



A Structure Activity Relationship (SAR) Based Case Study for a Cosmetic Ingredient



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Presentation Outline

- Background to P&G Structure Activity Relationship (SAR) approach
- PEG-Cocamine Case Study



Definitions

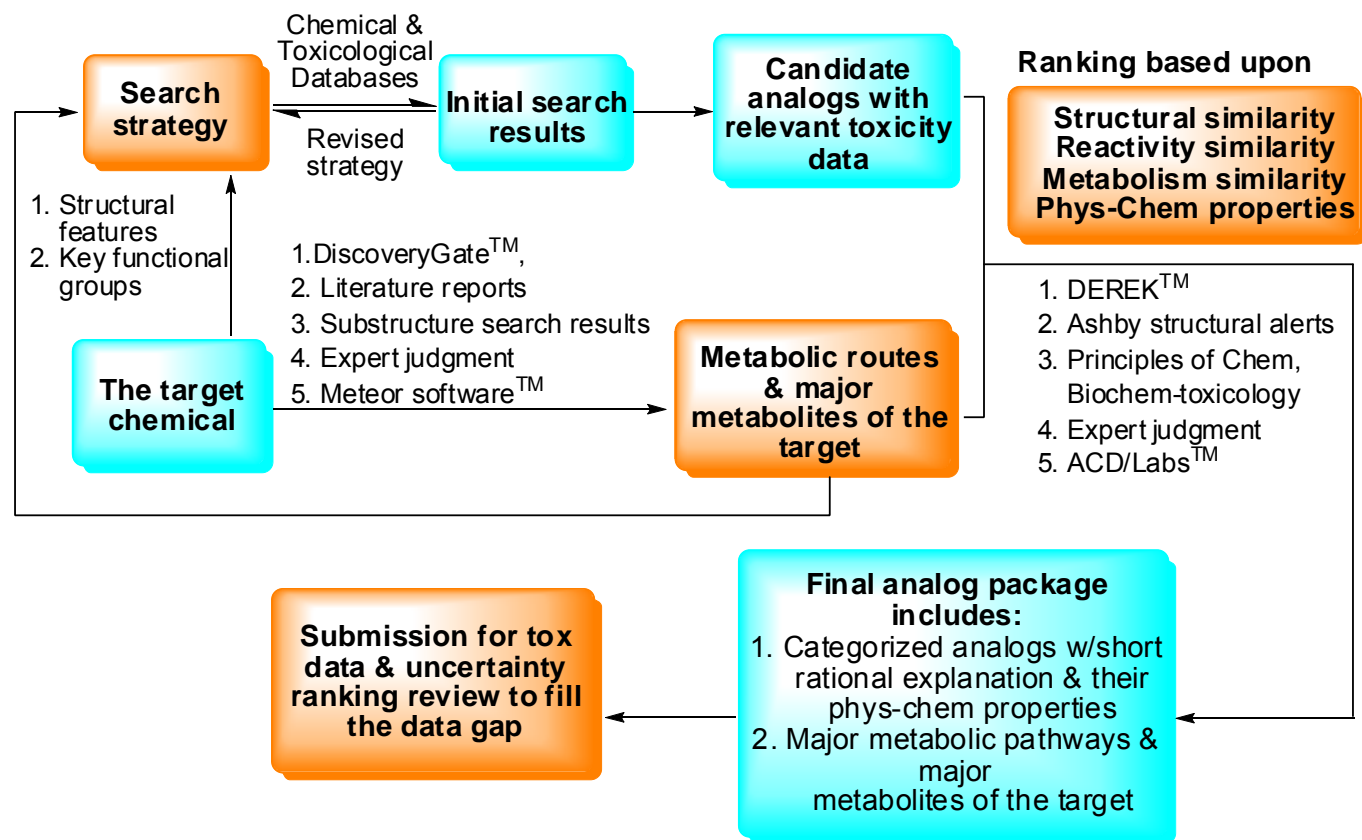
SAR versus QSAR

SAR: qualitative, involves expert judgment of structural features, assessment of factors that affect ADME and consideration of other supportive information (chemical and biological activity) as a basis for prediction

QSAR: quantitative methods which involve development of mathematical models that relate the biological activity of molecules to their chemical structures and corresponding physicochemical properties and other molecular properties



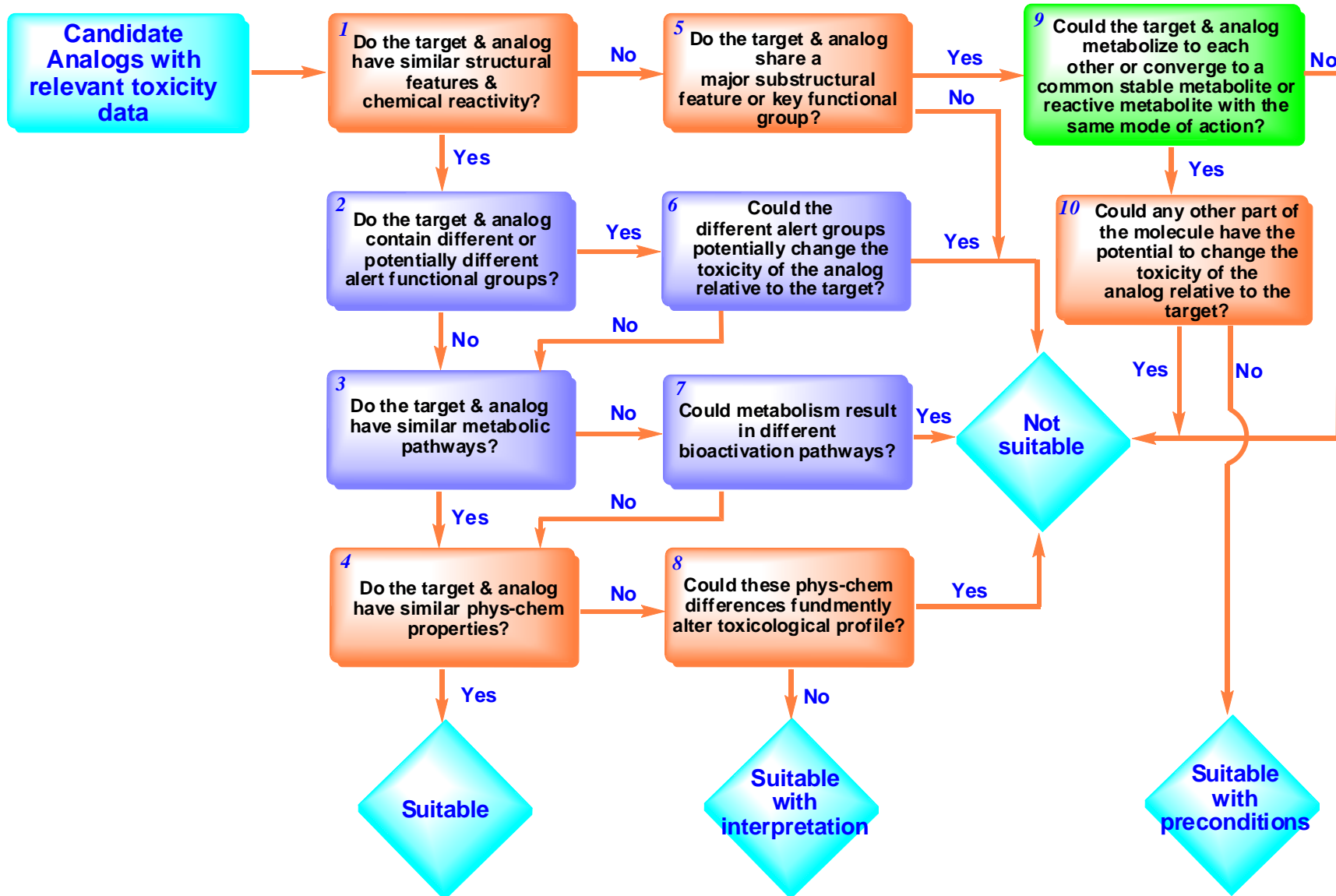
Basis for P&G SAR Approach



Wu, S. et al. 2010. A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. *Regulatory Toxicology and Pharmacology* 56, 67-81



Decision Tree for Categorizing Analogs



Evaluation of Candidate Analogs

- Chemistry evaluation
- Metabolism evaluation
- Toxicity data review and uncertainty ranking

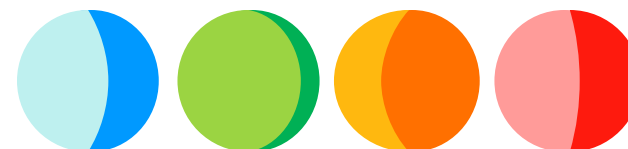


Evaluation Steps

- **Step 1 Chemistry Evaluation:**

Evaluating the following similarity areas:

- 1) Structural alerts (e.g. DEREK™ alerts, Ashby structural alerts).
- 2) Key functional groups (Ester, Aldehyde, Amide, Amine etc.) & Core sub-structures (e.g. phenyl ring, alkyl chain, etc.).
- 3) Position of double bonds (particularly, conjugation or relative to functional groups).
- 4) Effects of additional functional group (e.g. multi-functional analogs).
- 5) Phys-Chem properties (e.g. MW, pKa, log P, log D and Solubility).



Evaluation Steps

- **Step 2 Metabolism Evaluation:**

Evaluating following aspects related to metabolism:

- 1) The potential for the analog to metabolize to the target or a highly related compound (vice versa)
- 2) The potential for the metabolism of the analog and the target to converge on a common metabolite or reactive metabolite with the same mode of action.
- 3) The number of steps before metabolic convergence and the potential formation of other metabolites along the way that could change the toxicological profile of the analog or target.



Evaluation of Candidate Analogs

- The evaluation of metabolism is initially based upon expert judgment combined with a literature search to locate metabolic data on the SOI, analogs and/or closely related chemicals.
 - The DiscoveryGate™ database is the primary tool we use for locating literature on metabolism (supports structure and substructure searching)
- METEOR™ is used to evaluate possible routes of metabolism in the absence of literature reports.



Toxicity data review and uncertainty ranking

High Uncertainty Read Across Not Recommended	Moderate Uncertainty Read Across may be possible for some endpoints- larger MOE required	Low Uncertainty Read Across does not require a larger MOE
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Uncertainty Rankings

- Number of Analogs and their suitability ratings
- Biological concordance across analogs
- Corroborating metabolism data
- Corroborating data on structure of interest for one or more endpoints
- Mode of action for analogs
- Quality of the study data



Case Studies

Blackburn, K et. al. 2011. Case studies to test: A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. *Reg. Tox. Pharm.* 60: 120-135.



Published Case Studies

- 14 blinded case studies predicting
 - Genetic Toxicity +/- (all correct predictions)
 - Repeat Dose Toxicity (surrogate NOAEL estimate) (no under estimates)
 - Developmental Toxicity (critical effect +/-, if yes surrogate NOAEL) (no under estimates)
 - Reproductive Toxicity (critical effect +/-, if yes surrogate NOAEL) (no under estimates)

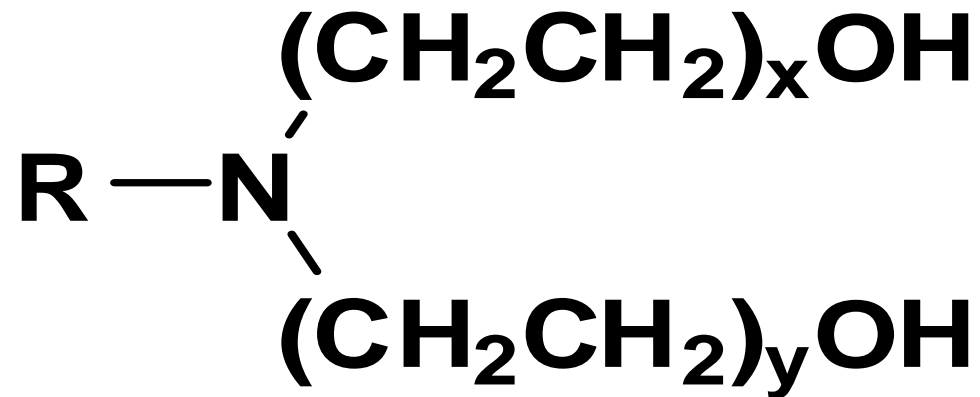


Case Study Conclusions

- The process that we have developed for read across can be successfully applied to develop surrogate values for risk assessment
- Successful application of the approach requires significant expertise as well as discipline to not over step the boundaries of the defined analogs and the rating system.
- The end result of this rigor may be the inability to read across all endpoints for all chemicals resulting in data gaps that cannot be filled using read across.
- The read across results supported by the process were shown for these blinded case study chemicals to be protective when compared to the bon fide toxicity data on the case study chemicals.



PEG-Cocamines Case Study



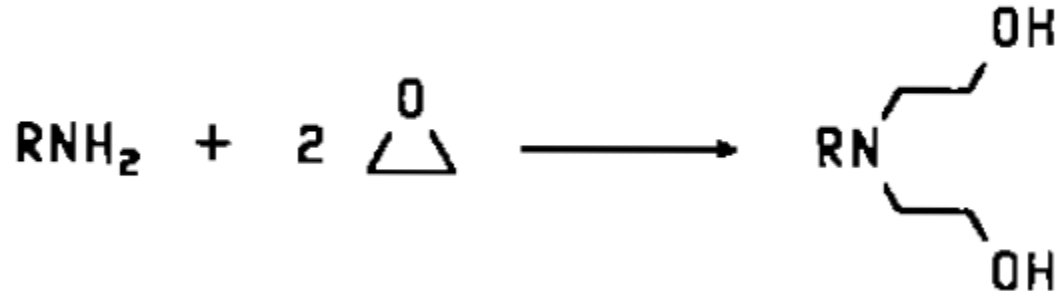
R represents the alkyl groups derived from the fatty acids of coconut oil

Polyethylene glycol groups where $x+y$ has an average value equal to the number in the chemical name



Ethoxylated Fatty Amines Synthesis

Step 1

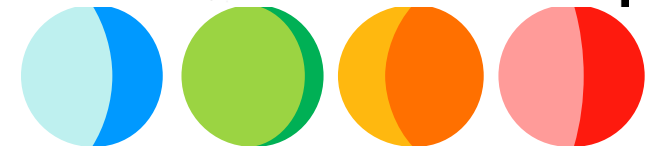
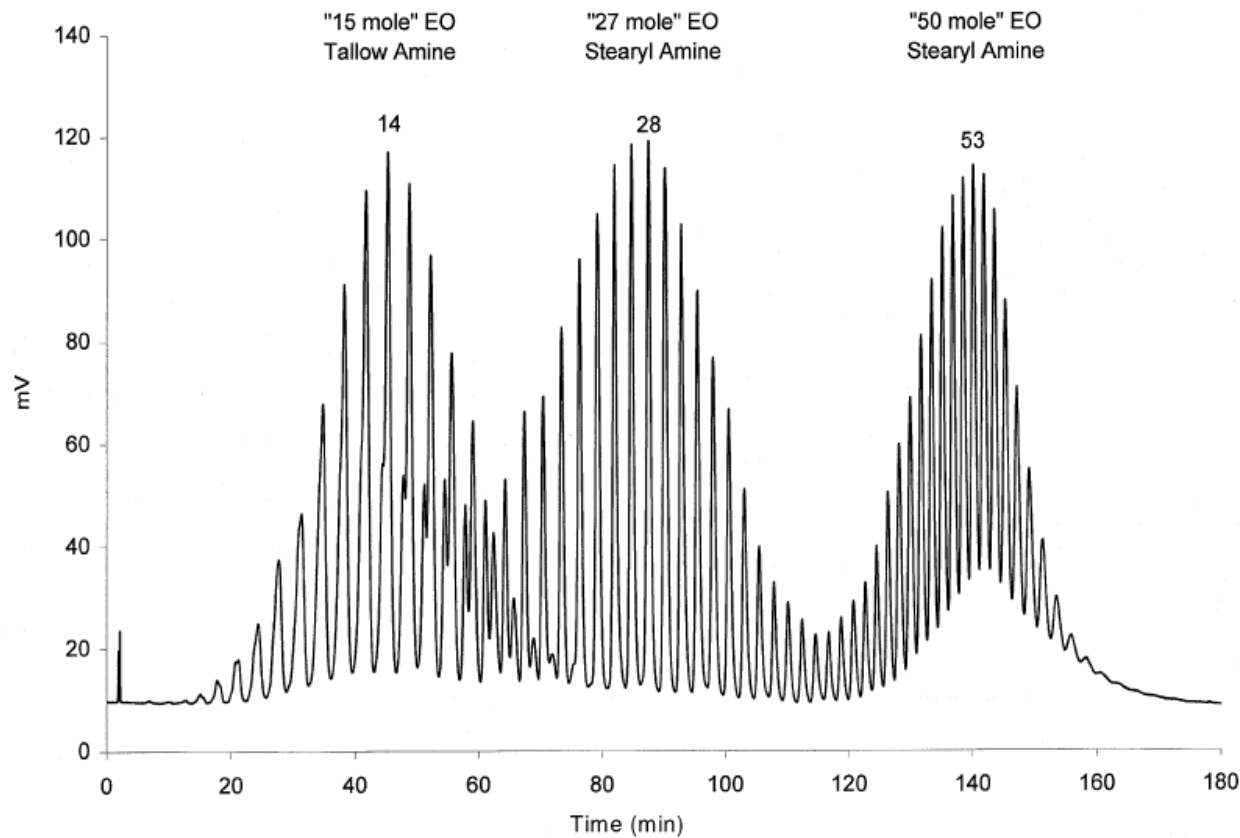


This reaction proceeds until all of the primary and secondary amine is consumed-result is that both X and Y will always be greater than 0

Step 2 requires catalyst, chain length related to reaction time and the two chains may not be symmetrical



All of the Commercially Produced Ethoxylated Amines Are Mixtures Of Oligomers



Ethoxylated Fatty Amines Are Produced From Coco, Lauryl, Tallow, Oleyl, And Stearyl Amines

Chain Length	Un-saturation	Coco %	Tallow %
C6		0-1	
C8		5-9	
C10		5-10	
C12 (Lauryl)			1
C14		13-19	3
C14	1		1
C15			0.5
C16		8-11	29
C17			1
C18 (Stearyl)		1-3	23
C16	1	0-1	3
C18 (Oleyl)	1	5-8	37
C18	2	1-3	1.5

Segmenting the PEG-Cocamines

- There are sufficient differences in potential reactivity, metabolism and phys/chem across the group that our methods did not allow all members of the group to be considered together initially.
- The group was segmented into discrete sub-groups, each with their own spectrum of analogs, for the initial assessment.



Segmenting the PEG-Cocamines: Medicinal Chemist Guidance

- Initial grouping primarily based on the ethylene glycol chains (rather than the fatty amine) because of potential impact on reactivity, metabolism and physicochemical properties (impact of amine chain length is not ignored, but secondary).
- No evidence of metabolism for ethylene glycol chain >8 units.



Segmenting the PEG-Cocamines

- In the following slides the PEG-Cocamine members are shown in red.
- As we progress through the sub-groups we will see that there is overlap between adjacent groups in terms of analogs and whether or not one PEG-cocamine is considered an appropriate analog for other PEG-cocamines.

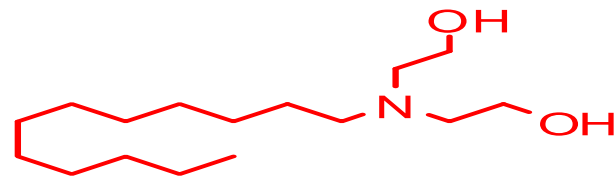


Segmenting PEG-Cocamines

- Keep in mind that the majority of the structures are representative structures that in reality represent a distribution of chain lengths.



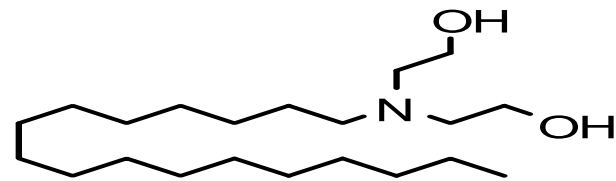
Segmenting the PEG-Cocamines-Group 1



PEG-2 Cocamine
C8-C16

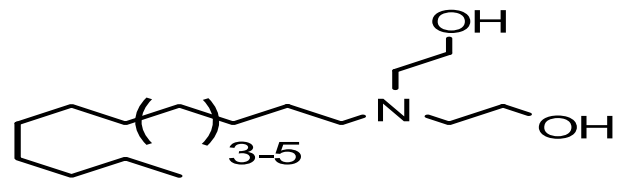
$$x+y=2$$

CAS# 61791-31-9 (SOI)



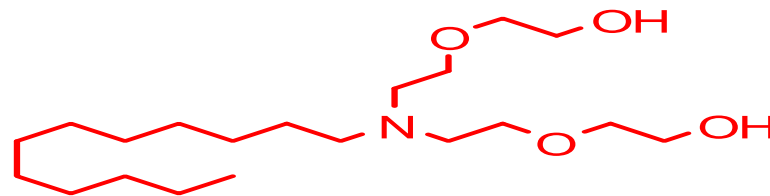
Tallow bis(2-hydroxyethyl)amine
C16-C18

CAS# 61791-44-4
(Sui table)



amines, C13-15-alkyl, ethoxylated

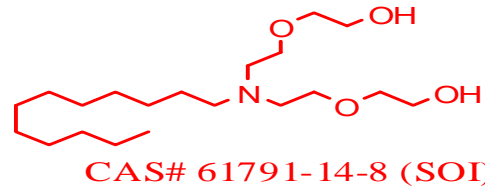
CAS# 70955-14-5
(Sui table)



PEG-4 cocamine

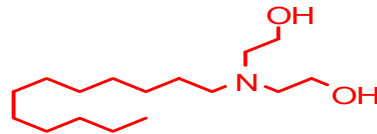
CAS# 61791-14-8 (suitable with interpretation)

Segmenting the PEG-Cocamines-Group 2

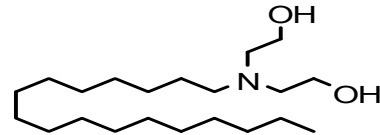


PEG-4 Cocamine

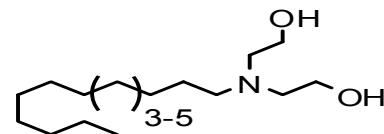
$$x+y=4$$



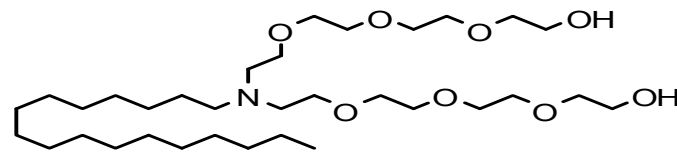
PEG-2 Cocamine



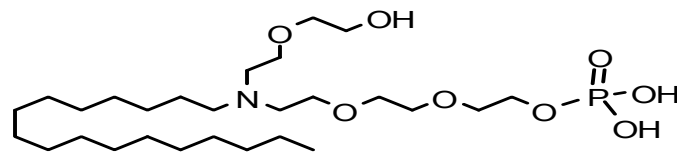
Tallow bis(2-hydroxyethyl)amine



amines, C13-15-alkyl, ethoxylated

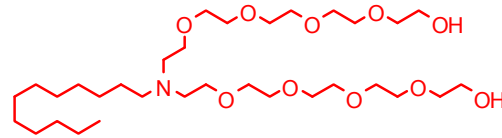


PEG-8 hydrogenated
Tallow amine



Tallow amine,
phosphate ester

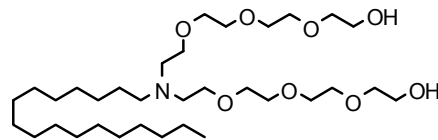
Segmenting the PEG-Cocamines-Group 3



PEG-10 Cocamine

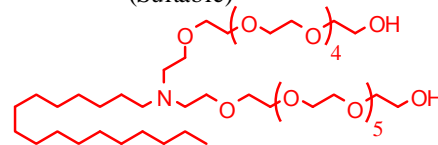
$x+y=10$

CAS# 56049-72-0
(SOI)



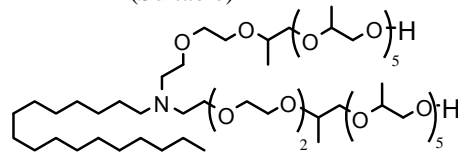
PEG-8 Hydrogenated
Tallow amine

CAS# 26635-92-7
(Suitable)



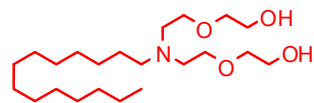
PEG-15 Tallowamine

CAS# 61791-26-2
(Suitable)



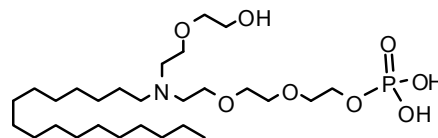
POE-5/POP-12 Tallowamine

CAS# 68213-26-3
(Suitable with interpretation)



PEG-4 cocamine

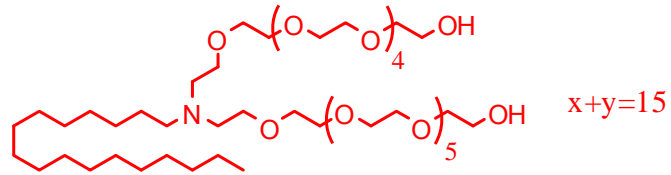
CAS# 61791-14-8
(Suitable with interpretation)



Tallow amine, phosphate ester

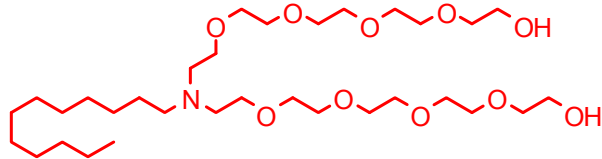
CAS# 68308-48-5 Representative structure for analog rating
(Suitable with precondition)

Segmenting the PEG-Cocamines-Group 4



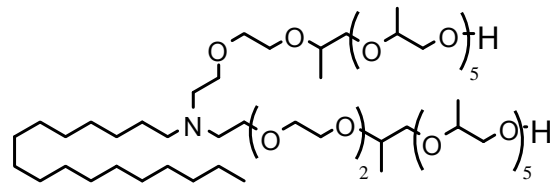
PEG-15 cocamine

CAS# 61791-26-2
(SOI)



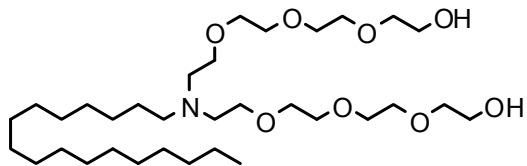
PEG-10 Cocamine

CAS# 56049-72-0
(Suitable)



POE-5/POP-12 Tallowamine

CAS# 68213-26-3
(Suitable with interpretation)



PEG-8 Hydrogenated Tallow amine

CAS# 26635-92-7
(Suitable with interpretation)

DEREK Alerts

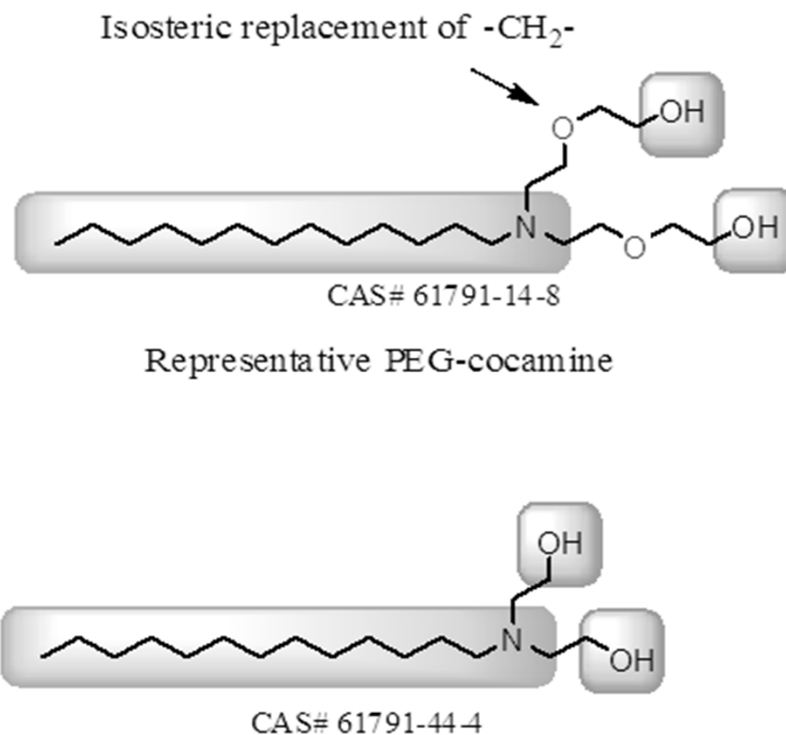
- Ethoxylated group members: 1,2-Ethyleneglycol or derivative. Nephrotoxicity.
- If the ethoxyl chains are metabolized as the PEGs are to yield acid metabolites it is reasonable that these chemicals could show high dose nephrotoxicity.
- Comparing the tested doses of the current alkyl amine ethoxylates to the dataset on the PEGs suggests that the GI irritancy of the alkyl amine ethoxylates precludes testing at doses that could be reasonably anticipated to produce nephrotoxicity.

Phys/Chem Properties

Across the class of PEG-cocamines and their analogs there are significant differences in physico/chemical properties that could impact bioavailability specifically with longer carbon amine chains and longer polyethoxy chains molecular weight increases which may decrease bioavailability. In addition, greater ethoxylation will increase polarity which may offset impact of increasing molecular weight. These factors can impact both bioavailability and excretion. These differences are best evaluated in the context of the toxicology data set.



Comparative Reactivity



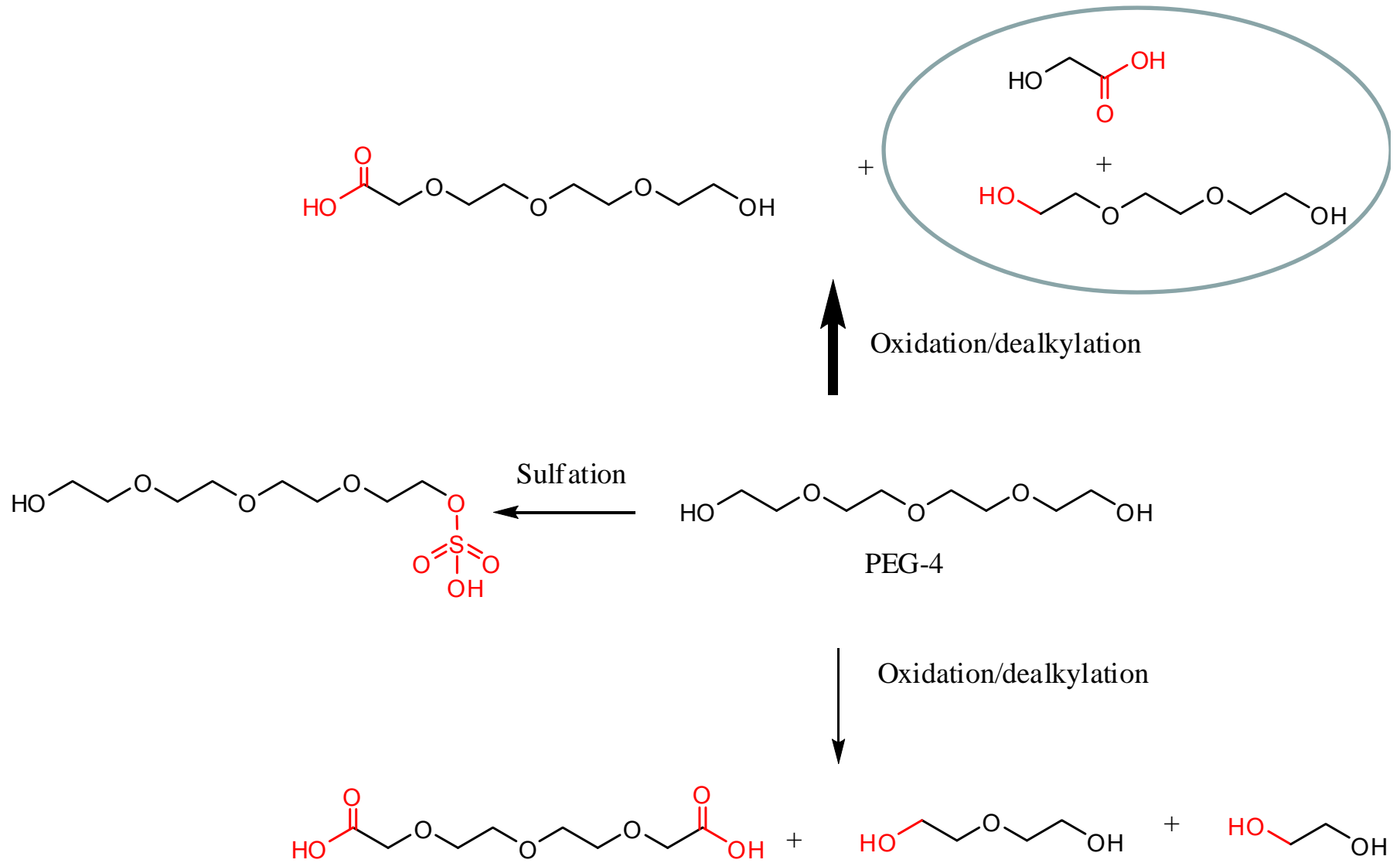
Ether linkage is an isosteric replacement of $-\text{CH}_2-$ linkage. Isosteric substituents have very similar molecular shape and volume, approximately the same distribution of electrons, and hence would not change reactivity

O-Dealkylation Producing Ethylene Glycol:

By analogy to the PEGs this is not anticipated to be a major route of metabolism because:

- PEGs are excreted mainly unchanged in the urine.
- Only very small amounts of oxalic acid are formed after the metabolism of the smaller PEGs (ethylene glycol chain ≤ 8 units), the current evidence supports that oxalic acid is formed as a result of acid metabolites of alcohol oxidation and not primarily via ethylene glycol
- Ethylene glycol has not been shown to be formed as a metabolite of PEGs

PEG-Metabolism



Comparative Reactivity of PEG-Cocamines and Analogs

Tallow vs Coconut

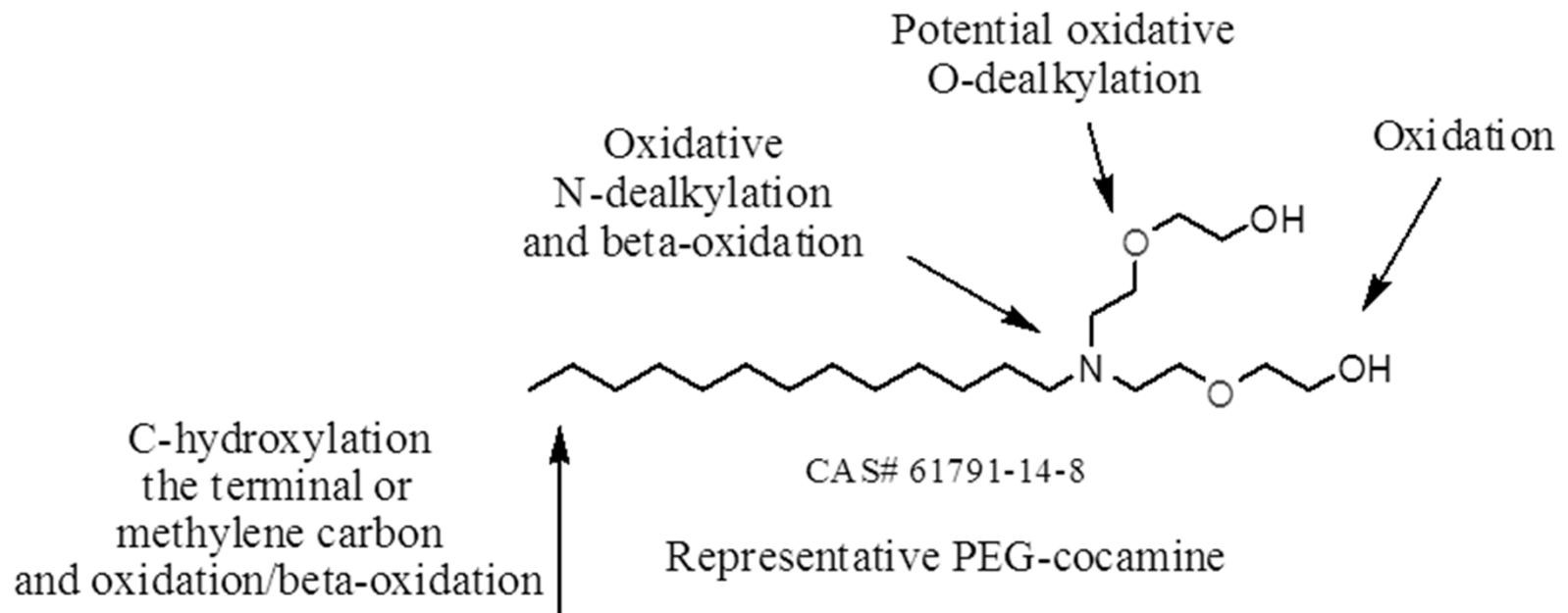
- Chain length mix skews longer with tallow
- Degree of unsaturation $>$ in tallow (hydrogenated tallow has least). Unsaturated fatty acids may form epoxides when metabolized

Metabolism

- Lack of direct data on metabolism of alkyl amine ethoxylates
- Metabolism of fatty amines and PEGs are well understood-these pathways are considered relevant to prediction of metabolism of alkyl amine ethoxylates



Metabolism



Metabolism

Potential Differences Across Analogs

- Hydroxyethyl- groups rather than polyethoxy-groups. PEG-2 cocaine and the analogs with EO=2 lack polyethoxy-groups and therefore O-dealkylation (scission of the ether linkage) cannot occur.
- Predicted little to no metabolism of polyethoxy chain lengths >8 units.
- Tallow-epoxide formation

Toxicology Data For Analogs

- In addition to data from the previous PCPC submission additional data are cited from:
 - US EPA. 2009. Alkyl Amine Polyethoxylates. Human Health Risk Assessment to Support Proposed Exemption from Requirement of a Tolerance When Used as Inert Ingredients in Pesticide Formulations
 - US EPA. 2010. Phosphate Ester, Tallowamine Ethoxylated. Human Health Risk Assessment to Support Proposed Exemption from Requirement of a Tolerance When Used as Inert Ingredients in Pesticide Formulations

Toxicology Data for Analogs

- To facilitate easier visualization of data across analogs for purposes of presentation:
 - only rodent data are shown
 - NOELs are shown avoiding discussion of adverse versus non-adverse for the purposes of the presentation and **not** implying the presented values are the appropriate ones for risk assessment.



Chemical	R	X+Y	Genetox	Rodent Repeat Dose	DART
amines, alkyl-ethoxylated 70955-14-5	13-16	2		NOEL 15 mg/kg (90 day)	
PEG-2 cocamine 61791-31-9	8-16	2			NOEL 23 mg/kg
bis(2-hydroxyethyl)tallow amine 61791-44-4	16-18	2	Ames – Mouse lymphoma – In vivo MN-	NOEL 50 mg/kg NOEL12 mg/kg (90 day)	
Tallow amine, phosphate ester 68308-48-5	16-18	5			NOEL 100 mg/kg
PEG-8 hydrogenated tallow amine 26635-92-7	16-18	8	Ames -		
PEG-15 Tallowamine 61791-26-2	16-18	15	Ames – In vivo MN -	NOEL 33 mg/kg (90 day)	NOEL 15 mg/kg
POE-5/POP-12 Tallowamine 68213-26-3	16-18	17		NOEL 75 mg/kg (28 day)	

Toxicological Effects Summary for Analogs

- Regardless of degree of ethoxylation or chain length/saturation of fatty amine group:
 - NOELs for repeat dose toxicity are similar in magnitude and primary effects are GI irritation, foamy macrophages/histiocytosis, decreases in body weight/food consumption
 - Genetic toxicity is not seen
 - Teratogenicity not seen.
 - Reproductive toxicity studies on two category members show effects on reproductive performance at levels marginally lower than those associated with parental toxicity and on one category member at levels associated with parental toxicity.

PEG-Cocamines Compared to Ethylene Glycol

- Lower molecular weight PEGs and Ethylene Glycol share common acid metabolites(linked to renal toxicity of PEGs and developmental toxicity of ethylene glycol).
 - In theory, if PEG-cocamines with polyethoxy-groups >2 and ≤ 8 could be dosed at sufficiently high doses similar toxicity might be seen, however, dosing is limited by irritancy.

US EPA Conclusions for Alkyl Amine Polyalkoxylate Class

“The toxicology database is adequate to support the use of the alkyl amine polyalkoxylates when used as inert ingredients.There is no evidence that the AAPs are neurotoxic, mutagenic, or clastogenic..... There is no clear target organ identified across the AAPs. Following subchronic exposure to rats, some gastrointestinal irritation was observed, but no specific target organ toxicity or neurotoxicity was seen.”

Weight of the Evidence Assessment

- The available analogs cover the chemical space of the PEG-cocamines in terms of showing a range of fatty amine chain lengths and degrees of saturation and a range of ethoxyl chain lengths.
- Despite significant differences in physicochemical properties the analogs show consistent biological responses, both quantitatively and qualitatively.
- There are studies of reasonable quality for a range of analogs for all major systemic endpoints



Uncertainty Characterization

- The uncertainty associated with read across for the PEG-Cocamines group using data from alkyl amine polyethoxylates is considered to be low, considering the consistency of the biological response data across a diverse group of analogs.

