

- Tallow bis(hydroxyethyl) amine C16-C18 (x+y=2); Tables 7 and 8
- PEG-8 Hydrogenate tallow amine (x+y=8); Tables 8, 9 and 10
- PEG-15 tallow amine (x+y=15); Table 9
- PEG-20 tallow amine (x+y=20); Table 10

The studies include mammalian and bacterial test systems, and address gene mutation and clastogenicity. The results consistently show an overall lack of evidence of genotoxicity across assays and analogs.

PEG-20 tallow amine was negative in an Ames test, an *in vitro* mouse lymphoma assay, and an *in vitro* unscheduled-DNA synthesis (UDS) assay (Table 10). An *in vitro* chromosome aberration assay for this analog was negative without metabolic activation, but was positive with metabolic activation. However, PEG-20 tallow amine was negative in an *in vivo* chromosome aberration assay in mice (Table 7). The authors also noted that tallow bis(hydroxyethyl) amine C16-C18 (x+y=2) was negative in an *in vivo* mouse micronucleus assay (Tables 7 and 8).[4]

The structure of PEG-4 cocamine shown below was evaluated for potential genotoxicity using the DEREK® and TIMES® prediction models.




The TIMES® software, in particular, enables the evaluation of liver metabolites likely to be formed from the structure. There were no structural alerts for genotoxicity using the DEREK® system. In addition, PEG-4 cocamine was predicted to be non-mutagenic and to not be a precursor of chromosomal aberrations using the TIMES® model.

The authors noted that the overall negative results of genotoxicity tests and computational predictions are consistent with the data reported in Appendix A of US EPA's FND) Amines Category HPV Chemical Challenge).[3,31] The latter presents the results of over 60 genotoxicity tests (including *in vitro*, *in vivo*, bacterial, and mammalian tests) on more than 30 FND amines and FND amides. Only the *in vitro* chromosome aberration assay for PEG-20 tallow amine and one Ames test were positive, among all of these chemicals.

***Reproductive and Developmental Toxicity***

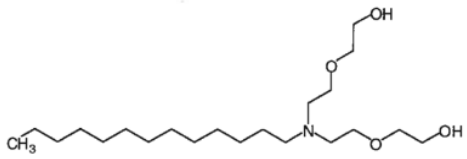
Reproductive and developmental toxicity data are available for:

- PEG-2 cocamine (x+y=2) Table 7
- Tallow amine phosphate ester ((x+y=5); Table 8
- PEG-15 tallow amine (x+y=15); Tables 9 and 10

No evidence of a teratogenic effect was observed in any of the studies. Reproductive toxicity studies of the analogs showed effects on reproductive performance at doses that were generally comparable to doses causing maternal toxicity. In the reproductive studies, the findings included smaller litter size and reduced body weight. In one of these studies, the effects were associated with frank maternal toxicity.

***Dermal Sensitization***

An evaluation of the PEG-4 cocamine structure illustrated below, using the TIMES®, indicated that this ingredient has the potential to be a weak sensitizer, because of potential formation of hydroperoxides by autoxidation of the ethoxylate chains.




This result is consistent with a report that ethoxylated alcohols were susceptible to autoxidation when exposed to air at ambient temperatures, in daylight, with stirring for 1 hour four times a day for 18 months. [34] Hydroperoxides were the primary oxidation products formed.

The potential for peroxide formation in PEGs has been considered by the CIR Expert Panel, and some literature on the quantitation of peroxides in PEGs of various molecular weights has been cited in CIR safety assessment reports.[7,8] In the Amended Safety Assessment for triethylene glycol and polyethylene glycols (June 29, 2010), the Panel concluded that the PEGs were not sensitizers in individuals with normal skin, and that sensitization is not a significant concern in individuals with damaged skin.[8]

No other alert for sensitization potential was noted in the PEGs cocamine structure. The PEG-4 cocamine structure mentioned above was also predicted to be non-mutagenic, not a precursor of chromosomal aberrations and not phototoxic, using TIMES®.

## SUMMARY

In a report published in 1999, the CIR Expert Panel found that the data were insufficient to support a safety assessment of several PEGs cocamine ingredients. Among the data gaps identified, data specifically on PEG-2 cocamine were needed to demonstrate that relevant exposures to the ingredient with the lowest molecular weight in this group of would not be toxic.

In 2011 and 2012, the CIR SSC presented information to the CIR, contending that these data needs can be met through the application of an SAR-based framework for identifying and evaluating structural analogs for read-across assessments. The framework is based on the assessment of structure activity (SAR) relationships, and enables the incorporation of information from the literature and from predictive computational tools on physicochemical properties, chemical reactivity, metabolism and toxicity to identify suitable analogs and develop an overall weight-of-evidence safety assessment.

The PEGs cocamine and related ingredients represent a series of mixtures of mostly tertiary amines that have alkyl groups derived from plant or animal fatty acids and an average number of polyethylene glycol groups equal to the number in the chemical name. The structures of the smallest members of the group (e.g., PEG-2 cocamine) may have two hydroxyethyl groups, rather than polyethoxyl, or one hydroxyethyl group and one polyethoxyl group. The possibility of similar structural variations is notable for PEG-3, -4, and -5 cocamine and

related ingredients. Each PEGs cocamine is a mixture of compounds with the fatty-acid derived chain lengths ranging from about C6 to C20.

The PEG-n cocamine and related ingredients are manufactured by condensing fatty acid with the ingredient's corresponding number of moles (n) of ethylene. The chain length of the polyethylene glycol groups depend on the duration of the reaction, and these groups may not be symmetrical; typically, this reaction yields a range of polyethylene glycol chain lengths.

The PEGs cocamine and related ingredients are mixtures of tertiary alkyl amines that may also contain some secondary or primary amines. Thus, nitrosamines can be produced in formulations that contain nitrosating agents. Additionally, the ingredients may contain traces of 1,4-dioxane, which is a by-product of ethoxylation, and ethylene oxide as impurities. Aflatoxin contamination of raw and dried copra have been reported. Copra is the dried coconut kernels from which the fatty acids may be obtained to produce the PEGs cocamine ingredients

The PEGs cocamine and related ingredients function primarily as surfactants and antistatic agents in cosmetic formulations.

VCRP and Industry survey data obtained in 2014 indicate that 10 of the ingredients included in this report are used in cosmetic formulations. PEG-2 rapeseedamine has the most reported uses, with a total of 255 uses in rinse-off hair-coloring preparations. No use concentrations were reported for PEG-2 rapeseedamine. PEG-2 oleamine has the second greatest number of uses, with a total of 239 uses in rinse-off hair-coloring preparations. The highest maximum use concentration for PEG-2 oleamine was 3.5%. Some of the ingredients are reported to be used in body and hand sprays and powder products, and could possibly be inhaled. There were 37 PEGs-cocamine ingredients that do not appear to be in use.

PEG cocamine absorption and metabolism data were not available.

The oral LD50 of PEG-15 cocamine in rats was 1.2 g/kg, and the LD50 of PEG-2 cocamine ranged from 0.75 g/kg to 1.3 g/kg. PEG-2 cocamine was classified as a moderate cutaneous irritant, and PEG-15 cocamine was considered a mild irritant. PEG-2 cocamine was considered an ocular irritant, and PEG-15 cocamine caused corneal irritation. PEG-15 cocamine was negative in mutagenicity studies. The CIR safety assessment report published in 1999 indicated that the PEGs cocamine would not be likely to cause reproductive or teratogenic effects, based on their structural characteristics.

No dermal sensitization studies were found or submitted for PEG-2 cocamine. In one HRIPT, a hair styling formulation containing 1.0% PEG-15 cocamine was not sensitizing in 212 subjects. In another HRIPT, an adult sunscreen formulation containing 2.9% PEG-15 cocamine was not sensitizing in 201 subjects. Summary data from a photoallergy study (116 subjects) and a phototoxicity study (22 subjects) indicated that there were no photoallergic or other phototoxic effects in the skin after exposure to an adult sunscreen formulation containing 2.9% PEG-15 cocamine (no details of these studies were provided)

The framework is a systematic, expert-driven method developed to identify and evaluate the suitability of analogs, based on similarities in chemical structure, reactivity, and metabolic and physicochemical properties, for use in read-across assessments. The framework is amenable to incorporating the results of (Q)SAR analyses to fill data gaps for specific endpoints or to inform the overall weight of evidence analysis that is integral to the exercise of the framework. The framework enables classifying candidate analogs in a manner that reflects the assumptions and uncertainties associated with their use in a safety assessment, based on structural, reactive, metabolic and physicochemical similarities to the SOI (i.e., the chemical with missing toxicological data), and differences in physicochemical properties. The results include the classification of each candidate analog as suitable, suitable with interpretation, suitable with a precondition or not suitable. All of the relevant toxicological data available for the SOI and analogs classified as "suitable," "suitable with interpretation" or "suitable with precondition" are then compiled and reviewed for consistency or concordance of toxicological responses and mechanisms or modes of action across multiple endpoints. All of the data are then taken together to develop an overall weight of evidence assessment, including a detailed review for consistency of the toxicology data for the analogs and the SOI, to develop a statement of confidence in the read-across assessment.

The framework performed well in a series of blinded case studies for all of the endpoints examined. The case studies showed that applying the framework can enable or facilitate the conduct of transparent, reproducible, and conservative read-across assessments. However, the successful application of the approach requires substantial expertise and discipline to avoid stepping over the boundaries of the defined analogs and the suitability rating system.

Four PEGs cocamine were selected as the structures of interest (SOIs) to cover the range of polyethylene glycol side-chain lengths for identifying analogs, including PEG-2 cocamine (Analog Group 1), PEG-4 cocamine (Analog Group 2), PEG-10 cocamine (Analog Group 3), and PEG-15 cocamine (Analog Group 4).

The analogs showed consistent biological responses, including the absence of genotoxicity and teratogenicity, and yield comparable NOAELs or NOELs in toxicology studies. In addition, several computational models were used to develop predictions for several major toxicological endpoints, as well as for the potential metabolic fate of the PEGs cocamine and, thus, inform the safety assessment of this ingredient group. For example, the potential for PEG-4 cocamine to induce dermal sensitization was evaluated using predictive software. PEG-4 cocamine was predicted to be a weak sensitizer, using predictive software, because of the potential autoxidation of PEG-4 cocamine to yield sensitizing hydroperoxides.

Many of the analogs identified are the larger tallow derivatives, rather than the smaller cocamine derivatives, which will generally have greater degrees of unsaturation as well as longer alkyl chain lengths than the cocamine derivatives. The tallow amines are potentially more toxic than the cocamines and the hydrogenated tallow amines because the unsaturated fatty acid moieties are susceptible to epoxidation and hydroperoxidation. Hydrogenated tallow will be saturated, but PEGs hydrogenated tallow amines will still have larger alkyl groups than the corresponding PEGs cocamine.

No structural alerts were found for genotoxicity when the SOIs and analogs were evaluated using the DEREK® and TIMES® prediction models.

The SOIs and analogs with ethoxylated chains consistently yielded a "rapid prototype" DEREK® alert for nephrotoxicity, which is associated in the software with the structural description of "1,2-ethyleneglycol or derivative." In previous safety assessments, the CIR Expert Panel determined that the PEGs are not metabolized to ethylene glycol, at least under normal homeostasis, and oral and dermal toxicity studies of the PEGs yielded no evidence of the type of nephrotoxicity produced by ethylene glycol and diethylene glycol. PEGs-induced nephrotoxicity has been observed only in patients with severe burns over large surface areas of the body. The Panel concluded that there was no reason for concern for PEGs in rinse-off products, and there is a large margin of safety for leave-on products containing PEGs, after reviewing PEG-4 dermal penetration data for normal skin and skin in which the stratum corneum was removed.

If the ethoxyl chains are metabolized to yield acid metabolites, then it would be reasonable to anticipate that the PEGs cocamine and related ingredients could cause nephrotoxicity at high doses. However, these materials are so irritating in the digestive tract that they cannot be tested at doses sufficiently high to cause nephrotoxicity.

There are substantial differences in physicochemical properties across the PEGs-cocamine SOIs and their corresponding analogs. These differences would undoubtedly affect bioavailability in a manner dependent upon the route of exposure. Generally, the longer alkyl chain-lengths derived from the fatty acids of tallow or hydrogenated tallow and longer polyethoxy chains would be expected to reduce bioavailability, compared to the shorter alkyl-chain lengths derived from the fatty acids of coconut oil and shorter polyethoxy chains. However, longer polyethoxy chain-lengths will be associated with greater polarity, which may offset the effect of the greater molecular weight of the tallow-derived analogs to some extent.

Another noteworthy difference among the SOIs and analogs is that some of them have hydroxyethyl side chains (and others have polyethoxyl side chains. However, the ether linkage is isosteric with a -CH2- linkage. Isosteric substituents have similar molecular shapes and volumes, approximately the same distributions of electrons and, thus, would not be expected to be very different in chemical reactivity.

There is likely to be some metabolism of the smaller PEGs cocamine and related ingredients with $x+y \leq 8$. Differences in chemical structure that could affect metabolism across the analogs include the presence of hydroxyethyl groups in SOIs and analogs for which $x+y \leq 5$.

The metabolism of the polyethoxylate groups in PEGs cocamine is anticipated to be similar to the metabolism of PEGs. PEGs are excreted mainly unchanged in the urine and feces after oral or intravenous exposure. None of the final metabolites of one PEG-4 cocamine structure were predicted to be of toxicological concern using computational tools.

The toxicological data available for the analogs identified for each of the four PEGs cocamine selected as SOIs can be summarized as follows.

Oral repeated-dose toxicity studies, including 28- and 90-day studies conducted in rats and dogs with tallow-derived analogs or C13-C15-alkyl, ethoxylated, showed local effects on the gastrointestinal tract, but little or no evidence of other treatment-related effects. No evidence of nephrotoxicity was observed in any of these studies. In several of the oral studies, histiocytosis (the presence of foamy macrophages) was noted in the small intestines and mesenteric lymph nodes of the test animals. The prevailing scientific opinion is that, without additional evidence of concurrent toxicity, the presence of foamy macrophages in organs such as the intestine should not be considered an adverse effect. The potential differences in chemical reactivity, physicochemical properties, or metabolism of the analogs that were identified during analog evaluation and categorization were not evident in the outcomes of these studies.

Analogs derived from tallow amine comprise the majority of the identified analogs with repeated-dose toxicity data. The higher degree of unsaturation in these analogs, compared with the PEGs cocamine, presents the potential for epoxide formation, suggesting that using these analogs for read-across analysis is a conservative approach to the safety assessment of these ingredients.

Percutaneous 28-day repeated-dose toxicity studies have been conducted in rabbits with tallow bis(2-hydroxyethyl) amine and PEG-20 tallow amine. Local skin irritant effects were noted in these studies, but there was no evidence of systemic toxicity.

Both in vitro and in vivo genotoxicity studies have been conducted with tallow amine analogs. The studies include mammalian and bacterial test systems, and address gene mutation and clastogenicity. The results consistently show an overall lack of evidence of genotoxicity across assays and analogs. There were no structural alerts for genotoxicity using the DEREK® system. PEG-4 cocamine was predicted to be non-mutagenic and to not be a precursor of chromosomal aberrations using the TIMES® model. The overall negative results of genotoxicity tests and computational predictions are consistent with the data reported in Appendix A of US EPA's FND) Amines Category HPV Chemical Challenge). The latter presents the results of over 60 genotoxicity tests (including in vitro, in vivo, bacterial, and mammalian tests) on more than 30 FND amines and FND amides. Only the in vitro chromosome aberration assay for PEG-20 tallow amine and one Ames test were positive, among all of these chemicals.

Reproductive and developmental toxicity data are available for PEG-2 cocamine, tallow amine phosphate ester, and PEG-15 tallow amine. No evidence of a teratogenic effect was observed in any of the studies. Reproductive toxicity studies of the analogs showed effects on reproductive performance at doses that were generally comparable to doses causing maternal toxicity.

An evaluation of one PEG-4 cocamine structure using the TIMES® indicated that this ingredient has the potential to be a weak sensitizer, because of potential formation of hydroperoxides by autoxidation of the ethoxylate chains. This result was consistent with a report that ethoxylated alcohols were susceptible to autoxidation when exposed to air at ambient temperatures, in daylight for 18 months. Hydroperoxides were the primary oxidation products formed. No other alert for sensitization potential was noted in the PEGs cocamine structure.

## DISCUSSION

The Expert Panel noted gaps in the available safety data for the PEGs cocamine and related ingredients in this safety assessment. However, the data available for some of these ingredients and their analogs, together with the SAR-based read-across analysis presented, can be used to support the safety of 32 of 47 ingredients addressed in this report. All of these 32 ingredients have $x+y > 5$, and they included PEGs cocamine, PEGs oleamine, PEGs tallow amine, PEGs hydrogenated tallow amine, PEGs soyamine, PEGs stearamine, and PEGs palmitamine.

In particular, the Panel agreed that gaps in genotoxicity and systemic toxicity data can be filled for these 32 ingredients by applying the SAR-based framework to identify and evaluate analogs for read across analyses. The selected analogs adequately covered the chemical space of these ingredients. The toxicology study summaries were sufficient to enable addressing all of the toxicology endpoints of potential concern for these ingredients in a safety assessment. Based on the toxicology data, the selected analogs showed sufficient concordance and consistency in biological responses (quantitative and qualitative) to support the read-across analysis. The read-across analysis was plausible and sufficiently persuasive to warrant a low or medium uncertainty rating.

The Panel noted that products containing these ingredients must be formulated to be non-irritating, because the potential exists for dermal irritation with the use of products containing these ingredients.

Additionally, the Panel noted that some or all of the fatty-acid moieties of these ingredients may be unsaturated or partially hydrogenated. The unsaturated fatty acid and trans-fatty acid moieties of these ingredients are subject to autoxidation, yielding hydroperoxides that are likely sensitizers. The Panel cautioned that products containing these ingredients should be formulated to minimize autoxidation and production of potentially allergenic hydroperoxides.

To assure the absence of a pathogenic agent in the ingredients, the PEGs tallow amine and PEGs hydrogenated tallow amine must be made from tallow containing a maximum level of insoluble impurities of 0.15% in weight.

Also of concern to the Expert Panel was the possible presence of 1,4-dioxane and ethylene oxide impurities. They stressed that the cosmetics industry should continue to use the necessary procedures to remove these impurities from PEGs cocamine and related ingredients before blending them into cosmetic formulations.

Plants are the source of the fatty acids used to manufacture some of the ingredients of this report. These ingredients are not expected to contain residual pesticides or heavy metals because the production of the ingredients involves significant processing. However, the Expert Panel stressed that the cosmetics industry should continue to

use current good manufacturing practices (cGMPs) to limit these impurities in these ingredients before blending into cosmetic formulations.

The Panel noted reports that raw and dried copra (i.e., dried coconut kernels from which the oil is obtained) can be contaminated with aflatoxin. The Panel believes PEGs cocamine ingredients manufactured using the fatty acids in coconut oil would not contain significant levels of aflatoxin; the Panel adopted the USDA designation of ≤ 15 ppb as corresponding to "negative" aflatoxin content.

PEGs cocamine and related ingredients should not be used in cosmetic products in which N-nitroso compounds can be formed.

The Panel discussed the issue of incidental inhalation exposure from PEGs cocamine and related ingredients. These ingredients are reportedly used at concentrations up to 3% in cosmetic products that may be aerosolized. There were no inhalation toxicity data available. However, the Panel noted that 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of the PEGs cocamine and related ingredients with $x+y > 5$. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

The Panel found that the information was insufficient to determine the safety of the 15 PEGs cocamine and related ingredients with $x+y \leq 5$, including PEG-2 rapeseedamine, PEG-2 lauramine, and others. They noted that each of these ingredients represents a distribution of molecules, some of which may be secondary or primary amines that may be glucuronidated or sulfated, and then undergo intramolecular cyclization to yield potentially sensitizing electrophilic quaternized intermediates. The prediction of metabolites using QSAR analysis of a single, idealized chemical structure for PEG-4 cocamine was not sufficient to address such possibilities.

In addition, the Panel noted that no quantitative basis was provided for the decision to include tallow amine phosphate ester as an analog for the SAR-based read across analysis of these ingredients. Tallow amine phosphate ester was categorized as "suitable with precondition" in the analysis, assuming that this compound can be metabolized on the skin or in the body to yield a "suitable" analog. However, no data was presented to show that tallow amine phosphate ester can be metabolized in this way. Further, the chemical structure presented for tallow amine phosphate ester was questioned.

The additional data requested for these 15 ingredients include:

- Physical and chemical properties, including impurities (especially nitrosamines and impurities that impart color to the ingredients)
- Manufacturing information sufficient to enable the characterization of the likely distributions of fatty acids in these ingredients, including the occurrence and distributions of unsaturated fatty acid and trans-fatty acid moieties
- Information sufficient to characterize the likely metabolism of PEGylated amines that have hydroxyl ethyl moieties
- Genotoxicity of PEG-2 cocamine in a mammalian test system (if the results are positive then a dermal carcinogenesis study may be needed)
- 28-day dermal toxicity using PEG-2 cocamine
- Dermal sensitization data for PEG-2 cocamine
- Use concentration data for PEG-2 rapeseedamine; in the absence of such data, the Panel may assume the 2-rapeseedamine is used in hair coloring products at the same concentrations as PEG-2 oleamine (e.g., 3.5% highest reported maximum concentration)

Generally, the Panel expressed support for developing the SAR-based framework as a systematic approach to identifying possible analogues for read-across assessments, and categorizing the analogues as suitable, suitable with interpretation, and suitable with precondition. However, the Panel emphasized the importance of developing quantitative measures for the key decision-making steps of the approach, characterizing the boundary conditions and

assumptions of the models applied, and using actual test data for the class of chemicals to which the ingredients belong to validate computational predictions.

## CONCLUSION

The CIR Expert Panel concluded that the following 32 ingredients are safe in cosmetics in the present practices of use and concentration when formulated to be non-irritating:

PEG-8 cocamine*
PEG-10 cocamine*
PEG-12 cocamine*
PEG-15 cocamine
PEG-20 cocamine*
PEG-8 hydrogenated tallow amine
PEG-10 hydrogenated tallow amine*
PEG-15 hydrogenated tallow amine*
PEG-20 hydrogenated tallow amine*
PEG-30 hydrogenated tallow amine*
PEG-40 hydrogenated tallow amine*
PEG-50 hydrogenated tallow amine*
PEG-6 oleamine*
PEG-10 oleamine*
PEG-15 oleamine*
PEG-20 oleamine*
PEG-25 oleamine*
PEG-30 oleamine*
PEG-12 palmitamine*
PEG-8 soyamine*
PEG-10 soyamine*
PEG-15 soyamine*
PEG-10 stearamine*
PEG-15 stearamine*
PEG-50 stearamine*
PEG-7 tallow amine*
PEG-11 tallow amine*
PEG-15 tallow amine*
PEG-20 tallow amine*
PEG-22 tallow amine*
PEG-25 tallow amine*
PEG-30 tallow amine*

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

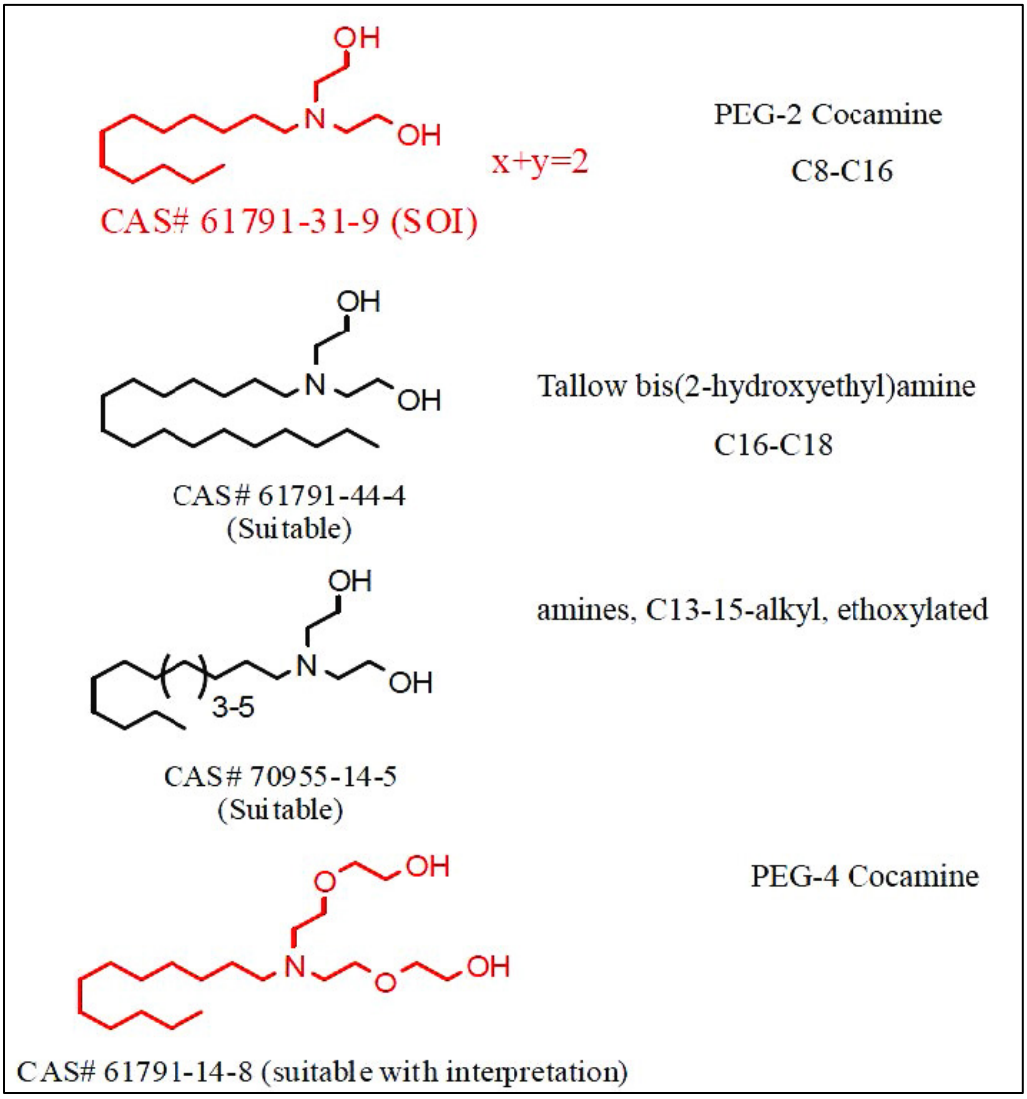
The CIR Expert Panel concluded that the available data are insufficient to make a determination that the following 15 ingredients are safe under the intended conditions of use:

PEG-2 cocamine
PEG-3 cocamine
PEG-4 cocamine
PEG-5 cocamine
PEG-2 hydrogenated tallow amine
PEG-5 hydrogenated tallow amine
PEG-2 lauramine
PEG-2 oleamine
PEG-5 oleamine
PEG-2 rapseedamine
PEG-2 soyamine
PEG-5 soyamine
PEG-2 stearamine
PEG-5 stearamine
PEG-2 tallow amine

This conclusion supersedes the earlier conclusion issued by the Expert Panel for PEG-2, -3, -4, -5, -10, -15 and -20 cocamine in 1999.

## TABLES AND FIGURES

**Figure 1.** Group 1 (PEG-2 Cocamine SOI)*



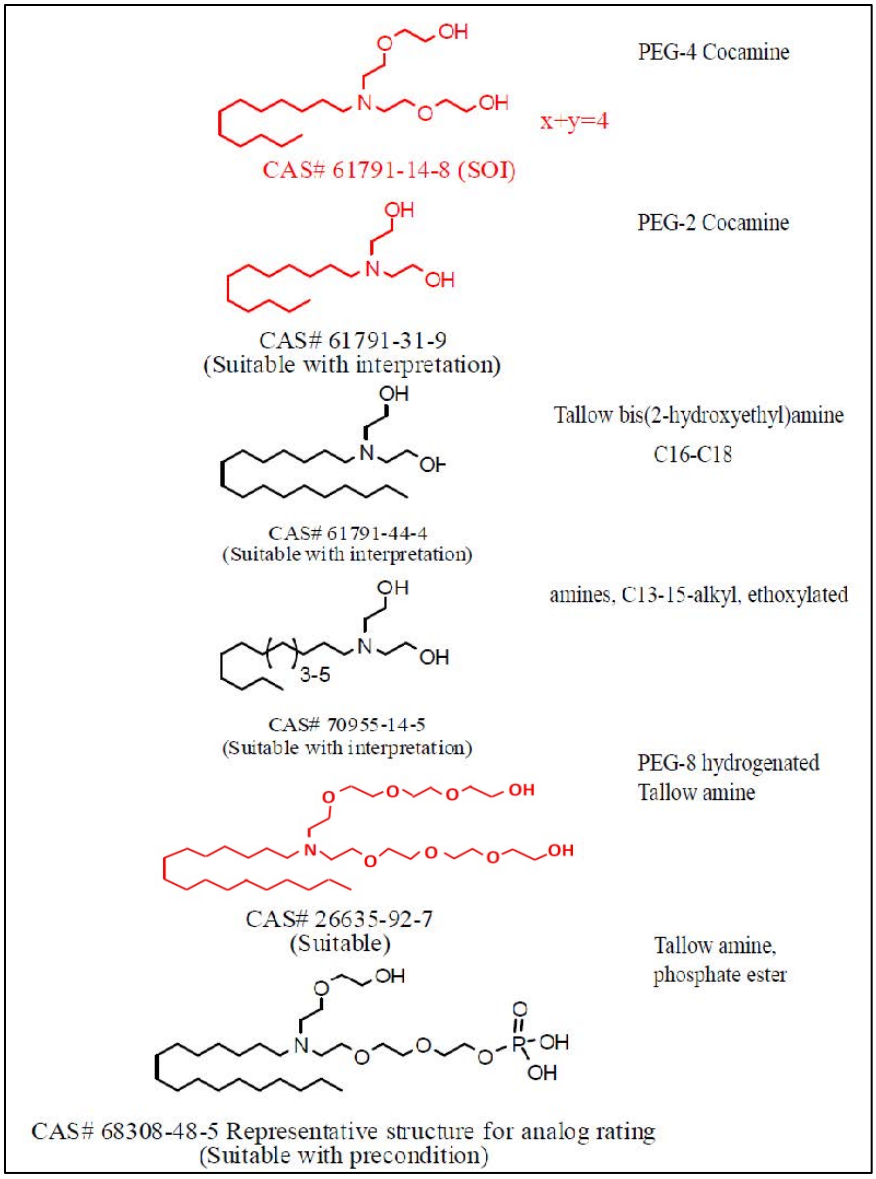

*Structures of PEGs Cocamine and related ingredients are shown in red
SOI = "structure of interest" for read across

Tallow bis(2-hydroxyethyl)amine is “suitable,” although it is longer than PEG-2 Cocamine and has some double bonds not present in PEG-2 Cocamine

Amines, C13-C15-alkyl, ethoxylated is “suitable” because, like PEG-2 Cocamine, it is not ethoxylated and it has a fatty-chain length similar to that of PEG-2 Cocamine

PEG-4 cocamine is “suitable with interpretation” because it has diethoxyl chains, rather than the hydroxyethyl chains of PEG-2 Cocamine, which may yield divergent metabolic pathways

**Figure 2**. Group 2 (PEG-4 Cocamine SOI)*




PEG-2 cocamine is “suitable with interpretation” because it has hydroxyethyl chains, rather than the diethoxyl chains of PEG-4 cocamine, which may yield divergent metabolic and distribution pathways

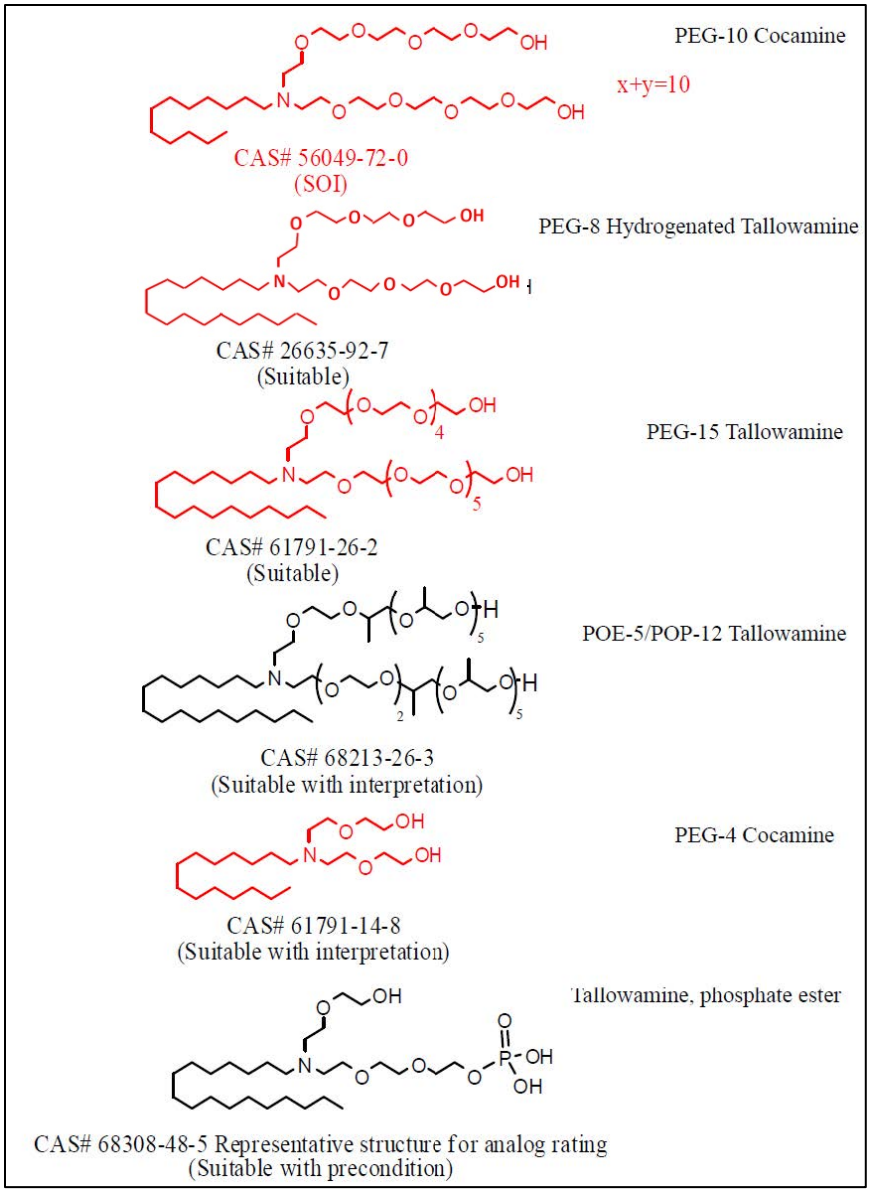
Tallow bis(2-hydroxyethyl)amine is “suitable with interpretation” because it has hydroxyethyl chains, rather than the diethoxyl chains of PEG-4 Cocamine, which may yield divergent metabolic and distribution pathways

Amines, C13-C15-alkyl, ethoxylated is “suitable with interpretation” because it has hydroxyethyl chains, rather than the diethoxyl chains of PEG-4 Cocamine, which may yield divergent metabolic and distribution pathways

PEG-8 hydrogenated tallow amine is a “suitable” analog because, among other reasons, its molecular size distribution overlaps that of PEG-4 Cocamine, which may yield similar metabolism, compared with PEG-4 Cocamine, and it is a hydrogenated tallow amine

Tallow amine, phosphate ester is “suitable with precondition” because of the requirement for metabolism by phosphatases to form a suitable analog

**Figure 3.** Group 3 (PEG-10 Cocamine SOI)*




PEG-8 hydrogenated tallow amine is a "suitable" analog because, among other reasons, its molecular size distribution overlaps that of PEG-10 Cocamine, some of which will be too long for significant metabolism

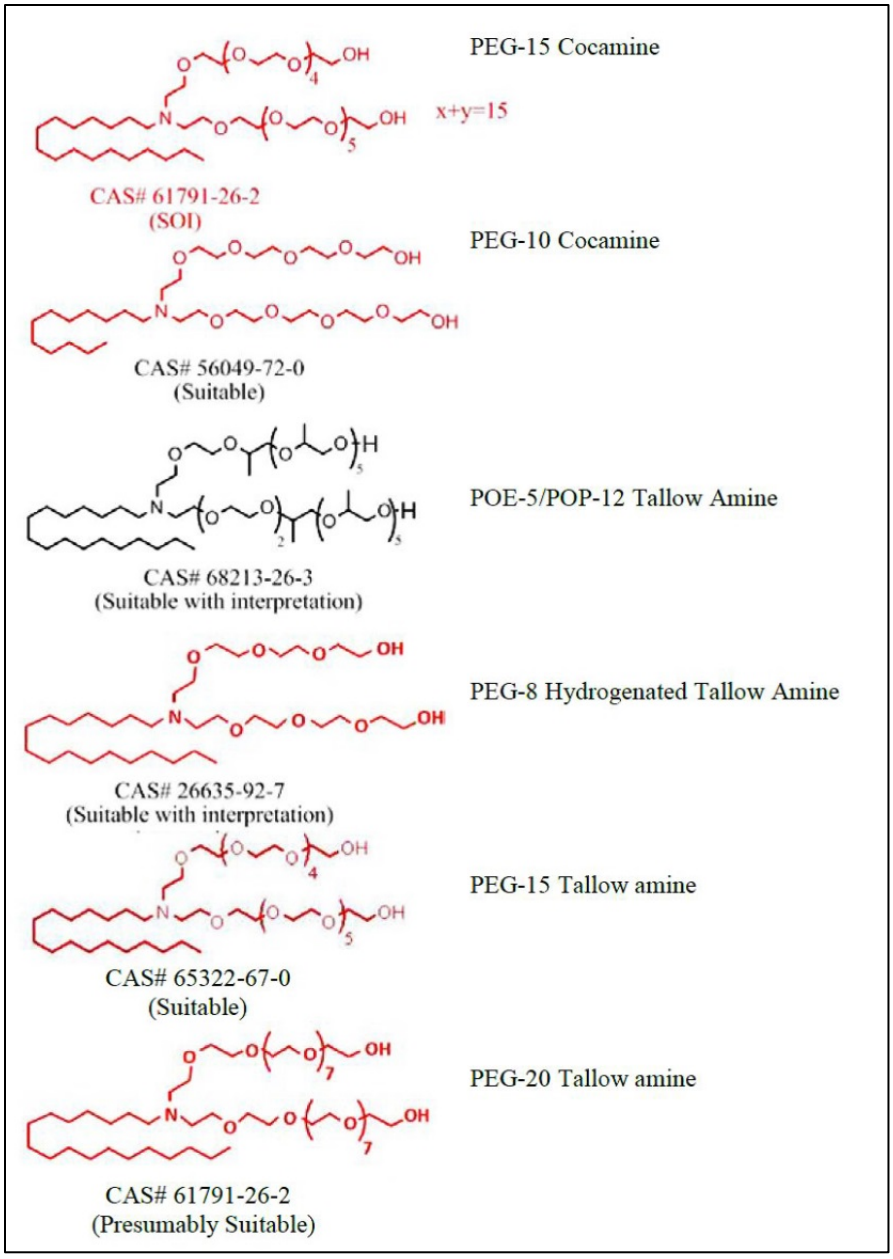
PEG-15 tallow amine is "suitable" because, among other reasons, its molecular size distribution overlaps that of PEG-10 Cocamine, some of which will be too long for significant metabolism

POE-5/POP-12 tallow amine is "suitable with interpretation" because it has both ethoxyl and propoxyl groups, which will yield substantial differences in physicochemical properties compared with PEG-10 Cocamine; this will not have much impact on reactivity in the judgment of the medicinal chemist

PEG-4 cocamine is "suitable with interpretation" because it has diethoxyl chains, rather than the polyethoxyl chains of PEG-10 Cocamine, which may yield divergent metabolic pathways

Tallow amine, phosphate ester is "suitable with precondition" because of the requirement for metabolism by phosphatases to form a suitable analog

**Figure 4.** Group 4 (PEG-20 Cocamine SOI)*




PEG-10 cocamine is “suitable” because, among other reasons, its molecular size distribution overlaps that of PEG-15 cocamine, although, unlike PEG-15 cocamine, some of the PEG-10 cocamine distribution may be short enough for significant metabolism

POE-5/POP-12 tallow amine is “suitable with interpretation” because it has both ethoxyl and propoxyl groups, which will yield substantial differences in physicochemical properties compared with PEG-10 Cocamine; this will not have much impact on reactivity in the judgment of the medicinal chemist

PEG-8 hydrogenated tallow amine is “suitable with interpretation” because it has shorter polyethoxyl chains and differences in physicochemical properties compared with PEG-15 cocamine

PEG-20 tallow Amine was not specified as to suitability rating, but is most probably “suitable”

**Table 1.** Chain length distribution and degree of unsaturation of the fatty acids in coconut oil

| Fatty Acid Chain Length | Degree of Unsaturation | Percent Composition |
|---|---|---|
| C6 | None | 0-1% |
| C8 | None | 5-9% |
| C10 | None | 5-10% |
| C12 | None | 44-53% |
| C14 | None | 13-19% |
| C16 | None | 8-11% |
| C18 | None | 1-3% |
| C16 | 1 | 0-1% |
| C18 | 1 | 5-8% |
| C18 | 2 | 1-3% |

**Table 2.** Chain length distribution and degree of unsaturation of the fatty acids in in tallow

| Fatty Acid Chain Length | Degree of Unsaturation | Percent Composition |
|---|---|---|
| C14 | None | 0-6% |
| C16 | None | 20-37% |
| C18 | None | 14-21% |
| C16 | 1 | 3-9% |
| C18 | 1 | 35-46% |
| C18 | 2 | 4-10% |
| C18 | 3 | 0-3% |

**Table 3.** Supplier specifications for PEGs cocamine and related ingredients[4]

| Property | Value |
|---|---|
| ***PEG-2 Cocamine*** | |
| Physical Appearance | Yellow to amber liquid |
| Color, Gardener | 2.0 max. |
| pH (10% in IPA/$H_2O$) | 9.0-11.0 |
| Tertiary Amine (%) | 97.0 min. |
| Moisture (%) | 0.5 max. |
| Neutralization Eq. | 290-310 |
| | |
| ***PEG-15 Cocamine*** | |
| Physical Appearance | Yellow to amber liquid |
| Color, Gardener | 9.0 max. |
| pH (10% in IPA/$H_2O$) | 9.0-11.0 |
| Tertiary Amine (%) | 96.0 min. |
| Moisture (%) | 1.0 max. |
| Neutralization Eq. | 825-905 |
| | |
| ***PEG-8 Hydrogenated Tallow Amine*** | |
| Physical Appearance | Amber Viscous Liquid (200 ºC) |
| % Activity | 93 min. |
| Solubility in water at 20°C | 0.4%; dispersion at > 0.4% |
| Specific Gravity | 1.027±0.050 (200 ºC) |
| % Ash | 0.05 max. |
| Iron ppm | 20 max. |
| Heavy Metals ppm | 5 max. |
| | |
| ***PEG-5 Oleamine*** | |
| Solubility | Water soluble |
| Specific Gravity at 25 ºC | 0.94 |
| Flash Point ºC | 158 |
| Ocular irritation test score | Not reported |
| Acute Toxicity | Oral $LD_{50}$ 1,000 mg/kg in rat |
| Skin Irritation | Not reported |

**Table 3.** Supplier specifications for PEGs cocamine and related ingredients[4]

| ***PEG-15 Oleamine*** | |
|---|---|
| Solubility | Water soluble |
| Specific Gravity at 25 ºC | 1.01 |
| Flash Point ºC | 79 |
| Ocular irritation test score | 18.8 (Rabbit, Draize, 10%) |
| Acute Toxicity | Not reported |
| Skin Irritation | Not reported |

| ***PEG-10 Stearamine*** | |
|---|---|
| Solubility | Water soluble |
| Specific Gravity at 25 ºC | 0.98 |
| Flash Point ºC | 160 |
| Ocular irritation test score | 18.8 (Rabbit, Draize, 10%) |
| Acute Toxicity | Not reported |
| Skin Irritation | Slight irritation (rabbit) |

**Table 4.** Frequency (2014) and concentration of use (2014) according to duration and type of exposure for PEGs-Cocamine ingredients. [10,11,35]

| | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|---|---|
| | **PEG-5 Hydrogenated Tallow Amine** | | **PEG-8 Hydrogenated Tallow Amine** | | **PEG-2 Oleamine** | | **PEG-2 Rapeseedamine** | |
| **Totals†** | **1** | **NR** | **4** | **NR** | **239** | **0.1-3.5** | **255** | **NR** |
| ***Duration of Use*** | | | | | | | | |
| Leave-On | NR | NR | NR | NR | NR | 0.16 | NR | NR |
| Rinse Off | 1 | NR | 4 | NR | 239 | 0.1-3.5 | 255 | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | | | |
| Eye Area | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR | NR | NR |
| Dermal Contact | NR | NR | NR | NR | NR | 0.16 | NR | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | NR | NR | NR | NR |
| Hair-Coloring | 1 | NR | 4 | NR | 239 | 0.1-3.5 | 255 | NR |
| Nail | NR | NR | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR | NR | NR |

| | **PEG-2 Soyamine** | | **PEG-5 Soyamine** | | **PEG-2 Tallow Amine** | |
|---|---|---|---|---|---|---|
| **Totals†** | **39** | **NR** | **6** | **4** | **30** | **NR** |
| ***Duration of Use*** | | | | | | |
| Leave-On | NR | NR | NR | NR | NR | NR |
| Rinse Off | 39 | NR | 6 | 4 | 30 | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | |
| Eye Area | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR |
| Dermal Contact | NR | NR | NR | NR | NR | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | NR | NR |
| Hair-Coloring | 39 | NR | 6 | 4 | 30 | NR |
| Nail | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR |

NR = Not reported.

† 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.

**Table 5.** Current and historical frequency and concentration of use of PEGs cocamine according to duration and exposure.[1,10,11,35]

| | *# of Uses* | | *Max Conc of Use (%)* | | *# of Uses* | | *Max Conc of Use (%)* | |
|---|---|---|---|---|---|---|---|---|
| | **2014** | **1996** | **2014** | **1995** | **2014** | **1996** | **2014** | **1995** |
| | **PEG-2 Cocamine** | | | | **PEG-3 Cocamine** | | | |
| **Totals†** | **107** | **15** | **0.33** | **NR*** | **NR** | **14** | **NR** | **NR*** |
| ***Duration of Use*** | | | | | | | | |
| Leave-On | NR | NR | 0.33 | NR | NR | NR | NR | NR |
| Rinse-Off | 107 | 15 | NR | NR | NR | 14 | NR | NR |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR | NR | NR |
| Eye Area | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | 0.33 | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | NR | NR | NR | NR | NR | NR | NR | NR |
| Dermal Contact | NR | NR | 0.33 | NR | NR | NR | NR | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | NR | NR | NR | NR |
| Hair-Coloring | 107 | 15 | NR | NR | NR | 14 | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | NR | NR | NR | NR |
| Baby Products | NR | NR | NR | NR | NR | NR | NR | NR |

| | **2014** | **1996** | **2014** | **1995** | **2014** | **1996** | **2014** | **1995** |
|---|---|---|---|---|---|---|---|---|
| | **PEG-5 Cocamine** | | | | **PEG-15 Cocamine** | | | |
| **Totals†** | **1** | **NR** | **NR** | **NR*** | **4** | **25** | **3** | **0.8-1.3** |
| ***Duration of Use*** | | | | | | | | |
| Leave-On | NR | NR | NR | NR | 4 | 20 | 3 | 0.8-1.3 |
| Rinse-Off | 1 | NR | NR | NR | NR | 5 | NR | 0.8-1 |
| Diluted for (Bath) Use | NR | NR | NR | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | | | |
| Eye Area | NR | NR | NR | NR | NR | NR | NR | 1.3 |
| Incidental Ingestion | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | NR | NR | NR | NR | 1[a]; 2[b] | 3; 13[a]; 2[b] | 3 | 1.0; 0.8[a] |
| Incidental Inhalation-Powder | NR | NR | NR | NR | 2[b] | 1; 2[b] | NR | NR |
| Dermal Contact | NR | NR | NR | NR | 3 | 19 | 3 | 1-1.3 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | NR | NR | NR | NR | 1 | 6 | NR | 0.8-1 |
| Hair-Coloring | 1 | NR | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | NR | NR | NR | NR | NR | NR |
| Mucous Membrane | NR | NR | NR | NR | NR | 2 | NR | 1 |
| Baby Products | NR | NR | NR | NR | NR | NR | NR | NR |

**Table 5.** Current and historical frequency and concentration of use of PEGs cocamine according to duration and exposure.[1,10,11,35]

| | *# of Uses* | | *Max Conc of Use (%)* | | *# of Uses* | | *Max Conc of Use (%)* | |
|---|---|---|---|---|---|---|---|---|
| | **2014** | **1996** | **2014** | **1995** | | | | |
| | **PEG-20 Cocamine** | | | | | | | |
| **Totals†** | **NR** | **38** | **NR** | **NR*** | | | | |
| Leave-On | NR | NR | NR | NR | | | | |
| Rinse-Off | NR | 37 | NR | NR | | | | |
| Diluted for (Bath) Use | NR | 1 | NR | NR | | | | |
| Eye Area | NR | NR | NR | NR | | | | |
| Incidental Ingestion | NR | NR | NR | NR | | | | |
| Incidental Inhalation-Spray | NR | NR | NR | NR | | | | |
| Incidental Inhalation-Powder | NR | NR | NR | NR | | | | |
| Dermal Contact | NR | 1 | NR | NR | | | | |
| Deodorant (underarm) | NR | NR | NR | NR | | | | |
| Hair - Non-Coloring | NR | 2 | NR | NR | | | | |
| Hair-Coloring | NR | 35 | NR | NR | | | | |
| Nail | NR | NR | NR | NR | | | | |
| Mucous Membrane | NR | 1 | NR | NR | | | | |
| Baby Products | NR | NR | NR | NR | | | | |

†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.

NR – no reported use

*Unspecified PEGs cocamine ingredient was reported to have a concentration of 8%-20% in hair coloring products.

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

[b] 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 6.** Ingredients that are not reported to be in use.

| | |
|---|---|
| PEG-3 Cocamine | PEG-25 Oleamine |
| PEG-4 Cocamine | PEG-30 Oleamine |
| PEG-8 Cocamine | PEG-12 Palmitamine |
| PEG-10 Cocamine | PEG-8 Soyamine |
| PEG-12 Cocamine | PEG-10 Soyamine |
| PEG-20 Cocamine | PEG-15 Soyamine |
| PEG-2 Hydrogenated Tallow Amine | PEG-2 Stearamine |
| PEG-10 Hydrogenated Tallow Amine | PEG-5 Stearamine |
| PEG-15 Hydrogenated Tallow Amine | PEG-10 Stearamine |
| PEG-20 Hydrogenated Tallow Amine | PEG-15 Stearamine |
| PEG-30 Hydrogenated Tallow Amine | PEG-50 Stearamine |
| PEG-40 Hydrogenated Tallow Amine | PEG-7 Tallow Amine |
| PEG-50 Hydrogenated Tallow Amine | PEG-11 Tallow Amine |
| PEG-2 Lauramine | PEG-15 Tallow Amine |
| PEG-5 Oleamine | PEG-20 Tallow Amine |
| PEG-6 Oleamine | PEG-22 Tallow Amine |
| PEG-10 Oleamine | PEG-25 Tallow Amine |
| PEG-15 Oleamine | PEG-30 Tallow Amine |
| PEG-20 Oleamine | |

**Table 7.** Analog Group 1: PEG-2 Cocamine as a Structure of Interest (SOI)

| Chemical | CAS No. | R | x + y | Genotoxicity | Repeated-dose Toxicity | Developmental & Reproductive Toxicity (DART) | Ref. |
|---|---|---|---|---|---|---|---|
| **SOI** | | | | | | | |
| PEG-2 Cocamine | 61791-31-9 | 8-16 | 2 | No data | No data (other than DART screening data) | Rat DART Screen: 2, 8, 23, 134 mg/kg/day (males) or 3, 9, 26, 148 mg/kg/day (females) via diet for 69-72 days. Developmental NOAEL = 23 mg/kg/day. Decreased postnatal survival, live litter size, # of pups born, & implantation sites. Reproductive NOAEL = 134 mg/kg/day (highest dose tested). Parental NOAEL = 23 mg/kg/day. | 36 |
| **Analogs** | | | | | | | |
| Tallow bis (2-hydroxyethyl)amine C16-C18 | 61791-44-4 | 14-18 | 2 | Ames test: (-)<br><br>*In vivo* mouse micronucleus test: (-) | Rat 90-Day Oral Study. 15, 50 or 150 mg/kg/day via diet; NOEL = 50 mg/kg/day. Palatability of diet decreased at high dose. Gross macroscopic observations: yellow coloration & thickening of mucosa in small intestine & regional mesenteric lymph nodes at high dose; histiocytosis in small intestine & mesenteric lymph nodes at mid & high dose.<br><br>Rat 90-Day Study. 0.8, 12 or 400 mg/kg/day via diet; NOEL = 12 mg/kg/day (based on body-weight gain) or 40 mg.kg/day (based on histiocytosis). Food consumption in all treated groups similar to control. Small decrease in body-weight gain in mid-dose males & high dose males & females; histiocytosis in small intestine & mesenteric lymph nodes at high dose. | No data | 37 |
| Tallow bis (2-hydroxyethyl)amine C16-C18 (Contd.) | | | | | Dog 90-Day Study. 13, 40 or 120 mg/kg/day via diet; NOEL = 13 mg/kg/day. Palatability issues at mid & high dose. GI clinical signs at mid & high dose (vomiting); histiocytosis in small intestine & regional lymph nodes at mid & high dose.<br><br>Rabbit 28-Day Percutaneous Study. 0.1% or 0.5% aqueous dispersion (2 or 10 mg/kg/day), 5 days/week for 4 weeks. Slight-to-moderate skin irritation at both concentrations; no evidence of systemic toxicity. | | |

**Table 7.** Analog Group 1: PEG-2 Cocamine as a Structure of Interest (SOI)

| Chemical | CAS No. | R | x + y | Genotoxicity | Repeated-dose Toxicity | Developmental & Reproductive Toxicity (DART) | Ref. |
|---|---|---|---|---|---|---|---|
| Amines, Cl3-Cl5- alkyl, ethoxylated | 70955-14-5 | 13-15 | 2 | No data | Rat 90-Day Oral Study. 15, 30 or 150 mg/kg/day via gavage; NOAEL = 15 mg/kg/day. Macro & microscopic changes in non-glandular stomach.<br><br>Dog 90-Day Oral Study. 15, 30 or 100 mg/kg/day via capsule; NOAEL = 30 mg/kg/day. GI clinical signs: Increased alanine aminotransferase (ALT) females only; increased pigment accumulation in Kupffer cells & bile canaliculi females only. | | 36 |
| PEG-4 Cocamine | 61791-14-8 | 8-16 | 4 | No data | No data | No data | - |

**Table 8. Analog Group 2: PEG-4 Cocamine as a Structure of Interest (SOI)**

| Chemical | CAS No. | R | x + y | Genotoxicity | Repeated-dose Toxicity | Developmental & Reproductive Toxicity (DART) | Ref. |
|---|---|---|---|---|---|---|---|
| **SOI** | | | | | | | |
| PEG-4 Cocamine | 61791-14-8 | 8-16 | 4 | No data | No data | No data | - |
| **Analogs** | | | | | | | |
| PEG-8 Hydrogenated Tallow Amine | 26635-92-7 | 16-18 | 8 | Ames test: (-) | No data | No data | [37] |
| Tallow Bis (2-hydroxyethyl)amine, C16-C18 | 61791-44-4 | 16-18 | 2 | Ames test: (-)<br><br>*In vivo* mouse micronucleus test: (-) | Rat 90-Day Oral Study. 15, 50 or 150 mg/kg/day via diet; NOEL = 50 mg/kg/day. Palatability of diet decreased at high dose. Gross macroscopic observations: yellow coloration & thickening of mucosa in small intestine & regional mesenteric lymph nodes at high dose; histiocytosis in small intestine & mesenteric lymph nodes at mid & high dose.<br><br>Rat 90-Day Study. 0.8, 12 or 400 mg/kg/day via diet; NOEL = 12 mg/kg/day (based on body-weight gain); 40 mg/kg/day (based on histiocytosis). Food consumption in all treated groups similar to control. Small decrease in body-weight gain in mid-dose males & high-dose males & females; histiocytosis in small intestine & mesenteric lymph nodes at high dose. | No data | [37] |
| Tallow Bis (2-hydroxyethyl)amine, C16-C18 (Contd.) | | | | | Dog 90-Day Oral study. 13, 40 or 120 mg/kg/day via diet; NOEL = 13 mg/kg/day. Palatability issues at mid- & high dose. GI clinical signs at mid & high dose (vomiting); histiocytosis in small intestine & regional lymph nodes at mid & high dose.<br><br>Rabbit 28-Day Percutaneous study. 0.1% or 0.5% aqueous dispersion (2 or 10 mg/kg/day), 5 days/week. Slight (to moderate) skin irritation at both concentrations. No evidence of systemic toxicity. | | |

**Table 8. Analog Group 2: PEG-4 Cocamine as a Structure of Interest (SOI)**

| Chemical | CAS No. | R | x + y | Genotoxicity | Repeated-dose Toxicity | Developmental & Reproductive Toxicity (DART) | Ref. |
|---|---|---|---|---|---|---|---|
| Amines, C13-C15, alkyl, ethoxylated | 70955-14-5 | 13-15 | 2 | No data | Rat 90-Day Oral study. 15, 30 or 150 mg/kg/day via gavage; NOAEL=15 mg/kg/day. Macro & microscopic changes in non-glandular stomach.<br><br>Dog 90-Day Oral study. 15, 30 or 100 mg/kg/day via capsule; NOAEL 30 mg/kg/day. GI clinical signs: Increased ALT in females only; Increased pigment accumulation in Kupffer cells & bile canaliculi in females only. | No data | 36 |
| Tallow amine, phosphate ester | 68308-48-5 | 16-18 | 5 | No data | No data | Rat DART Screen: 25, 100 or 200 mg/kg/day via gavage. Reproductive/developmental NOAEL = 100 mg/kg/day. Decrease in *corpora lutea*, implantation sites, litter size, pup body-weight gain. Notable parental toxicity. Parental systemic NOAEL = 100 mg/kg/day. | 38 |
| PEG-2 Cocamine | 61791-31-9 | 8-16 | 2 | No data | No data | Rat DART Screen: 2, 8, 23, 134 mg/kg/day (M) or 3, 9, 26, 148 mg/kg/day (F) via diet for 69-72 days via diet; Developmental NOAEL 23 mg/kg/day; decreased postnatal survival, live litter size, # of pups born, implantation sites; Reproductive NOAEL 134 mg/kg/day (highest dose tested); Parental NOAEL 23 mg/kg/day | 36 |

**Table 9. Analog Group 3: PEG-10 Cocamine as a Structure of Interest (SOI)**

| Chemical | CAS No. | R | x + y | Genotoxicity | Repeated-dose Toxicity | Developmental & Reproductive Toxicity (DART) | Ref. |
|---|---|---|---|---|---|---|---|
| **SOI** | | | | | | | |
| PEG-10 Cocamine | 56049-72-0 | 8-16 | 10 | No data | No data | No data | - |
| **Analogs** | | | | | | | |
| PEG-15 Tallow Amine | 61791-26-2 | 16-18 | 15 | Ames test :(-)<br><br>*In vivo* mouse micronucleus test: (-) | Rat 90-Day Oral study. 33, 99 & 292 mg/kg/day via diet; NOEL=33 mg/kg/day. GI irritation (hypertrophy & vacuolation of histiocytes in the *lamina propria* of the small intestine); histiocytosis in small intestine & mesenteric lymph nodes at mid & high dose. | Rat Developmental Toxicity Test. 15, 100 or 300 mg/kg/day via gavage on GD 6-15; NOAEL = 300 mg/kg/day (Highest dose tested); Maternal NOAEL = 100 mg/kg/day.<br><br>Rat 2-generation DART screen. 100, 300 or 1000 in diet. Reproductive / developmental NOAEL = 15 mg/kg/day; LOAEL = 53 mg/kg/day. Litter loss, decreased litter size, & postnatal survival. | [36] |
| PEG-8 Hydrogenated Tallow Amine | 26635-92-7 | 16-18 | 8 | Ames test: (-) | No data | No data | [37] |
| POE-5/POP-12 Tallow Amine | 68213-26-3 | 16-18 | 17 | No data | Rat 4-Week Oral Study: 15, 75 or 200 mg/kg/day via gavage. NOAEL=75 mg/kg/day; decreased body-weight gain & food consumption at high dose. | No data | [36] |
| Tallow amine, phosphate ester | 68308-48-5 | 16-18 | 5 | No data | No data | Rat DART Screen: 25, 100 or 200 mg/kg/day via gavage. NOAEL = 100 mg/kg/day. Decreases in corpora lutea, implantation sites, litter size, pup body-weight gain. Notable parental toxicity. | [38] |
| PEG-4 Cocamine | 61791-14-8 | 8-16 | 4 | No data | No data | No data | - |

**Table 10. Analog Group 4: PEG-15 Cocamine as a Structure of Interest (SOI)**

| Chemical | CAS No. | R | x + y | Genotoxicity | Repeated-dose Toxicity | Developmental & Reproductive Toxicity (DART) | Ref. |
|---|---|---|---|---|---|---|---|
| **SOI** | | | | | | | |
| PEG-15 Cocamine | 61791-26-2 | 8-16 | 15 | No data | No data | No data | - |
| **Analogs** | | | | | | | |
| POE-5/POP-12 Tallow Amine | 68213-26-3 | 16-18 | 17 | No data | Rat 4-Week Oral Study. 15, 75 or 200 mg/kg/day via gavage. NOAEL = 75 mg/kg/day. Decreased body-weight gain & food consumption. | No data | 36 |
| PEG-8 Hydrogenated Tallow Amine | 26635-92-7 | 16-18 | 8 | Ames test: (-) | No data | No data | 37 |
| PEG-15 Tallow Amine | 65322-67-0 | 16-18 | 15 | Ames test: (-)<br><br>*In vivo* mouse micronucleus test: (-) | Rat 90-Day Oral Study. 33, 99 & 292 mg/kg/day via diet. NOEL = 33 mg/kg/day. GI irritation, histiocytosis in small intestine & mesenteric lymph nodes at mid & high dose. | Rat Developmental Toxicity Study: 15, 100 or 300 mg/kg/day via gavage on gestation days 6-15. NOAEL 300 = mg/kg/day.<br><br>Rat 2-Generation DART Study. NOAEL = 15 mg/kg/day; LOAEL = 53 mg/kg/day. Litter loss, decreased litter size & postnatal survival. | 36 |
| PEG-20 Tallow Amine | 61791-26-2 | 16-18 | 20 | Ames test: (-)<br><br>*In vitro* mouse lymphoma test: (-)<br><br>*In vitro* UDS test: (-)<br><br>*In vitro* chromosome aberration test: (-) without S-9; (+) with S-9<br><br>*In vivo* mouse chromosome aberration test: (-) | Rabbit 28-Day Percutaneous Study: 10% aqueous dispersion, reduced to 2% aqueous dispersion after 2 treatments (200 mg/kg/day reduced to 40 mg/kg/day), 5 days/week for 4 weeks. Severe skin irritation at 10% leading to reduction in concentration to 2%. No evidence of systemic toxicity.<br><br>Rabbit 28-Day Percutaneous Study: 2% aqueous dispersion (40 mg/kg/day), 5 days/week for 4 weeks. Severe skin irritation. No evidence of systemic toxicity. | No data | 37 |
| PEG-10 Cocamine | 56049-72-0 | 8-16 | 10 | No data | No data | No data | - |

# Appendix

## for the Safety Assessment of PEGs Cocamine and Related Ingredients as Used in Cosmetics

## Robust Summaries from the US EPA Fatty Nitrogen Derived Amines Category High Production Volume Chemical Challenge

| **Table 11. Repeated-Dose Toxicity**[39] | **Tallow bis(2-hydroxyethyl)amine (C16-C18) (CAS No. 61791-44-4) (x+y=2)** |
|---|---|
| **Test Substance** | |
| Identity: | Ethomeen T/12<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs. |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Not stated |
| Test type: | Oral |
| GLP: | No |
| Year: | 1965 |
| Species: | Rat |
| Strain: | SPF Wistar |
| Route of administration: | Oral (feed) |
| Duration of test: | 90 days |
| Doses/concentration levels: | 0, 170, 500, 1500 and 4500 ppm |
| Sex: | Male and female |
| Exposure period: | 90 days |
| Frequency of treatment: | *ad libitum* |
| Control group and treatment: | Yes; concurrent, untreated diet |
| Post exposure observation period: | None |
| Statistical methods: | Not stated |
| Remarks: | Groups of 25 young adult male and female rats were fed diets containing the test substance at concentrations of 0, 170, 500 and 1500 ppm (approximately 15, 50 and 150 mg/kg/day). A group of ten male and ten female rats were fed a diet containing test substance at a concentration of 4500 ppm. In addition, a further group of seven male and seven female rats were fed a diet containing 4500 ppm of the test substance and killed at intervals up to six weeks from the beginning of the experiment. Tissues from these animals were examined for sudanophilic material. Diets were prepared at the laboratory and contained powdered stock diet, malt extract and corn. Test substance was added to experimental diets via corn oil, in which it was dissolved by gentle heating at 40°C. The ingredients were mixed mechanically and water added to produce a dough, which was then formed into pellets and dried at a temperature of not more than 40°C. Food and water were available ad libitum. Body weights were recorded at study initiation and weekly during the treatment period. Hemoglobin concentrations, packed-cell volumes, white-cell counts and differential white-cell counts were measured prior to treatment and immediately prior to sacrifice at the end of the 90-day test period. These hematologic parameters were evaluated on individual samples from five male and five female rats from each group except that blood was examined from all animals fed diet containing 4500 ppm of the test substance. At the time of sacrifice, the liver, heart, lung, adrenals, kidneys and spleen were weighed and organ/body weight ratios calculated from random selection of animals in each group. Tissues and organs from the remaining animals were fixed and examined microscopically. The following tissues and organs were examined: liver, kidney, spleen, heart, lung, adrenals, gonads, thymus, thyroid, pancreas, stomach, duodenum, jejunum, ileum, cecum, colon, salivary gland, mesenteric lymph nodes, spinal cord and brain (cerebrum, cerebellum and medulla) |

.

**Table 11. Repeated-Dose Toxicity (Contd.)**

**Results**

| | |
|---|---|
| NOAEL (NOEL) | NOEL = 500 ppm (approximately 50 mg/kg/day) |
| LOAEL (LOEL): | LOEL = 1500 ppm |
| Actual dose received: | Not determined |
| Toxic response/effects: | Described below |
| Statistical results: | None |
| Remarks: | No unscheduled deaths occurred and males and females responded similarly. Rats fed diet containing 4500 ppm of the test substance lost hair and generally were lethargic throughout the study. No clinical observations were noted in rats at any other dietary level. Body weight gain was inhibited at the 4500 ppm dietary level and partly inhibited in the 1500 ppm dietary level. There was no apparent affect on body weight for rats in the 1500 ppm or 700 ppm groups. The palatability of the diet was decreased by the addition of 4500 and 1500 ppm of the test substance. No definite hematological abnormality was detected at any dose level of the test substance. No significant differences were seen between test and control group organ weights. Gross macroscopic observations at necropsy were seen only in the 4500 ppm group and comprised of yellow coloration of the stomach and bowel contents, and thickening and yellow coloration of the mucosa of the small intestine and the regional mesenteric nodes. Microscopic findings, which were documented in rats treated at dietary levels of 1500 and 4500 ppm, were confined to the small intestine and regional mesenteric nodes. All rats in the 4500 ppm group showed engorgement of the *villi* and *lamina propria* of the small intestine with swollen foamy macrophages. Similar macrophages occasionally were seen to a lesser degree in Peyer's patches and in the regional lymph nodes. Changes were most pronounced in the jejunum and upper ileum but were detected throughout the small intestine. The macrophages were sudanophilic and were presumed to contain deposits of the test substance. Similar findings were present to a lesser degree in 31 of the 40 rats fed 1500 ppm of the test substance. No findings were noted at any other dietary level. Reproductive organs were examined, meeting the requirements of SIDS/HPV reproductive screening. |

**Conclusions**

| | |
|---|---|
| Remarks: | The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |

**Data Quality**

| | |
|---|---|
| Reliability (Klimisch): | 1B |
| Remarks: | Reliable without restriction; comparable to guideline study. |

**References:**

Goater T, Griffiths OD and McElligott TF. 1965. Ninety-Day Oral Toxicity of Ethomeen T/12 – Albino Rats. Report No. IHR/173. Industrial Hygiene Research Laboratories, Macclesfield, Cheshire

**Other Available Reports:**

Goater T, Griffiths OD, McElligott TF and Swan, AAB. 1970. Summary of Toxicology Data – Acute Oral Toxicity and Short-Term Feeding Studies on Polyoxyethylene Tallow Amine in Rats and Dogs. Food & Cosmetics Toxicol. 8:249-252.

**Other**

| | |
|---|---|
| Last changed: | June 7, 2002 |
| Remarks: | None. |

**Table 11. Repeated-Dose Toxicity (Contd.)**

| | |
|---|---|
| **Test Substance** | Ethomeen T/12 |
| Identity: | CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs.) |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Not stated |
| Test type: | Oral |
| GLP: | No |
| Year: | 1965 |
| Species: | Dog |
| Strain: | Beagle |
| Route of administration: | Oral (feed) |
| Duration of test: | 90 days |
| Doses/concentration levels: | 0, 13, 40, and 120 mg/kg |
| Sex: | Male and female |
| Exposure period: | 90 days |
| Frequency of treatment: | *ad libitum* |
| Control group and treatment: | Yes; concurrent, untreated diet |
| Post exposure observation period: | None |
| Statistical methods: | Not stated |
| Remarks: | Groups of four male and female dogs were fed diets containing the test substance at concentrations to yield doses of 0, 13, 40 and 120 mg/kg. Diets were prepared at the laboratory and contained a meat preparation, dry pelleted diet and corn oil. Test substance was added to experimental diets. The main meal was offered to each dog daily at noon and a dog biscuit was offered late each afternoon. Water was available ad libitum. Body weights were recorded at study initiation and weekly during the treatment period. Hemoglobin concentrations, packed-cell volumes, white-cell counts and differential white-cell counts were measured in all animals prior to treatment and immediately prior to sacrifice. Blood urea, serum alkaline phosphatase, liver function and urine analysis also were tested. At the end of the test period, dogs were sacrificed, and the following organ weights were recorded: heart, liver, kidneys, adrenals, spleen, thyroid, testes, epididymides, brain and pituitary. For microscopic examination, representative sections were taken from the following organs: brain (cerebrum, cerebellum and medulla), spinal cord, pituitary, submaxillary gland, thyroid, thymus, heart, lung, aorta, stomach, duodenum, jejunum, ileum, colon, liver, spleen, kidney, bladder, adrenal, ovary and uterus or testes and epididymis, and sciatic nerve. |
| **Results** | |
| NOAEL (NOEL) | NOEL = 13 mg/kg/day |
| LOAEL (LOEL): | LOEL = 50 mg/kg/day |
| Actual dose received: | Not determined |
| Toxic response/effects: | Described below |
| Statistical results: | None |
| Remarks | No unscheduled deaths occurred and males and females responded similarly. Rats fed diet containing 4500 ppm of the test substance lost hair and generally were lethargic throughout the study. No clinical |

**Table 11. Repeated-Dose Toxicity (Contd.)**

observations were noted in rats at any other dietary level. Body weight gain was inhibited at the 4500 ppm dietary level and partly inhibited in the 1500 ppm dietary level. There was no apparent affect on body weight for rats in the 1500 ppm or 700 ppm groups. The palatability of the diet was decreased by the addition of 4500 and 1500 ppm of the test substance. No definite hematological abnormality was detected at any dose level of the test substance. No significant differences were seen between test and control group organ weights. Gross macroscopic observations at necropsy were seen only in the 4500 ppm group and comprised of yellow coloration of the stomach and bowel contents, and thickening and yellow coloration of the mucosa of the small intestine and the regional mesenteric nodes. Microscopic findings, which were documented in rats treated at dietary levels of 1500 and 4500 ppm, were confined to the small intestine and regional mesenteric nodes. All rats in the 4500 ppm group showed engorgement of the *villi* and *lamina propria* of the small intestine with swollen foamy macrophages. Similar macrophages occasionally were seen to a lesser degree in Peyer's patches and in the regional lymph nodes. Changes were most pronounced in the jejunum and upper ileum but were detected throughout the small intestine. The macrophages were sudanophilic and were presumed to contain deposits of the test substance. Similar findings were present to a lesser degree in 31 of the 40 rats fed 1500 ppm of the test substance. No findings were noted at any other dietary level. Reproductive organs were examined, meeting the requirements of SIDS/HPV reproductive screening.

**Conclusions**

Remarks: The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group)

**Data Quality**

Reliability (Klimisch): 1B

Remarks: Reliable without restriction; comparable to guideline study.

**References:**

Goater T, Griffiths OD and McElligott TF. 1965. Ninety-Day Oral Toxicity of Ethomeen T/12 – Beagle Dogs. Report No. IHR/175. Industrial Hygiene Research Laboratories, Macclesfield, Cheshire.
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**Other Available Reports:**

Goater T, Griffiths OD, McElligott TF and Swan, AAB. 1970. Summary of Toxicology Data – Acute Oral Toxicity and Short-Term Feeding Studies on Polyoxyethylene Tallow Amine in Rats and Dogs. Food & Cosmetics Toxicol. 8:249-252.

**Other**

Last changed: June 10, 2002

Remarks: None

**Table 11. Repeated-Dose Toxicity (Contd.)**

| | |
|---|---|
| **Test Substance** | Test article E1095.01 |
| Identity: | CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs. |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Not stated |
| Test type: | Oral |
| GLP: | Yes |
| Year: | 1981 |
| Species: | Rat |
| Strain: | Crl:CD(SD)BR |
| Route of administration: | Oral (feed) |
| Duration of test: | 13 weeks |
| Doses/concentration levels: | 0.001, 0.015 and 0.5% w/w (approximately 0.8, 12 and 400 mg/kg/day) |
| Sex: | Male and female |
| Exposure period: | 13 weeks |
| Frequency of treatment: | *ad libitum* |
| Control group and treatment: | Yes; untreated powdered diet |
| Post exposure observation period: | None |
| Statistical methods: | Not stated |
| Remarks: | Four groups of 40 rats (20 males and 20 females) were fed diets containing the test substance at concentrations of 0, 0.001, 0.015 and 0.5% w/w for 13 weeks, or until necropsy. The test substance was added to experimental diets as solutions in corn oil (1%). Rats at approximately 6-½ weeks of age, weighing 136 to 188 g (males) and 119 to 165 g (females), were acclimated to the laboratory for 19 days prior to test initiation. With the exception of an overnight fasting period before necropsy, food and water were available ad libitum. All animals were examined at least once daily for signs of ill health, overt toxicity or behavioral changes. Individual body weights and group food consumption were recorded weekly throughout the study. Hematology analyses and necropsy were performed on all rats. Organ weights (adrenals, kidneys, lungs, testes, heart, liver and ovaries) were determined at necropsy. Histopathology, including reproductive organs, was conducted for all animals in the control and high dose groups. In addition, jejunum and mesenteric lymph nodes were examined for animals in Groups 2 and 3. The "no effect" dose level was determined on the basis of evidence of systemic toxicity at the respective dosage levels. |
| **Results** | NOEL = 0.015% (approximately 12 mg/kg/day) |
| NOAEL (NOEL) | Not stated |
| LOAEL (LOEL): | Not stated |
| Actual dose received: | Described below |
| Toxic response/effects: | Not applicable |
| Statistical results: | |

**Table 11. Repeated-Dose Toxicity (Contd.)**

| | |
|---|---|
| Number of Deaths: | Control = 0/20 males; 1/20 females (during blood sampling)<br>0.001% w/w = 0/20 males; 0/20 females<br>0.015% w/w = 0/20 males; 0/20 females<br>0.5% w/w = 0/20 males; 0/20 females |
| Remarks: | A high incidence of hair loss observed across all groups within each sex (70-90% males; 35-70% females) was not considered to be treatment related. Body weight gain was slightly reduced in the 0.5% w/w treatment group and the 0.015% male treatment group. Food consumption was similar among all groups relative to the control. There were no biologically significant differences in hematology or organ weights between treatment and control groups during Week 13. Histiocytosis, characterized by aggregations of macrophages with foamy cytoplasm, in the jejunum and mesenteric lymph nodes in the 0.5% w/w treatment group was the only treatment related histopathological finding in this study. Histiocytosis was not observed in these organs of the lower dose groups. No treatment-related effects on organ weights or histopathology of the reproductive organs were seen. |
| **Conclusions** | |
| Remarks: | The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |
| **Data Quality** | |
| Reliability (Klimisch): | 1B |
| Remarks: | Reliable without restriction; comparable to guideline study. |
| **Reference:** | Sheppard, DB. 1982. 13 Week Oral (Dietary) Toxicity Study in the Rat: ECM BTS 306, E1095.01. Unpublished report (No. 2913-110/369), for Procter and Gamble, Ltd., Longbenton, Newcastle-upon-Tyne, England; from Hazleton Laboratories Europe, Ltd., Harrogate, England. |
| **Other** | |
| Last changed: | September 21, 2003 |
| Remarks: | None |

**Table 11. Repeated-Dose Toxicity (Contd.)**

| | |
|---|---|
| **Test Substance** | ECM BTS 306, E1069.02 |
| Identity: | CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs. |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Not stated |
| Test type: | 4-Week Percutaneous Toxicity |
| GLP: | Yes |
| Year: | 1980 |
| Species: | Rabbit |
| Strain: | New Zealand White |
| Route of administration: | Dermal |
| Duration of test: | 4 weeks |
| Doses/concentration levels: | 2.0 ml/kg; 0.1 or 0.5% w/v (2 and 10 mg/kg/day) |
| Sex: | Male and female |
| Exposure period: | 4 weeks |
| Frequency of treatment: | Daily, 5 days/week |
| Control group and treatment: | Yes; distilled water |
| Post exposure observation period: | None |
| Statistical methods: | Not stated |
| Remarks: | Five young adult rabbits of each sex, weighing 2.5 to 3.3 kg, were administered distilled water (control) or the liquid test substance as 0.1 or 0.5% w/v aqueous dispersions at a dosage volume of 2.0 ml/kg daily, 5 days per week for 4 weeks. The test dispersion (or distilled water) was applied to the shaved dorso-lumbar region of each animal through a syringe and left for 7 hours before removal by washing. All rabbits were examined at least once daily for signs of ill-health or overt toxicity. Skin irritation was assessed daily using a Draize scoring procedure. Individual body weights were measured at initiation and weekly through the study period. Hematology analyses and a complete necropsy were conducted at termination. Organ weights (adrenals, heart, liver, kidneys, lungs, and ovaries/testes) were weighed at necropsy. Histopathology was performed for tissues, including treated skin and reproductive organs, of all rabbits in the control and high dose groups. |
| **Results** | Not stated |
| NOAEL (NOEL) | Not stated |
| LOAEL (LOEL): | Not stated |
| Actual dose received: | Described below |
| Toxic response/effects: | Not applicable |
| Statistical results: | |
| Number of Deaths: | Control group: 1/5 males, 0/5 females;<br>0.1% w/v aqueous dispersion: 2/5 males, 2/5 females;<br>0.5% w/v aqueous dispersion: 0/5 males, 1/5 females |

**Table 11. Repeated-Dose Toxicity (Contd.)**

| | |
|---|---|
| Remarks: | Three animals of each sex died or were killed because of illness before study termination, none of which were deemed treatment related. Skin irritation developed in all rabbits of the 0.5% w/v treatment group within 24 hours and persisted throughout the study. Slight erythema and edema developed into moderate erythema in most rabbits in this group after the second treatment. Slight to moderate fissuring and atonia with wrinkled skin and slight desquamation also developed during the first half of the study, although the presence of a thick layer of skin prevented assessment of edema and atonia in one rabbit in this group. Skin irritation in the lower concentration, 0.1% w/v, treatment group was characterized by slight erythema 2 days after treatment, which developed into moderate erythema 2 days later. Slight edema, desquamation and wrinkled skin also developed in most animals in this group. No reaction to treatment was observed in the control group.<br><br>There were no treatment-related effects on body weights, organ weights or hematology. The skin reaction found in all rabbits in the 0.5% w/v treatment group was assessed histologically as slight to moderate and was characterized by slight to moderate acanthosis, hypergranulosis and hyperkeratosis accompanied by slight congestion, edema and leukocyte infiltration in the superficial dermis. One rabbit in this group had an acute inflammatory reaction at the exposure site and died during the study. A few rabbits in the control group had a few minor changes in the treated skin site. While infrequent, minor pathological findings were noted in surviving rabbits in both treatment groups, there was no evidence of systemic toxicity. |
| **Conclusions** | |
| Remarks: | Repeated topical application of the test substance at 0.1 and 0.5% w/v to the non-abraded skin of rabbits elicited overt slight and moderate irritation, respectively. There was no evidence of systemic toxicity from mortalities, clinical changes, hematological measurements, body and organ weights or pathological findings.<br><br>This study is useful in the overall evaluation of repeated-dose toxicity of the test substance. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |
| **Data Quality** | 1D |
| Reliability (Klimisch): | Reliable without restriction; only two dose groups were examined. |
| Remarks: | None |
| **Reference:** | Shaw, DC. 1982. E1069.02: A 4 Week Percutaneous Toxicity Study in the Rabbit, ECM BTS 306. Unpublished report no. 2827-110/366, for The Procter and Gamble Limited, Longbenton, Newcastle- Upon-Tyne, England, from Hazleton Laboratories Europe, Ltd., Harrogate, North Yorkshire, England. |
| **Other** | |
| Last changed: | September 21, 2003 |
| Remarks: | None |

| **Table 12. Genotoxicity *In vitro*[39]** | **Tallow bis(2-hydroxyethyl)amine (C16-C18) (CAS No. 61791-44-4) (x+y=2)** |
|---|---|
| **Test Substance** | |
| Identity: | Genamin S080 (20% in water + H3PO4)<br>Alkylamine ethoxylate<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs.) |
| Purity: | 99.5% |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | *Salmonella*/Mammalian Microsome Mutagenesis Assay (Ames Test), 9/15/1980; modified from Ames, B.N. et al. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Research 31:347-364 |
| Test type: | Microsome mutagenicity assay (Ames test) |
| GLP: | Yes |
| Year: | 1981 |
| Species/Strain: | Salmonella typhimurium, strain TA100 (range-finding toxicity); and strains TA98, TA100, TA1535, TA1537, TA1538 |
| Metabolic activation: | With and without metabolic activation; Aroclor 1254-induced rat liver S-9 from Sprague-Dawley rats 4 weeks |
| Concentrations tested: | 0.0008, 0.02, 0.04 and 0.08 µl per plate |
| Statistical methods: | Not stated |
| Remarks: | A dose-range finding study indicated that a maximum of <0.1 µl of the test substance per plate be used for the mutagenicity assay. The maximum dose tested was 40 µl/plate. Results indicated that the background bacterial lawn was normal to moderately reduced at 0.003 to 0.1 µl/plate, with complete disappearance of bacterial lawn above 1.0 µl/plate. No precipitation was reported.<br><br>For the Salmonella mutagenesis assay, the test substance was diluted in water, which was also used as the vehicle control (50 µl per plate). 2-Aminoanthracene (1.0 µg/plate) was the positive control for strains TA98 and TA100 with S-9 activation. The positive controls utilized without S-9 activation were as follows: 2-nitrofluorene (10.0 µg/plate, TA98 and TA 1538); 1,2-propane sultone (0.4 µl/plate, TA 100 and TA 1535); and 9- aminoacridine (75 µg/plate [*sic*], TA 1537). The solvent controls and all test substance doses were plated in triplicate, while positive controls were tested with no replication. The S-9 homogenate and mix was prepared at the testing facility. The test substance or positive control, tester strain and S-9 mix, when applicable, were added to molten selective top agar in said order. The criteria for a valid test were: 1) A sterility check on the S-9 mix must yield les than two viable cells per plate; 2) A sterility check on al levels of test substances at conclusion must yield less than two viable colonies per plate; 3) the positive controls must produce at least at 3-fold increase in the number of revertant colonies; and 4) the average number of revertant colonies in the negative controls must fall within the historical limit for each strain. |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Results** | |
| Result | There was no increase in the number of revertant colonies in any tester strain at any dose. |
| Cytotoxic concentration: | Negative with and without S-9 activation |
| Genotoxic effects: | Negative with and without S-9 activation |
| Statistical results: | Not stated |
| Remarks: | None |
| **Conclusions** | |
| Remarks: | The results of this Salmonella/mammalian-microsome mutagenicity assay indicate that this test substance did not cause a significant increase in the number of revertants per plate of any of the tester strains with or without metabolic activation.<br><br>The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |
| **Data Quality** | |
| Reliability (Klimisch): | 2C |
| Remarks: | Reliable with restrictions; comparable to guideline study; no confirmatory assay. |
| **Reference:** | Haworth, SR. 1981. Salmonella/Mammalian-Microsome Mutagenesis Assay (Ames Test). Report No. 003-407-637-1; for The Procter and Gamble Company, Cincinnati, OH, USA; from EG&G Mason Research Institute, Rockville, MD, USA. |
| **Other** | |
| Last changed: | September 23, 2003 |
| Remarks: | None |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Test Substance** | |
| Identity: | "TAMET" Benzoate (20% in water)<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs.) |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | *Salmonella*/Mammalian Microsome Mutagenesis Assay (Ames Test), 9/15/1980; modified from Ames, B.N. et al. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Research 31:347-364<br>Microsome mutagenicity assay (Ames test) |
| Test type: | Yes |
| GLP: | 1981 |
| Year: | Salmonella typhimurium, strain TA100 (range-finding toxicity); and |
| Species/Strain: | strains TA98, TA100, TA1535, TA1537, TA1538<br>With and without metabolic activation; Aroclor 1254-induced rat liver |
| Metabolic activation: | S-9 from Sprague-Dawley rats 4 weeks<br>2.0, 10. 50. 100 and 200 µl per plate |
| Concentrations tested: | Not stated |
| Statistical methods: | A dose-range finding study indicated that a maximum of 200 µg of the |
| Remarks: | test substance per plate be used for the mutagenicity assay. Results indicated that the background bacterial lawn was reduced at concentrations 305[*sic*] µg/plate, with complete disappearance of bacterial lawn above 977 µg/plate. Moderate precipitation was reported only at the maximum dose tested (20,000 µg/plate).<br><br>For the Salmonella mutagenesis assay, the test substance was diluted in ethanol, which was also used as the vehicle control (50 µl per plate). 2- Aminoanthracene (1.0 or 4.0 µg/plate, depending on tester strain) was the positive control for all tester strains with S-9 activation. The positive controls utilized without S-9 activation were as follows: 2-nitrofluorene (10.0 µg/plate, TA98 and TA 1538); 1,2-propane sultone (0.4 µl/plate, TA 100 and TA 1535); and 9-aminoacridine (75 µg/plate, TA 1537). The negative and solvent controls and all test substance. doses were plated in triplicate, while positive controls were tested with no replication. In order to clarify erratic plate counts observed in tester strain TA1537 with the test substance without activation, this strain was retested. Additionally, tester strain TA100 was retested over an extended dose range (including 300 and 400 µg/plate) in order to clarify the corresponding initial plate counts. The S-9 homogenate and mix was prepared at the testing facility. The test substance or positive control, tester strain and S-9 mix, when applicable, were added to molten selective top agar in said order. The criteria for a valid test were: 1) A sterility check on the S-9 mix must yield les than two viable cells per plate; 2) A sterility check on al levels of test substances at conclusion must yield less than two viable colonies per plate; 3) the positive controls must produce at least at 3-fold increase in the number of revertant colonies; and 4) the average number of revertant colonies in the negative controls must fall within the historical limit for each strain. |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Results** | |
| Result | There was no increase in the number of revertant colonies in any tester strain at any dose. |
| Cytotoxic concentration: | Negative with and without S-9 activation |
| Genotoxic effects: | Negative with and without S-9 activation |
| Statistical results: | Not stated |
| Remarks: | None |
| **Conclusions** | |
| Remarks: | The results of this Salmonella/mammalian-microsome mutagenicity assay indicate that this test substance did not cause a significant increase in the number of revertants per plate of any of the tester strains with or without metabolic activation by Aroclor induced rat liver microsomes.<br><br>The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |
| **Data Quality** | |
| Reliability (Klimisch): | 2C |
| Remarks: | Reliable with restrictions; comparable to guideline study; no confirmatory assay. |
| **Reference:** | Haworth, SR. 1981. Salmonella/Mammalian-Microsome Mutagenesis Assay (Ames Test). Report No. 003-468-677-1; for The Procter and Gamble Company, Cincinnati, OH, USA; from EG&G Mason Research Institute, Rockville, MD, USA. |
| **Other** | |
| Last changed: | September 23, 2003 |
| Remarks: | None |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Test Substance** | |
| Identity: | $(POE)_{20}$ Tallowamine (Varonic T-220)<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-, N-tallow alkyl derivs. |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Salmonella/Mammalian Microsome Mutagenesis Assay (Ames Test), 11/1/1979; modified from Ames, B.N. et al. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Research 31:347-364. |
| Test type: | Microsome mutagenicity assay (Ames test) Bacterial |
| GLP: | Yes |
| Year: | 1980 |
| Species/Strain: | Salmonella typhimurium, strain TA100 (range-finding toxicity); and strains TA98, TA100, TA1535, TA1537, TA1538 |
| Metabolic activation: | With and without metabolic activation; Aroclor 1254-induced rat liver S-9 from Sprague-Dawley rats |
| Concentrations tested: | 0.0008, 0.004, 0.02, 0.04 and 0.08 µl/plate |
| Statistical methods: | Not stated |
| Remarks: | A dose-range finding study indicated that a maximum of 0.08 µl of the test substance per plate be used for the mutagenicity assay. Results indicated that the background bacterial lawn was normal to slightly reduced at 0.003 to 0.1 µl/plate, and extremely reduced from 0.3 to 10 µl/plate. No precipitation was reported.<br><br>For the Salmonella mutagenesis assay, the test substance was diluted in water, which was also used as the vehicle control (50 µl per plate). 2-Aminoanthracene (1.0 µg/plate) was the positive control for strains TA98 and TA100 with S-9 activation. The positive controls utilized without S-9 activation were as follows: 2-nitrofluorene (10.0 µg/plate, TA98 and TA 1538); 1,2-propane sultone (0.4 µl/plate, TA 100 and TA 1535); and 9- aminoacridine (75 µg/plate, TA 1537). The solvent controls and all test substance doses were plated in triplicate, while positive controls were tested with no replication. In order to clarify the reduced (81%)plasmid content in cells from the TA100 culture, indicated by a "halo" surrounding the Ampicillin disc, this strain was simply retested. The S-9 homogenate and mix was prepared at the testing facility. The test substance or positive control, tester strain and S-9 mix, when applicable, were added to molten selective top agar in said order. The criteria for a valid test were: 1)a sterility check on the S-9 mix must yield less than two viable cells per plate; 2) a sterility check on al levels of test substances at conclusion must yield less than two viable colonies per plate; 3) the positive controls must produce at least at 3-fold increase in the number of revertant colonies; and 4) the average number of revertant colonies in the negative controls must fall within the historical limit for each strain. |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Results** | |
| Result | There was no increase in the number of revertant colonies in any tester strain at any dose. |
| Cytotoxic concentration: | Negative with and without S-9 activation |
| Genotoxic effects: | Negative with and without S-9 activation |
| Statistical results: | Not stated |
| Remarks: | None |
| **Conclusions** | |
| Remarks: | The results of this Salmonella/mammalian-microsome mutagenicity assay indicate that this test substance did not cause a significant increase in the number of revertants per plate of any of the tester strains with or without metabolic activation by Aroclor induced rat liver microsomes.<br><br>The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |
| **Data Quality** | |
| Reliability (Klimisch): | 2C |
| Remarks: | Reliable with restrictions; comparable to guideline study; no confirmatory assay. |
| **Reference:** | Haworth, SR. 1981. Salmonella/Mammalian-Microsome Mutagenesis Assay (Ames Test). Report No. 003-468-677-1; for The Procter and Gamble Company, Cincinnati, OH, USA; from EG&G Mason Research Institute, Rockville, MD, USA. |
| **Other** | |
| Last changed: | September 23, 2003 |
| Remarks: | None |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Test Substance** | |
| Identity: | $(POE)_{20}$ Tallowamine (Varonic T-220)<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-, N-tallow alkyl derivs. |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Test for Chemical Induction of Mutation in Mammalian Cells in Culture, the L5178Y/$TK^{+/-}$ Mouse Lymphoma Assay, 9/15/1980; based on Clive, D. and Spector, J.F.S. 1975. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L517BY Mouse Lymphoma cells. Mutation Research 31:17-29.<br>Mouse lymphoma mutagenesis assay |
| Test type: | Yes |
| GLP: | 1980 |
| Year: | $TK^{+/-}$ L5178Y cells |
| Species/Strain: | With and without metabolic activation; Aroclor 1254- |
| Metabolic activation: | induced rat liver S-9 from Sprague-Dawley rats<br>0.33, 1.0, 3.3, 10, 33 and 100 µg per plate. |
| Concentrations tested: | Not stated |
| Statistical methods: | A preliminary toxicity test with and without S-9 activation indicated |
| Remarks: | that threshold levels of complete toxicity at 0.1 µl/ml of the test substance for non- activated cultures, and at about 10 µl/ml for the S-9 activated cultures. Based on these data, the test substance concentrations used in the mutagenesis assay ranged from 0.0013 to 0.µl/ml. The test substance, solubilized in ethanol, diluted to the prescribed test concentrations and added to tubes with and without the S-9 activation mix to yield a final cell suspension of $3x10^5$ cells/ml. Two additional tubes were prepared as solvent controls. Positive controls were treated with EMS (1.0 and 0.5 µl/ml) and 7,12-DMBA (7.5 and 5.0 µg/ml), each with and without duplicate solvent controls. After the initial 4-hour exposure to the test substance, the cells were washed, resuspended and incubated for two days with a cell population adjustments to maintain the $3x10^5$-cells/ml concentration for a continuous active growth state. After the 2-day expression period, cultures with and without activation (10 each) exhibiting 10 to 90% relative growth inhibition during the expression period were selected for cloning. The cultures were transferred to cloning medium for duplicate cloning, one with trifluoro thymidine (TFT)/ml as a selective agent and one for viable counts (V.C.). Cells from each culture were then plated in triplicate for both TFT and V.C. and were incubated at 37ºC in a humidified 5% $CO_2$ atmosphere for 10-12 days. Following incubation, both the TFT and V.C. plates were scored or the total number of colonies per plate and mutation frequency was calculated. |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Results** | |
| Result | None of the cloned cultures, treated in either the presence or absence of induced rat liver S-9, exhibited mutant frequencies which were significantly different from average mutant frequency for the corresponding solvent control cultures. The percent total growth ranged from 25 to 116% and 36 to 113% for the non-activated and S-9 activated cultures, respectively. |
| Cytotoxic concentration: | Negative with and without S-9 activation |
| Genotoxic effects: | Negative with and without S-9 activation |
| Statistical results: | Not stated |
| Remarks: | None |
| **Conclusions** | |
| Remarks: | This test substance was tested in the presence and absence of Aroclor induce rat liver S-9 in the L5178Y $TK^{+/-}$ Mutagenesis Assay, did not significantly increase the mutation frequency of treated cultures over that of the solvent control cultures. Under these test conditions, this test substance is considered negative in this mutagenicity assay.<br><br>The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |
| **Data Quality** | |
| Reliability (Klimisch): | 1B |
| Remarks: | Reliable without restrictions; comparable to guideline study. |
| **Reference:** | Kirby, PE. 1980. Test for Chemical Induction of Mutation in Mammalian Cells in Culture – the L5178Y $TK^{+/-}$ Mouse Lymphoma Assay. Report No. 003-692-420-7; for The Procter and Gamble Company, Cincinnati, OH, USA; from EG&G Mason Research Institute, Rockville, MD, USA. |
| **Other** | |
| Last changed: | September 23, 2003 |
| Remarks: | None |

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| **Test Substance** | |
| Identity: | $(POE)_{20}$ Tallowamine (Varonic T-220)<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-, N-tallow alkyl derivs. |
| Purity: | 100% |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Not stated |
| Test type: | Cytogenicity Study – Chinese Hamster Ovary (CHO) Cells *in vitro*. |
| GLP: | Yes |
| Year: | 1982 |
| Species/Strain: | Chinese hamster ovary (CHO) cells |
| Metabolic activation: | With and without metabolic activation; Aroclor 1254-induced rat liver S-9 from Sprague-Dawley rats |
| Concentrations tested: | 0.005, 0.007, 0.01, 0.013, 0.017, 0.023, 0.03 µl/ml (absence of S-9)<br>0.05, 0.07, 0.1, 0.13, 0.17, 0.23, 0.3 µl/ml (presence of S-9) |
| Statistical methods: | Chi-Square analysis using a 2x2 contingency table |
| Remarks | Approximately 7.4 x $10^6$ CHO cells/ flask were seeded for the assay and were incubated in a humidified atmosphere of 5 – 0.5% $CO_2$ in air for approximately 24 hours. The cells were harvested and resuspended to a final cell density of $5x10^6$ cells/ml. Based upon results of the initial cytotoxicity test, cultures in the chromosome aberrations assay were dosed with one of seven decreasing dose levels from 0.03 µl/ml in the non-activated system and from 0.3 µl/ml in the S-9 activated systems, respectively. TEM was used as the positive control in the non-activation study at a concentration of 0.5 µg/ml. CP was used as the positive control in the S-9 activated study at a concentration of 35 µg/ml. The solvent vehicle, ethanol, was used as the solvent control at the same concentration as that found in the test substance-treated groups Cultures were exposed to treatment for 4 hours in a 37ºC water bath, were washed and resuspended, incubated again for 16 hours at 37 – 1ºC in a humidified atmosphere of 5 – 0.5% $CO_2$ in air, treated with colcemid (1 µg /ml), and incubated for an additional 2 hours. The metaphase cultures were then harvested and cytotoxicity was estimated. Slides were prepared from fixed cells and scored. Fifty metaphase spreads were scored for each dose level. The cells that appeared intact with chromosomes spread symmetrically were used to obtain the final count. The following aberrations were scored at three dose levels with and without activation: number of metaphase chromosomes, gaps, chromatid breaks and fragments, chromosome breaks, exchange figures, dicentria, rings, polyploids, pulverization and severely damaged cells (>10 aberrations). |
| **Results** | |
| Cytotoxic concentration: | >0.01 µl/ml with S-9 activation<br>>0.03 µl/ml without S-9 activation |
| Genotoxic effects: | Negative without S-9 activation<br>Positive with metabolic activation |
| Statistical results: | Described below |

**Table 12. Genotoxicity *In vitro* (Contd.)**

Remarks: The following data for chromosome aberrations were collected:

Without Metabolic Activation (50 cells/analysis)

| Treatment | # of Aberrations/ Cell | # Cells with Aberrations | % of Cells with >1 Aberration |
|---|---|---|---|
| TA (0.01)* | 0.26 | 18 | 4 |
| TA (0.007) | 0.22 | 14 | 6 |
| TA (0.005) | 0.30 | 24 | 6 |
| Neg. Control | 0.16 | 10 | 6 |
| Pos. Control | 1.94 | 68 | 48 |
| Solvent Control | 0.16 | 12 | 2 |

* Test Article (µl/ml)

With Metabolic Activation (50 cells/analysis)

| Treatment | # of Aberrations/ Cell | # Cells with Aberrations | % of Cells with >1 Aberration |
|---|---|---|---|
| TA (0.17)* | 1.98 | 58 | 36 |
| TA (0.13) | 0.92 | 36 | 22 |
| TA (0.10) | 0.64 | 28 | 18 |
| Neg. Control | 0.1 | 12 | 6 |
| Pos. Control | 3.5 | 80 | 68 |
| Solvent Control | 0.14 | 12 | 2 |

* Test Article (µl/ml)

The original author stated the following: The cytotoxicity test conducted with the chromosome aberration assay did not yield the expected 50-90% toxicity at any of the dose levels without activation due to the narrow toxic range of this test substance. The cells treated with the test substance showed a significant increase in the frequency of chromosome aberrations with and without activation, relative to the negative control, although a definite dose response was only observed in the activated system.

**Conclusions**

Remarks: The original author concluded the following: "Under the conditions of the test, the test cultures which were treated with and without induced rat liver S-9 exhibited chromosome aberrations which were significantly higher than the frequency of aberrations in the negative control."

The Sponsor of the Study concluded: "I do not agree with the conclusions drawn by the Study Director. [The test substance] clearly is positive in the presence of metabolic activation. The three doses scored show a distinct dose-related increase in the number of chromosome aberrations. In the absence of

metabolic activation, an elevation in chromosome aberration occurs relative to the negative control, but there is no dose-response. Therefore, the results should be considered negative in the absence of metabolic activity."

**Table 12. Genotoxicity *In vitro* (Contd.)**

| | |
|---|---|
| | The test substance was positive with metabolic activation only. The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |
| **Data Quality** | |
| Reliability (Klimisch): | 1B |
| Remarks: | Reliable without restrictions; comparable to guideline study. |
| **Reference:** | Thiagar, A. 1982. Cytogenicity Study – Chinese Hamster Ovary (CHO) Cells *In vitro*. Study No. T1807.338; for The Procter & Gamble Company, Cincinnati, OH, USA; from Microbiological Associates Inc., Bethesda, MD, USA.. |
| **Other** | |
| Last changed: | September 23, 2003 |
| Remarks: | None |

**Table 12. Genotoxicity *In vitro* (Contd.)**

**Test Substance**

| | |
|---|---|
| Identity: | $(POE)_{20}$ Tallowamine (Varonic T-220)<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-, N-tallow alkyl derivs. |
| Purity: | Not stated |
| Remarks: | None |

**Method**

| | |
|---|---|
| Method/guideline followed: | Test for Chemical Induction of Unscheduled DNA Synthesis in Primary Cultures of Rat Hepatocytes (by autoradiography), 5/1/1981; based on Williams, G.M. 1977.<br><br>Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Research* 37:1845-1851; Williams, G.M. 1978. |
| Test type: | Unscheduled DNA synthesis |
| System of testing | Hepatocyte primary cell culture |
| GLP: | Yes |
| Year: | 1982 |
| Species/Strain: | Sprague-Dawley rat |
| Metabolic activation: | Not applicable |
| Concentrations tested: | $0.008x10^{-4}$ to $0.23x10^{-4}$ µl/ml (10 concentrations) |
| Statistical methods: | Not stated |
| Remarks: | Complete cytotoxicity (0% relative viability) in a preliminary toxicity and dose-range finding assay was observed at all but the lowest dose tested, $1.0x10^{-4}$ µl/ml. Ten test substance concentrations ranging from $0.035x10^{-4}$ to $1.0x10^{-4}$ µl/ml were chosen for use in the first UDS assay. Due to excessive toxicity (only the two lowest concentrations, $0.035x10^{-4}$ µl/ml and $0.051x10^{-4}$ µl/ml did not exceed acceptable toxicity) and higher than normal grain counts in the controls, this test was considered invalid. A second study with doses ranging from $0.008x10^{-4}$ to $0.23x10^{-4}$ µl/ml was therefore conducted and was considered valid. The test substance was dissolved in absolute ethanol. The positive control, 7,12- dimethylbenzanthracene (DMBA) was dissolved in dimethyl sulfoxide (DMSO). Only the ethanol was included as a solvent control in the UDS assay, as DMSO was known to not induce UDS at the levels used in this study. Primary cultures for the second of two UDS assays were prepared from $3.7x10^{8}$ cells from the liver of a male Sprague-Dawley rat, which were estimated to be 92% viable by exclusion of tryptophan blue. Following a 1.5-2 hour period allowing for culture attachment to each coverslip, cultures were exposed to both test substance, or control, and 10 µCi/ml $^{3}$H-thymidine for 18-20 hours at 37ºC under an atmosphere of 5% $CO_2$ in air. Cultures were then scored for toxicity or processed for autoradiography, viability was estimated again by exclusion of tryptophan blue and $^{3}$H-thymidine incorporation was quantified in 25 randomly selected but normal appearing cells from at least two coverslips per dose group (total of 50 cells/group). |

**Table 12. Genotoxicity *In vitro* (Contd.)**

**Results**

| | |
|---|---|
| Result: | This test substance was tested twice for the induction of unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes, as results of the first UDS assay were deemed equivocal. In the first UDS assay, the mean net nuclear grain counts of treated samples were elevated with respect to the negative control. However, the standard deviations of the means were very large. Moreover, there were morphological signs of cytotoxicity throughout the dose range and the net nuclear grain count of the solvent control was above the normal cutoff point for an acceptable assay.<br><br>The second UDS assay was considered to be a valid test, as the standard deviations of mean net nuclear grain counts were reduced, significant cytotoxicity was observed in the five highest dose levels in the wider dose range employed, and the net nuclear grain counts of the solvent and positive controls were in the acceptable range. The DMBA positive control did induce a response which indicates that the cells were capable of DNA repair.<br>$0.052x10^{-4}$ µl/ml<br>None observed |
| Cytotoxic concentration: | Not stated |
| Genotoxic effects: | None |
| Statistical results: | |
| Remarks: | Based on the results of the second assay, this test substance did not induce unscheduled DNA synthesis in freshly prepared primary cultures of rat hepatocytes under the conditions employed in this assay. |

**Conclusions**

| | |
|---|---|
| Remarks: | The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group) |

**Data Quality**

| | |
|---|---|
| Reliability (Klimisch): | 1B |
| Remarks: | Reliable without restrictions; comparable to guideline study. |

**Reference:** Coppinger, WJ. 1983. Unscheduled DNA Synthesis Assay in Primary Cultures of Rat Hepatocytes. Report No. M0021, The Procter & Gamble Company, BTF – Miami Valley Laboratories, Cincinnati, OH, USA.

**Other**

| | |
|---|---|
| Last changed: | September 24, 2003 |
| Remarks: | None |

| **Table 13. Genotoxicity *In vivo*[39]** | **Tallow bis(2-hydroxyethyl)amine (C16-C18) (CAS No. 61791-44-4) (x+y=2)** |
|---|---|
| **Test Substance** | |
| Identity: | Tallow Amine Ethoxylate<br>15% TAMET solution with 5% H3PO4 in water<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs. |
| Purity: | 15% |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | OECD Guideline 474; EEC Directive 79/831 (Annex V, Part B) |
| Test type: | Micronucleus assay |
| GLP: | Yes |
| Year: | 1981 |
| Species/Strain: | Mouse |
| Strain: | CD-1 |
| Sex: | Male and female |
| Route of administration: | Oral gavage |
| Dose/concentrations tested: | Single dose/10,860 mg/kg (concentration=543 mg/ml) |
| Exposure duration: | Single administration |
| Statistical methods: | Mann-Whitney |
| Remarks: | Groups of 30 mice (15 of each sex) were administered a single dose of the test substance by oral gavage. Based upon results of a preliminary toxicity study, a dosage of 10860 mg/kg body weight was chosen for this micronucleus test. Two additional groups of mice (15 of each sex/group) were used as the negative control and positive control. The negative control group received sterile distilled water by gavage. The positive control, mitomycin C, was injected IP as a 0.2 mg/ml solution in 0.9% saline. The animals were examined regularly for mortality or clinical signs of reaction to the test substance following dosing. Five males and five females from each group were sacrificed 24, 48 and 72 hours after dosing. One bone marrow smear was prepared per animal from the tissue cleared from each femur. Stained smears were examined by light microscopy for incidence of micronucleated cells per 1000 polychromatic erythrocytes per animal and the ratio of polychromatic to normochromatic erythrocytes was assessed by the examination of at least 1000 erythrocytes. |
| **Results** | |
| Genotoxic effects: | Negative |
| NOAEL (NOEL): | Not determined |
| Statistical results: | Described below |
| Remarks<br>: | One male animal died approximately 30 hours after treatment. Clinical signs reported during the 72 observation period included slight pallor to the extremities and diarrhea, slight to moderate piloerection, lethargy, decreased respiratory rate and ptosis, walking on toes, and greasy fur. Animals showed no reaction to the vehicle control and positive, mitomycin C, control treatments.<br><br>Increases in the number of micronucleated polychromatic erythrocytes at the 48- or 72-hour kills were significant; however, a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was obtained at the 24- hour kill. These increases were |

**Table 13. Genotoxicity *In vivo* (Contd.)**

concluded to be unrelated to treatment, as both the individual and group results fell well within the historical negative control range. Significant decreases were observed in the ratio of polychromatic to normochromatic erythrocytes at all three kill times, suggesting treatment-related bone marrow cell toxicity.

The positive control compound, mitomycin C, produced significantly increased frequencies of micronucleated polychromatic and normochromatic erythrocytes, and decreased ratios of polychromatic to normochromatic erythrocytes.

*Mean number of micronucleated polychromatic cells/1000 cells (vehicle control, mitomycin C control)*
At 24 hours: 1.6 (0.6; 69.2)
At 48 hours: 1.7 (0.9; 62.8)
At 72 hours: 0.2 (0.9; to few erythrocytes to count)

*Mean number of micronucleated polychromatic cells/1000 cells (vehicle control, mitomycin C control)*
At 24 hours: 1.0 (0.9; 2.1)
At 48 hours: 1.6 (1.0; 4.2)
At 72 hours: 0.9 (0.8; 4.5)

**Conclusions**

Remarks: The <1% mortality and increased incidence of micronucleated polychromatic erythrocytes at 24 hours were concluded to be unrelated to treatment. However, it was also concluded that this test substance resulted in bone marrow cell toxicity, as evidenced by the significantly decreased ratios of micronucleated polychromatic to normochromatic erythrocytes.

The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group)

**Data Quality**

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

**Reference:** Allen, JA, Proudlock, RJ, McCaffrey, K. 1984. Micronucleus Test on E-2352.01 (ECM BTS 902/01) Tamet. Unpublished Report No. P+G 1114/84560; for Procter and Gamble N.V., Stroombeek-Bever, Belgium; from Huntingdon Research Centre plc, Huntingdon, England.

**Other**

Last changed: September 24, 2003
Remarks: None

**Table 13. Genotoxicity *In vivo* (Contd.)**

| | |
|---|---|
| **Test Substance** | |
| Identity: | $(POE)_{20}$ Tallowamine (Varonic T-220)<br>CAS RN 61791-44-4<br>Ethanol, 2,2'-iminobis-,N-tallow alkyl derivs.) |
| Purity: | Not stated |
| Remarks: | None |
| **Method** | |
| Method/guideline followed: | Not stated |
| Test type: | Cytogenicity study – Rat bone marrow *in vivo* |
| GLP: | Yes |
| Year: | 1982 |
| Species/Strain: | Rat |
| Strain: | Sprague-Dawley |
| Sex: | Male and female |
| Route of administration: | Oral gavage |
| Dose/concentrations tested: | 39, 130, 390 g/kg/day |
| Exposure period: | Daily for 5 consecutive days |
| Statistical methods: | Not stated |
| Remarks: | Groups of rats (five of each sex), weighing 150 to 200 g, were administered the test substance in water by oral gavage at one of three dose levels, 39, 130 or 390 mg/kg body weight. Two additional groups of rats (five of each sex) were treated in the negative control and positive control groups. The negative groups received distilled water by gavage . The positive control, methylmethane sulfonate (MMS), was administered by gavage at a concentration of 80 mg/kg/day. Animals were examined twice daily during the 5-day treatment period for mortality, moribund or signs of adverse reaction to treatment.<br><br>An intraperitoneal injection of colchicine (1mg/kg) was given to inhibit mitosis in each animal approximately 20 hours after the last treatment and animals were sacrifice 2-4 hours later. Following sacrifice, the bone marrow of both femurs of each animal was prepared for chromosomal analysis. Approximately 50 metaphase spreads were analyzed per animal. Cytogenetic abnormalities such as deletions, exchanges, rings, gaps and breaks were scored and the mitotic index on each animal was determined |
| **Results** | |
| Genotoxic effects: | Negative |
| NOAEL (NOEL): | Not determined |
| Statistical results: | Described below |
| Remarks: | All animals in the high dose group, 390 mg/kg/day, developed diarrhea, and only 2 females in the lower dose groups displayed similar signs. Some of the treated animals developed red-brownish exudates around the eyes and mount, but these signs were not considered treated related. Pale brown feces was observed in some of the animals in the positive control, MMS, group. |

**Table 13. Genotoxicity *In vivo* (Contd.)**

The following Total Aberrations (including gaps) were recorded:

| Group | Treatment | Males | Females |
|---|---|---|---|
| Control | Water | 0.4 | 1.6 |
| + Control | MMS | 18.0 | 18.0 |
| TS | 390 g/kg | 0.4 | 1.6 |
| TS | 130 g/kg | 0 | 0.4 |
| TS | 39 g/kg | 0 | 0.4 |

TS = Test Substance

It was concluded that a significant number of chromosomal aberrations were not induced by this test substance.

**Conclusions**
Remarks:

Based on the results of this cytogenicity study, this test substance has no mutagenic potential.

The endpoint has been adequately characterized. (American Chemistry Council, Fatty Nitrogen Derivatives Panel, Amines Task Group).

**Data Quality**
Reliability (Klimisch):
Remarks:

1B
Reliable without restriction; comparable to guideline study.

**Reference:**

Esher, HJ. 1982. *In vivo* Cytogenetics Study in Rats. Unpublished Report No. MRI-182-PG-82-58; for The Procter and Gamble Company, Cincinnati, OH, USA; from EG&G/Mason Research Institute, Worcester, MA, USA.

**Other**
Last changed:
Remarks:

September 24, 2003
None

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