PINK BOOK 4

CAPB

CIR EXPERT PANEL MEETING

AUGUST 30-31, 2010

Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons

From: Christina L. Burnett, Scientific Writer/Analyst

Date: July 28, 2010

Subject: Draft Final Amended Report on Cocamidopropyl Betaine and Related Amidopropyl Betaines

At the April 2010 meeting, the CIR Expert Panel tabled the report on Cocamidopropyl Betaine (CAPB) in order for the report to be reorganized and to allow for the incorporation of new data.

Those things have been done. In addition, the term "active" in regards to CAPB concentrations has been clarified in the Chemistry section of the report and the new paragraph in which it is defined has been highlighted. In the toxicity and clinical sections of the report, the activity has been calculated . In some cases, the study was not clear as to the activity, so a range is presented (e.g., it might be 10% active or it might be 3% (10% of 30% active)). The good news is that the uncertainty is only a factor of 3.

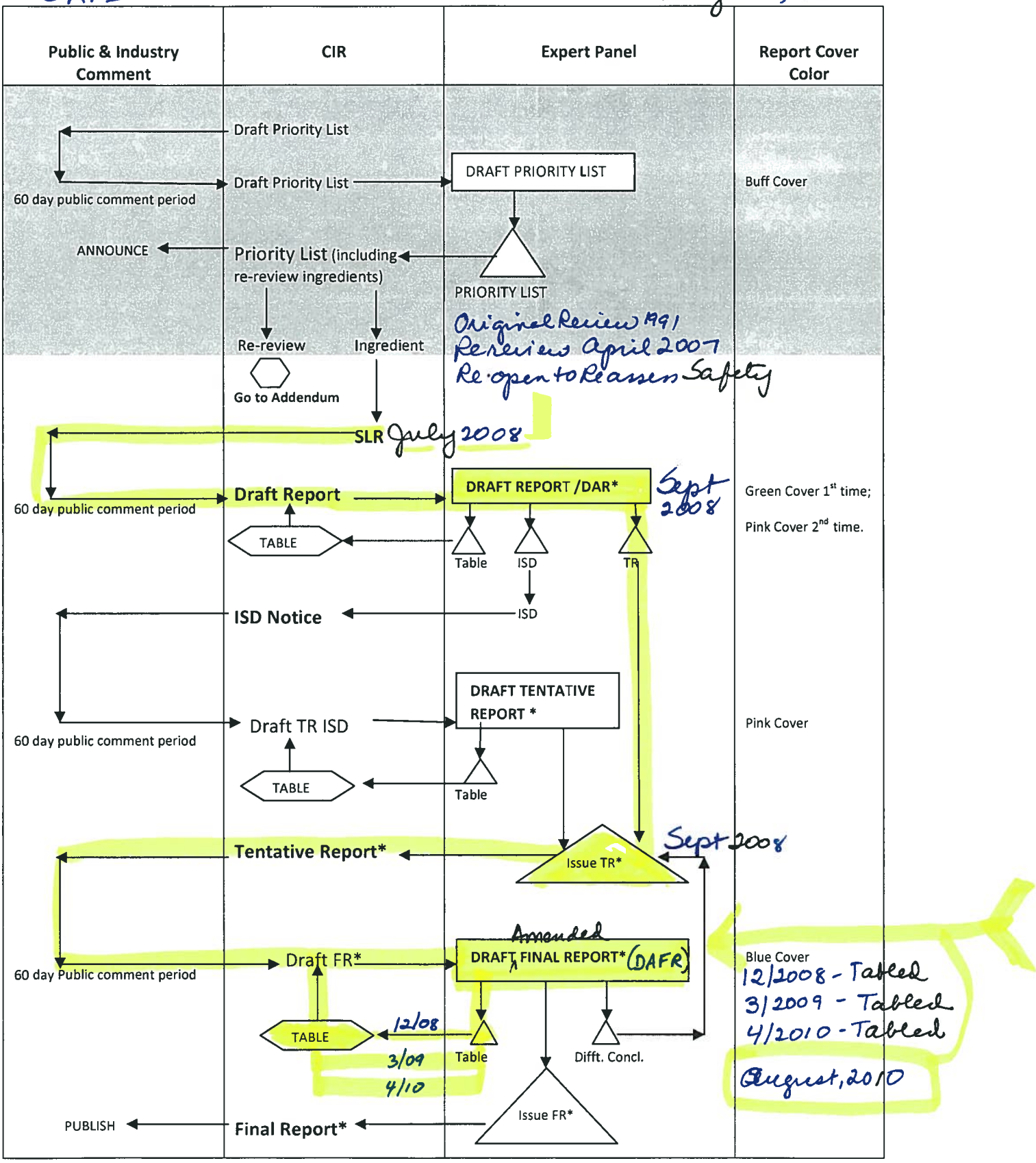
Previously unincorporated unpublished data relating to sensitization studies of CAPB as well as a few studies on impurities, including the LLNA study on amidoamine, have been incorporated into the study and highlighted. A brief summary of the Council's QRA findings have also been included and highlighted. At the April Panel meeting, the teams were in agreement that these ingredients were safe with qualifications, but were divided on the wording of the "qualifications" in the conclusion. Two versions of the conclusion have been prepared. The Panel should review the discussion and these proposed conclusions of this report to determine which version is most appropriate. If there are no major issues, this report should proceed as a Final Amended Safety Assessment.

For your convenience, the materials for this report can also be found at http://www.cir-safety.org/aug10.shtml.

SAFETY ASSESSMENT FLOW CHART

CAPB

August, 2010



* For ingredient groups originating as Re-Reviews, add word "Amended" before Report; (DAR: Draft Amended Report).

△ Expert Panel Decision

▭ Document for Panel Review

Ingredients	Toxline &PubMed	ChemIDplus	Multidatabase (See legend*)	DART	Household Products	Beilstein	Registry	Kosmet	Napralert	RTECS	CAplus
CAPB	√	√	√	√	√	√	√	√	√	√	√
AB	√	√	√	√	√	√	√	√	√	√	√
AlB	√	√	√	√	√	√	√	√	√	√	√
ApB	√	√	√	√	√	√	√	√	√	√	√
AvB	√	√	√	√	√	√	√	√	√	√	√
BaB	√	√	√	√	√	√	√	√	√	√	√
BeB	√	√	√	√	√	√	√	√	√	√	√
CaB	√	√	√	√	√	√	√	√	√	√	√
CaprB	√	√	√	√	√	√	√	√	√	√	√
CoB	√	√	√	√	√	√	√	√	√	√	√
CuB	√	√	√	√	√	√	√	√	√	√	√
IsB	√	√	√	√	√	√	√	√	√	√	√
LaB	√	√	√	√	√	√	√	√	√	√	√
MeB	√	√	√	√	√	√	√	√	√	√	√
MilB	√	√	√	√	√	√	√	√	√	√	√
MinB	√	√	√	√	√	√	√	√	√	√	√
MyB	√	√	√	√	√	√	√	√	√	√	√
OaB	√	√	√	√	√	√	√	√	√	√	√
OleB	√	√	√	√	√	√	√	√	√	√	√
OliB	√	√	√	√	√	√	√	√	√	√	√
PaB	√	√	√	√	√	√	√	√	√	√	√
PalB	√	√	√	√	√	√	√	√	√	√	√
PaKB	√	√	√	√	√	√	√	√	√	√	√
RiB	√	√	√	√	√	√	√	√	√	√	√
SeB	√	√	√	√	√	√	√	√	√	√	√
ShBB	√	√	√	√	√	√	√	√	√	√	√
SoB	√	√	√	√	√	√	√	√	√	√	√
StB	√	√	√	√	√	√	√	√	√	√	√
TaB	√	√	√	√	√	√	√	√	√	√	√
UnB	√	√	√	√	√	√	√	√	√	√	√
WGB	√	√	√	√	√	√	√	√	√	√	√
Impurities of Concern											
DMAPA	√	√	√	√	√	√	√	√	√	√	√
AA	√	√	√	√	√	√	√	√	√	√	√

Multidatabase = HSDB, CCRIS, ITER, IRIS, Gene-Tox, and LacMed;

Search Updated on 7-23-2010

Ingredients

CAPB – Cocamidopropyl Betaine OR CAPB OR 61789-40-0 OR 86438-79-1

AB – Amidopropyl Betaine
AlB – Almondamidopropyl Betaine
ApB – Apricotamidopropyl Betaine OR 133934-08-4
AvB – Avocadamidopropyl Betaine
BaB – Babassuamidopropyl Betaine
BeB – Behenamidopropyl Betaine OR 84082-44-0
CaB – Canolamidopropyl Betaine
CaprB – Capryl/Capramidopropyl Betaine
CoB – Coco/Oleamidopropyl Betaine
CosB – Coco/Sunfloweramidopropyl Betaine
CuB – Cupuassamidopropyl Betaine OR 657350-94-2
IsB – Isostearamidopropyl Betaine OR 63566-37-0
LaB – Lauramidopropyl Betaine OR 4292-10-8 OR 86438-78-0
MeB – Meadowfoamamidopropyl Betaine
MilB – Milkamidopropyl Betaine
MinB – Minkamidopropyl Betaine
MyB – Myristamidopropyl Betaine OR 59272-84-3
OaB – Oatamidopropyl Betaine
OleB – Oleamidopropyl Betaine OR 25054-76-6
OliB – Olivamidopropyl Betaine
PaB – Palmamidopropyl Betaine
PalB – Palmitamidopropyl Betaine OR 32954-43-1
PaKB – Palm Kernelamidopropyl Betaine
RiB – Ricinoleamidopropyl Betaine OR 71850-81-2
SeB – Sesamidopropyl Betaine
ShBB – Shea Butteramidopropyl Betaine
SoB – Soyamidopropyl Betaine
StB – Stearmidopropyl Betaine OR 6179-44-8
TaB – Tallowamidopropyl Betaine

UnB – Undecyleneamidopropyl Betaine

WGB – Wheat Germamidopropyl Betaine OR 133934-09-5

Impurities of Concern
DMAPA – 3-dimethylaminopropylamine OR 109-55-7
AA – amidoamine OR cocamidopropyl dimethylamine OR dimethylaminopropyl cocamide OR 68140-01-2

CIR Expert Panel History with Cocamidopropyl Betaine (CAPB)

1991 - The Final Report on the Safety Assessment for Cocamidopropyl Betaine (CAPB) was published in the Journal of the American College of Toxicology with the conclusion: "Cocamidopropyl Betaine is safe for use in rinse-off cosmetic products at the current levels of use. The concentration of use for products designed to remain on the skin for prolonged periods of time should not exceed 3.0%. The latter is expressed as a 10% dilution of a full-strength Cocamidopropyl Betaine solution that has an activity of 30%."

April 2007 -The CIR Expert Panel reopened the final report on CAPB based on new published data that described sensitization in patients from use of rinse-off products, new uses in aerosol products, and a substantial increase in the number of uses. Sensitization may be due to impurities found in CAPB.

July 2008 - CIR issued a Scientific Literature Review.

September 2008 - The CIR Expert Panel discussed at the length the issue of the impurities DMAPA and amidoamine in CAPB. The Panel considered placing concentration limits on these two sensitizing impurities, but the available data gave conflicting accounts as to thresholds of sensitization, especially for the amidoamine. The Panel concluded that CAPB is safe for use in cosmetic formulations in the present practices of use and concentration, provided that the content of 3-dimethylaminopropylamine (DMAPA) and cocamidopropyl dimethylamine (amidoamine) are not high enough to induce sensitization. The CIR Expert Panel advised that human repeat insult patch tests be undertaken by industry to demonstrate conformance with this conclusion. A Tentative Amended Report was issued.

December 2008 - The CIR Expert Panel decided to table the Draft Final Amended Report on CAPB in order to gather more safety information on the impurities DMAPA and amidoamine. The Panel decided to consider potentially expanding the report with the addition of related amidopropyl betaines.

March 23, 2009 - The CIR Expert Panel expanded the CAPB report to include the following ingredients:

almondamidopropyl betaine, apricotamidopropyl betaine, avocadamidopropyl betaine, babassuamidopropyl betaine, behenamidopropyl betaine, canolamidopropyl betaine, capryl/capramidopropyl betaine, coco/oleamidopropyl betaine, coco/sunfloweramidopropyl betaine, cupuassuaidopropyl betaine, isostearmidopropyl betaine, lauramidopropyl betaine, meadowfoamamidopropyl betaine, milkamidopropyl betaine, minkamidopropyl betaine, myristamidopropyl betaine, oatamidopropyl betaine, oleamidopropyl betaine, olivamidopropyl betaine, palmamidopropyl betaine, palmitamidopropyl betaine, palm kernelamiodpropyl betaine, ricinoleamidopropyl betaine, sesamidopropyl betaine, shea butteramidopropyl betaine, soyamidopropyl betaine, stearamidopropyl betaine, tallowamidopropyl betaine, undecyleneamidopropyl betaine, and wheat germamidopropyl betaine.

The Draft Final Amended Report was still considered tabled while Industry conducts LLNA studies on amidoamine.

April 6, 2010 - The CIR Expert Panel tabled the Draft Final Amended Report on CAPB and related amidopropyl betaine ingredients in order to allow reorganization of the report to include clarification of the reporting of active use concentrations versus full strength concentration. The Expert Panel also discussed the quantitative risk assessment (QRA) approach used by the Personal Care Products Council Task Force to assess amidoamine and asked the Council to consider using the approach on DMAPA.

ONE HUNDRED EIGHTH MEETING
OF THE
EXPERT PANEL
September 22-23, 2008

REPORTS ADVANCING – Full Panel Discussion
Cocamidopropyl Betaine

A CIR Final Report with the following conclusion on Cocamidopropyl Betaine was published in 1991: Based on the available data included in this report, the Expert Panel concludes that Cocamidopropyl Betaine is safe for use in rinse-off products at the current levels of use. The concentration of use for products designed to remain on the skin for prolonged periods of time should not exceed 3.0%. The latter is expressed as a 10% dilution of a full-strength Cocamidopropyl Betaine solution that has an activity of 30%.

Dr. Belsito stated that, at the April 14-15, 2008 Expert Panel meeting, the Expert Panel reopened the published CIR Final Report on this ingredient to allow the Panel an opportunity to review new skin sensitization data, new uses in aerosols, and overall increased ingredient usage. He noted that, after reviewing these data, his Team determined that the issues relating to sensitization seem to vary from country to country. In the U.S., it seems to be due more to amidoamine. In Europe, particularly in Italy, it seems to be due more to 3-dimethylaminopropylamine (DMAPA). Dr. Belsito said that both chemicals appear to be the sensitizing agents that were responsible for the problems with Cocamidopropyl Betaine.

Dr. Belsito added that, after reviewing all of the data, his Team determined that it could be concluded that Cocamidopropyl Betaine is safe as a cosmetic ingredient in the practices of use and concentrations described in this safety assessment, if the levels of 3-dimethylaminopropylamine or cocamidopropyl dimethylamine (amidoamine) in formulation are not greater than 100 ppm in leave-on products and, using a 10-fold dilution product, not greater than1000 ppm in rinse-off products.

Dr. Marks said that it appears that the 100 ppm limit is based on the dose-response that was observed. He added that his Team determined that the conclusion should be stated as follows: Cocamidopropyl Betaine is safe when formulated to be non-sensitizing in the present practices of use and concentration. He added that his Team was not sure of what the minimum dose for the two contaminants should be; however, if Dr. Belsito's Team is comfortable with the 100 ppm limit, then his Team concurs.

Dr. Belsito said that his Team is fairly comfortable with the 100 ppm limit. He noted that, probably, the Italian group addressed the issue best. Patients who were allergic to Cocamidopropyl Betaine were patch tested with DMAPA at reduced doses. This was also done, somewhat, in the publication that followed; but, this study did not result in a minimal elicitation threshold of 10. However, Dr. Belsito noted that his Team agreed that by keeping the level of both DMAPA and amidoamine below 100 pm in a leave-on product and using a 10-fold dilution factor for rinse-off products (i.e., up to 1000 ppm in rinse-off products), this would constitute a safe exposure for consumers.

Dr. Bergfeld wanted to know if the use of a 10-fold dilution factor for establishing the safe limit for rinse-off products is considered a standard.

Dr. Belsito agreed that this is a standard.

Dr. Marks said that he believes that the experience of sensitivity has mainly been associated with rinse-off products. With this in mind, he wanted to know whether Dr. Belsito is comfortable with having the higher threshold for rinse-off products.

Dr. Belsito said that how the subjects were sensitized in the study remains unknown. However, he said that it is known that the expression of sensitization is largely from shampoos, but that these subjects may have been sensitized to a leave-on product, coupled with the fact that their more frequent exposure is to a shampoo.

Dr. Belsito said that his Team also dealt with the issue of different exposures for a hairdresser, but acknowledged that the Panel has not typically looked at occupational exposure. He added that, for the consumer, the concentration limits for leave-on and rinse-off products established by his Team should be acceptable. Dr. Belsito said that the limits will still be restricting significantly the DMAPA and amidoamine below the levels that have been reported as being present in some cosmetic products, that is, based on the wide ranges of what Cocamidopropyl Betaine may contain in terms of DMAPA and amidoamine. He recalled that amidoamine could range from 0.3 % to 3.0% of the product supplied by the manufacturer.

Dr. Shank wanted to know the origin of the 100 ppm limit for the two impurities. He then referred to the following statement on page 38 of the safety assessment: The authors concluded that the concentration limit for DMAPA in 1% Cocamidopropyl Betaine (CAPB) or 1% sodium lauryl ether sulfate (SLES)/CAPB 3:1 should be 0.5 ppm, corresponding to 15 ppm and 60 ppm, respectively, in commercial alkylamidopropyl betaines at 30% active ingredient for DMAPA-sensitized subjects (Angelini et al. 1998).

Dr. Belsito said that 0.5 ppm refers to the level in 1% CAPB.

Dr. Shank noted that the concentration limit that is being proposed is 100 ppm.

Dr. Belsito said the limit is 100 ppm in formulation, and that many of the formulations do not contain more than 1% CAPB. He added that the limit for DMAPA in 1% CAPB or 1% SLES/CAPB 3:1 should be 0.5 ppm.

Following Dr. Belsito's explanation, Dr. Shank said that the math does not seem to work for him.

Dr. Belsito referred Dr. Shank to the following statements (from Angelini et al. 1996b) on page 37 of the safety assessment: Eighteen subjects had positive reactions to DMAPA in water at 0.1%. No positive reactions were noted for DMAPA in water at 0.01% to 0.00005%. Dr. Belsito then noted that 0.01% is equivalent to 100 ppm. He added that, for amidoamine, the basis for the concentration limit is taken from the publication by Hunter and Fowler.

Dr. Bergfeld said that, throughout her tenure on the Expert Panel, she has not heard the Panel reflect on patients with dermatitis in setting a standard according to reactive skin. She noted that, usually, standards are set based upon provocative testing and repeat insult patch testing of non-dermatitic skin, and not patients.

Dr. Belsito said that a limit is being established based on the information that the Panel has. He then stated that if industry wants to raise the level and decides to perform tests that would allow the Panel to determine a threshold for sensitization for DMAPA and amidoamine, this would be looked favorably upon, but may prove to be an expensive effort.

Dr. Belsito said that in reviewing the data in the safety assessment relating to the purity of CAPB, i.e., in terms of being free of DMAPA and amidoamine (thought to be sensitizers), his Team agrees that the proposed limits are realistic based on the available information, which includes data on patients allergic to CAPB who were patch tested, the results of which indicated a threshold dose where elicitation was either 0 (in the case of DMAPA) or a minimal elicitation threshold approaching 10 (which is what the Europeans use when they set standards for nickel and chrome, looking at sensitized patients).

Dr. Belsito acknowledged that, typically, the Expert Panel considers human repeated insult patch test data on normal skin, which, in this case, do not exist. However, he noted that his Team agrees that the Panel does have data that are sufficient for establishing safe levels.

Dr. Bailey wanted to know whether it would be better to accept the statement by Dr. Marks' Team for the conclusion, that the finished product should be nonsensitizing, and include the details relating to concentration limits and sensitization potential (stated by Dr. Belsito) in the Discussion section of the report.

Dr. Marks said that this would be another approach, noting that this has been done for ingredients in the past. Specifically, he recalled statements to the effect that ingredients are safe when formulated to be nonirritating in the Conclusion and including the rationale for these statements in the Discussion section. Dr. Marks also stated that the limits proposed by Dr. Belsito's Team are based on clinical science and are reasonable, and that this guidance with respect to how the ingredient could be nonsensitizing could be incorporated into the Discussion.

Dr. Belsito said that when the Panel has said that products should be formulated to be nonirritating, the issue has not only been ingredient concentration, but also has taken into consideration pH, other ingredients that would make an acid a salt, and other variables. With this in mind, he stated that the issue of sensitization is different from that of irritation in that regard.

Dr. Marks said that the object is that of having CAPB as free of DMAPA and amidoamine contaminants as possible. So, rather than setting a 0 level (which is probably unattainable), the Panel is basically saying that products containing CAPB should be formulated to be nonsensitizing.

Dr. Marks also noted that his Team had expressed concern over what was actually being patch tested after reviewing the conclusion in the Final Report on CAPB that was published in 1991: Based on the available data included in this report, the Expert Panel concludes that Cocamidopropyl Betaine is safe for use in rinse-off cosmetic products at the current levels of use. The concentration of use for products designed to remain on the skin for prolonged periods of time should not exceed 3.0%. The latter is expressed as a 10% dilution of a full-strength Cocamidopropyl Betaine solution that has an activity of 30%.

Dr. Marks said that the objective of his Team is to minimize the contaminants, such that products containing Cocamidopropyl Betaine are nonsensitizing. He added that how this is to be accomplished is stated specifically in the Conclusion or Discussion is basically a judgement call.

Considering the Panel's conclusion on Methylisothiazolinone that was approved earlier in the day, Dr. Belsito noted that the Panel could have stated that products containing this ingredient should be formulated to be nonsensitizing, but concluded that Methylisothiazolinone is safe for use in cosmetic products at concentrations up to 100 ppm (0.01%). He also said that it is his understanding that, for all sensitizers that have reviewed by the Panel, a concentration limit appears in the Conclusion.

Dr. Marks noted that the Panel is actually limiting contaminants of CAPB, not the actual ingredient.

Dr. Shank said that he favors the qualification of nonsensitizing rather than stating a numerical limit in the Conclusion. He added that, at this point, he does not have much confidence in establishing a numerical limit.

Dr. Belsito wanted to know whether Dr. Shank would have confidence in industry's establishment of a nonsensitizing limit.

Dr. Shank indicated that he would favor such a limit.

Dr. Andersen said that there is one example of where the Panel has gone beyond "when formulated to be nonirritating". This was done for the Glyceryl Diesters in a conclusion stating that these ingredients are safe, provided that the content of the 1,2-diesters is not high enough to induce epidermal hyperplasia. He noted that this conclusion placed the burden on companies to use the available technology to achieve this. Similarly, he noted that use of the terminology "when formulated to be nonsensitizing" would place the burden on companies to perform human repeated insult patch tests to demonstrate this.

Specifically, Dr. Andersen noted that the Final Report conclusion states that the Glyceryl Diesters reviewed are safe as cosmetic ingredients in the practices of use and concentrations as described in the safety assessment, provided that the content of 1,2-diesters is not high enough to induce epidermal hyperplasia.

Dr. Belsito said that he would be comfortable with this type of conclusion in the current report, which would state that Cocamidopropyl Betaine is safe as a cosmetic ingredient in the practices of use and concentration described in this safety assessment, provided that the content of dimethylaminopropylamine
and cocamidopropyl dimethylamine (amidoamine) is not high enough to induce sensitization.

The Panel voted unanimously in favor of issuing a Tentative Amended Final Report on Cocamidopropyl Betaine with the conclusion that is stated in the preceding paragraph.

Referring to the report Discussion that will be developed, Dr. Belsito said that a statement to the effect that Cocamidopropyl Betaine should not be used with nitrosating agents should be included.

Dr. Marks said that the aerosols boilerplate should also be included in the Discussion.

Dr. Bergfeld noted that the amended statement should be included at the beginning of the report and in the Conclusion.

Dr. Bailey confirmed with Dr. Bergfeld that the Discussion section of the report will be amended to reflect today's discussion. He also said that actual numbers should be presented to show the basis for the 100 ppm concentration limit that was proposed.

Dr. Belsito said that the numbers are better for DMAPA, where a 0 level at 100 ppm was reported, when compared to aminoamine, whereby a 0 level was not achieved. Thus, for amidoamine, Dr. Belsito noted that he is taking into consideration practices in Europe, considering that data on sensitized patients are being used by the Panel. Typically, in Europe, to set safe levels for consumer exposure, they have looked at what is called the MET 10 (i.e., the minimal elicitation threshold for 10% of the sensitized population). Dr. Belsito noted that the numbers taken from the publication by Hunter and Fowler were close to an MET of 10 at 100 ppm.

Dr. Andersen noted that there is a consistency between the two, even though the publication by Hunter and Fowler did not go below 100 ppm.

ONE HUNDRED NINTH MEETING
OF THE
EXPERT PANEL
December 8-9, 2008

APPROVAL OF FINAL REPORTS – Full Panel Discussion

Cocamidopropyl Betaine

A CIR Final Report with the following conclusion was published in 1991: Based upon the available data included in this report, the Expert Panel concludes that Cocamidopropyl Betaine is safe for use in "rinse-off" cosmetic products at the current levels of use. The concentration of use for products designed to remain on the skin for prolonged periods of time should not exceed 3.0%. The latter is expressed as a 10% dilution of a full strength Cocamidopropyl Betaine solution which has an activity of 30%.

Dr. Belsito stated that the Expert Panel reopened the published Final Report on this ingredient at the April 16-17, 2007 Expert Panel meeting. This was done because of increasing reports of sensitization in patients due to the use of Cocamidopropyl Betaine (detergent) in rinse-off products, new uses in aerosolized products, and a substantial increase in the number of uses. Dr. Belsito recalled that, at the September 22-23, 2008 Expert Panel meeting, the Panel had a discussion on how to limit what were thought to be the impurities in Cocamidopropyl Betaine (dimethylamidopropylamine [DMAPA] and amidoamine) that caused sensitization reactions, and the following statement was proposed for addition to the conclusion: The products should be formulated to be nonsensitizing.

Dr. Belsito added that his Team is requesting that someone who was involved in the development of this QRA, such as Dr. Anne Marie Api or one of the other authors in the article, be invited to give a presentation to the Expert Panel on what the QRA entails and how it has worked. Dr. Belsito noted that there is a recent publication by Dr. Api showing that, had the QRA been in effect, sensitization to some of the fragrance ingredients could have been reduced.

Dr. Belsito said that one of the data points that is needed if the Panel is going to accept the QRA was not available for amidoamine, and industry was suggesting that the Panel substitute stearylamine. He noted that some of the members of his Team were not comfortable with this suggestion, and expressed a preference for a local lymph node assay on amidoamine in order to establish a safe level. Dr. Belsito reiterated that the reopened safety assessment should be tabled, pending more information on the QRA.

The Panel voted unanimously in favor of tabling the reopened safety assessment on Cocamidopropyl Betaine.

Dr. Bergfeld wanted to know whether the report is being tabled until the Panel hears the QRA presentation, after which the review of Cocamidopropyl Betaine will continue.

Dr. Belsito said that the reason for tabling the report is based on the understanding that the Panel's prior conclusion cannot go forward with the language, when formulated not to be sensitizing and that the Panel is trying to establish levels for the impurities. He added that the Panel has good data on DMAPA, but not on amidoamine, as to what is considered a non-sensitizing concentration. Dr. Belsito said that, by using a QRA and obtaining the actual LLNA for amidoamine, this approach could be used as a worst case scenario in establishing levels for these impurities in cosmetic products and the Panel could proceed with an appropriate conclusion.

Dr. Bergfeld said that, based on today's discussion, it seems as though Cocamidopropyl Betaine is being tabled and probably will be considered again during the latter part of next year.

Dr. Andersen said that CIR will have to negotiate with RIFM as to when a staff person would be available to make the presentation on QRA, and the report would then have to be revised to include the findings. Actually, Dr. Api would make the presentation, the Panel's feed back would be solicited, and information from both sources would be subsequently incorporated into the report. Dr. Andersen said that if the presentation were to be made at the March 2009 Panel meeting, the report could be considered again at the June 2009 Panel meeting.

Dr. Bergfeld noted that the local lymph node assay was also mentioned.

Dr. Andersen said that industry should be on alert because it is the sense of Dr. Belsito's Team that data from an LLNA on amidoamine would be very useful in establishing a safe level for this impurity. These are data that would input into the QRA.

Dr. Bailey said that this concern will be brought before his committee, to make sure that there is sufficient interest and agreement to perform the test.

Dr. Marks said that the QRA conclusion will significantly change the hypothesis that amidoamine is a moderate sensitizer. However, he added that, if it is a strong sensitizer, then that result is markedly different in terms of the risk assessment.

Dr. Belsito said that, in order to perform a QRA, one would need either an LLNA or a human repeated insult patch test on the chemical. He noted that the Panel is being asked to accept values for a chemical that is similar, and that some members of his Team are not comfortable with this proposal. Dr. Belsito added that an LLNA could be completed in 5 or 6 days, and is not a very expensive assay.

Dr. Marks said that the results of this assay could change the safety assessment. He added that, if one were to assume that amidoamine is a strong sensitizer, it is likely that the Panel's conclusion would change. Dr. Marks asked Dr. Belsito whether his Team had discussed the list of ingredients that potentially could be added to the safety assessment.

Dr. Belsito said that his Team had discussed this and that it is his understanding from the industry representatives that the method of manufacture and impurities would be the same for ingredients on this list. In other words, there would be concern over amidoamine and DMAPA as impurities in these ingredients. Dr. Belsito said that if this is true, then the ingredients should be added; however, data on methods of manufacture and impurities would be needed to confirm this.

Dr. Marks said that the available literature on the list of ingredients that may be added needs to be obtained so that the Panel may make an informed decision.

Dr. Bergfeld noted that the Draft Amended Final Report on Cocamidopropyl Betaine has been tabled and that the Panel is awaiting the outcome of some of the suggestions that were made.

ONE HUNDRED TENTH MEETING
OF THE
EXPERT PANEL
March 23-24, 2009

March 23, 2009 Panel Meeting-Team Minutes

Cocamidopropyl Betaine (CAPB)

Belsito Team

Dr. Belsito reminded the team that Cocamidopropyl Betaine (CAPB) that the Panel is still awaiting further information on the QRA approach for assessing the contaminants DMAPA and amidoamine. The task of this Panel session is to determine what related ingredients should be added to the CAPB safety assessment. Industry provided method of manufacturing data on a few of the related betaines. Many ingredients are still lacking information.

Dr. Eisenmann noted that compositions are missing from milk, oat, and apricot betaines, among others.

The team noted the issues regarding the review of the botanical Rice and were concerned that similar problems could arise.

Dr. Belsito asked the team if ingredients that are still lacking information should be struck from the report or if it should be assumed that the composition and method of manufacturing would be similar. What should the Panel do with ingredients where the non-betaine half of the molecule has not been reviewed, like milk, oat, etc.?

Dr. Eisenmann noted that if composition of the betaines are provided, it may be enough information for the Panel.

Dr. Bailey asked if milk is an ingredient exempt from CIR review. It was noted that the fatty acids in milk are found in cosmetics.

Dr. Belsito noted that the assumption of similar methods of manufacture for the betaine ingredients could be part of the report's discussion. The impurities of the betaines are the main concern, not the different fatty acids. The different fatty acids that have not been reviewed may be included in the safety assessment.

The team looks forward to Dr. Anne Marie Api's QRA presentation in June.

Dr. Eisenmann told the team that the LLNA study on amidoamine was still in the planning stages.

Dr. Snyder noted to Ms. Burnett that information on non-cosmetic uses for the betaines still needs to be added to the safety assessment.

The team recommends that all of the proposed related betaines be added to the safety assessment. Fatty acid constituents of the new ingredients should be provided when possible. The report should return to the Panel with the LLNA study on amidoamine is ready.

Dr. Liebler would like to see validation on the assays being performed in order to be sure impurities are not from decomposition. Artificial numbers are possible from improper methods.

Marks Team

Ms. Burnett went through the various handouts the Panel received that morning.

Dr. Shank noted that the Panel's task at hand is to decide which of the proposed additional betaines should be incorporated into the report. Dr. Shank felt that all of the ingredients could be incorporated.

Dr. Hill had concerns about the ricinoleamidopropyl, undecyleneamidopropyl, and tallowamidopropyl betaines. Fatty acid compositions and biochemistry may be too different from the other proposed betaines.

Dr. Andersen reminded the team that industry is still developing data on the amidoamine issue, specifically how to quantify amidoamine.

Dr. Hill was concerned that the biological activities of amidoamine may vary from ingredient to ingredient.

Dr. Marks noted that the Panel is waiting on QRA information.

Dr. Andersen said that a NOEL will be needed to predict the sensitization risk of amidoamine with the QRA method.

Dr. Hill would like a thorough review of amidoamine toxicity, specifically mitogenic activity.

Dr. Marks reminded the team that the potential additions to the report should not pose any new safety issues. Additions should be "no-brainers".

Dr. Bergfeld asked if a literature search would be performed on the additional ingredients.

Dr. Andersen and Ms. Burnett responded that yes, literature searches would be performed on the additional ingredients.

Dr. Marks said that the team would recommend that all ingredients should be added to the current safety assessment of CAPB. The Panel awaits information on amidoamine and DMAPA. A literature search will be performed on the new ingredients. If no information is found on a specific ingredient, the available information on the parent compound should be able to extend to the ingredient that is lacking information.

Dr. Andersen stated that the structure of the meadowfoam seed betaine was clearer to interpret than the structures provided by the dictionary and used in the report. He asked the team if that kind of structure would be more useful.

Dr. Hill asked if the dermal penetration of the different betaines would differ.

Ms. Burnett told the team she did not know when the report would be back for the Panel to review.

Dr. Loretz told the team that the information on amidoamine was still be developed.

REPORTS ADVANCING – Full Panel Discussion

Cocamidopropyl Betaine

Dr. Marks stated that the Final Safety Assessment on Cocamidopropyl Betaine (surfactant) was reopened at the April 16-17, 2007 Expert Panel meeting because of increased use and an observed increase in skin sensitization data. He noted that his Team is particularly concerned about the sensitization capabilities of impurities that are present in Cocamidopropyl Betaine, namely DMAPA and amidoamine. Furthermore, the Panel is expecting data from industry, particularly on amidoamine and what would be considered a safe level for this impurity relative to its sensitization potential. Dr. Marks said that these data would be needed in order to proceed with this safety assessment.

Dr. Marks said that there is also a number of amidopropyl betaines (~30) that potentially could be added to this safety assessment based on their chemical similarities. He then stated that his Team agreed that the current safety assessment should be expanded to include these 30 ingredients and, also, that the Panel should wait for clarification of the impurities.

Dr. Belsito said that his Team also agreed that the 30 amidopropyl betaines should be added. He also noted that Dr. Liebler had expressed interest in learning more about validation of the assay method for DMAPA and amidoamine.

Dr. Liebler said that the issue was whether or not these impurities actually exist in the product or whether they could be artifactually generated during the analysis. He added that there should be information on validation of the assay, considering that the impurities could be hydrolyzed to the precursors. Dr. Liebler said that if the assay has not been validated, then this needs to be done because, if the result is artifactual, this would be problematic and misleading to the Panel. As an example, he noted that an isotope labeling strategy using a model compound could clearly establish the validity of the assay, done either by GC-MS or LC-MS.

Dr. Hill said that he had not thought about the artifactual issue, but that his Team had discussed amidoamine, particularly in the context of expanding the safety assessment to include additional ingredients. He then noted that he had not thought about the possible degradation of the betaene back to the amidoamine, which would be an elimination reaction, and the Panel did not have information about this chemistry in the safety assessment.

Dr. Bergfeld said that it is her understanding from Dr. Andersen that the Panel does not need to vote at this time, but that the Panel's concerns/requests relating to impurities will be conveyed to industry.

The Panel agreed that the Final Safety Assessment Cocamidopropyl Betaine should be reopened to include the following ingredients: Almondamidopropyl Betaine, Apricotamidopropyl Betaine, Avocadamidopropyl Betaine, Babassuamidopropyl Betaine, Behenamidopropyl Betaine, Canolamidopropyl Betaine, Capyrl/Capamidopropyl Betaine, Coco/Oleamidopropyl Betaine, Coco/Sunfloweramidopropyl Betaine, Cupuassuamidopropyl Betaine, Isostearamidopropyl Betaine, Lauramidopropyl Betaine, Meadowfoamamidopropyl Betaine, Milkamidopropyl Betaine, Minkamidopropyl Betaine, Myristamidopropyl Betaine, Oatamidopropyl Betaine, Oleamidopropyl Betaine, Olivamidopropyl Betaine, Palmamidopropyl Betaine, Palmitamidopropyl Betaine, Palm Kernelamidopropyl Betaine, Ricinoleamidopropyl Betaine, Sesamidopropyl Betaine, Shea Butteramidopropyl Betaine, Soyamidopropyl Betaine, Stearamidopropyl Betaine, Talowamidopropyl Betaine, Undecyleneamidopropyl Betaine, and Wheat Germamidopropyl Betaine.

Dr. Andersen said that the revised safety assessment will be reviewed at a future Expert Panel meeting.

ONE HUNDRED FOURTEENTH MEETING
OF THE
EXPERT PANEL
April 5-6, 2010

April 5, 2010 Panel Meeting-Team Minutes

Cocamidopropyl Betaine (CAPB)

Belsito Team

DR. BELSITO: Okay, so the last of the presumably contentious ingredients, cocoamidopropyl betaine -- well, in the second wave of data, we did get an LLNA, and then we got a risk assessment on the amidoamines in amidopropyl betaine ingredients. And the EC3 in the LLNA for amidoamine was 0.98 percent, so it was a moderate, not a strong sensitizer at all. And then we got the risk assessment.

One problem with the risk assessment is that we had stated before that we wanted C12, which was the predominant carbon group in amidoamine, and again they were trying to go back to the C18 data. But then they also included a report that indicated that the absorption of these increased with increasing chain length up to C20-something, I think they said, or C18 and it sort of goes against everything I'm used to thinking about. But I guess it has to do with lipophilic and hydrophilic as you increase the chain lengths, but -- so, the argument was that we were -- that if anything, they would be getting more across with the C18 than they would with the C12 and so they could use that.

I think regardless, the -- actually the NESIL that was determined using the data on HRIPT from the C18 was a lower NESIL than would have been calculated from the LLNA. It was done with amidoamine and it looks like that the data would support, based upon how this is put into formulation, that it's 20 percent cocoamidopropyl betaine and then at the highest it's diluted 20 percent in a face wash, that it would support a maximum level of 1.5 percent cocoamidopropyl betaine in the cosmetic product. But -- and that was sort of their argument, but I think we had stated that we weren't going to go with a level of amidoamine or DMAPA in the -- what was supplied to the manufacturer, we were going to go with a limit in the final product.

So, the only other thing I would like to point out is that if you look on the CIR supplement page 68, it's page four of the cocoamidopropyl betaine report, on the amidoamine II study that we had seen before, it still gives very quirky results. That's the study on the liquid fabric softener. I just don't understand whether those were all irritation or what because that ended up with a NESIL of 25 mg/cm2, which would have been lower than anything else we saw.

DR. LIEBLER: Yeah, almost tenfold.

DR. BELSITO: You know -- because the next day several subjects experienced mild to moderate erythema during induction patching. Eight subjects reacted at challenge with mild to moderate erythema and/or edema. Seven were re-challenged with 4 and 0.4 percent concentrations of the formula equal to 25 mg/cm2, and 2.5 mg/cm2 with a mixture. There was evidence of mild erythema and for the seven subjects re-challenged at the higher concentration, 25 mg/cm2. And then they say there's no evidence of sensitization during a re-challenge, so -- I mean, I thought I'd show you that that fact is the case. I still have problems -- we're now going to accept the fact that we're okay looking at the higher carbon chain lengths because in fact at that range they absorb more. It's still quite quirky data.

DR. SNYDER: Could you go back to where you were thinking of drawing the line for the sensitization limit? At what? Based on what?

DR. BELSITO: Well, I'm not sure based on what. The -- what industry has argued is -- and then, again, they weren't -- and I didn't go back and calculate. What they were saying is that the level of amidoamine contaminate in what's supplied to industry can be 1.5 percent or lower and what's supplied to industry is 30 percent and then the highest concentration of that 30 percent is 20 percent in a face wash, a rinse off, and they did the math and that all works out, but what we're saying is, we were going to set limits on the final impurity in the finished product, not on what was supplied to industry. So, you'd have to go back and calculate -- go back from the NESIL and calculate what would be in a finished product, and I didn't do that. Our original proposal was for 50 parts per million, I think.

DR. EISENMANN: At one point it was not sensitized, but --

DR. BELSITO: Amidoamine?

DR. EISENMANN: Yeah, I thought you had --

DR. BELSITO: We never said amidoamine was not sensitized.

DR. EISENMANN: No, no, no, were formulated not to be non-sensitizing. I thought at one point --

DR. BELSITO: No, that was the Marks Team. We were always setting limits for DMAPA and amidoamine. If you go into our –

DR. SNYDER: Well, here in the current version she has, she says, "to be nonsensitizing at a concentration up to 100 parts per million" for DMAPA, but we don't have a -- for the --

DR. BELSITO: DMAPA was never --

DR. SNYDER: I mean, I'm sorry -- yeah, (inaudible) and it was -- that's for amidoamine.

DR. LIEBLER: Well, the problem was with amidoamine we didn't have the information to draw a threshold.

DR. SNYDER: Right.

21 DR. BELSITO: We don't have all the minutes here in this report.

MS. BURNETT: (inaudible) further discussion on the limits?

DR. BELSITO: Yeah.

MS. BURNETT: (inaudible) September 2008.

DR. BELSITO: No, where Ron and I had that back and forth. Okay, yeah, September of 2008.

DR. SNYDER: 2008, yeah.

DR. BELSITO: Yeah.

MS. BURNETT: It's also in the second page here.

DR. BELSITO: Right.

MS. BURNETT: It looks like --

DR. BELSITO: So, DMAPA we had less than a 100 parts per million and I guess the industry had no problems with that. That was based off of the Angelini studies. In amidoamine, we have problems with and that's why we asked for the LLNA --

DR. LIEBLER: Right.

DR. BELSITO: And the QRA.

DR. LIEBLER: Right.

DR. BELSITO: I think we need to go with that.

DR. LIEBLER: So, your concern about the QRA is this study, too --

DR. BELSITO: Yeah, that according to the study --

DR. LIEBLER: -- (inaudible) much lower NESIL.

DR. BELSITO: Right.

DR. LIEBLER: Yeah, and it's hard for me to understand how to interpret these because, you know, I've only been on a year and so I haven't seen these as long as you guys have, but you know, when you're looking at the data you have basically a lot of zeroes and then some level of background and I don't know if there is any statistical basis for analyzing data like that. There must be. I haven't heard it described or cited here in (inaudible). I mean, this type of test must be, you know, susceptible to statistical analysis to give us an idea of what would be a chance finding versus what is likely not to be a chance finding.

DR. BELSITO: The numbers were -- I mean, the numbers for mild reactions in this study were fairly substantial, I mean, if you look at it, and, you know, I looked at the raw data before and it doesn't really help give me an explanation of what might be going on other than it's, you know, just a really quirky study.

The other thing that's interesting, and may be -- have a, you know, bearing, is that in the other study that was done on the stearamidopropyl dimethylamine, it says that the vehicle was mineral oil and then if you look at
page three of the manuscript it says that CAPB is insoluble in mineral oil. So, that study may not be a valid study. So -- but I don't understand how cocoamidopropyl betaine can be insoluble in mineral oil and a few chain lengths higher and purer, it's -- was able to be solubilized and put into a human repeat insult patch testing. Right at the top of page three it says, CAPB is soluble in water, ethanol, and isopropynol and insoluble in mineral oil. Which, again, confuses me because then we have one quirky study and one study that makes no sense because the vehicle, presumably, is an inappropriate vehicle, at least for CAPB.

DR. LIEBLER: So, which study claimed to have put the material in mineral oil?

DR. BELSITO: The first one, where they got the lower NESIL, the one with the stearamidopropyl dimethylamine, study one.

DR. LIEBLER: In the QRA's summary?

MS. BURNETT: Yes.

DR. BELSITO: And the human sensitization data right on the HRIPT where they were getting a NESIL –

DR. LIEBLER: Gotcha.

DR. BELSITO: It's page 4 of the report, it's page of the CIR supplement.

DR. BAILEY: Right.

DR. BELSITO: But it says here -- it doesn't say amidoamine, it says cocoamidopropyl betaine is insoluble in mineral oil, on page 3. It says, "CAPB is soluble in water, ethanol, and isopropynol and insoluble in mineral oil."

DR. BAILEY: But the betaine is charged, right?

DR. LIEBLER: Right, so that's charged, so the thing that they're saying is insoluble in mineral oil is charged and that doesn't make sense. And then if you go to the study, in the --

DR. BELSITO: Oh, what you're saying is the dimethylamine -- okay. I see what you're saying.

DR. LIEBLER: So, that's not necessarily an inconsistency for it --

DR. SNYDER: Well, the thing I was wondering about with the quirky study, is that's in a vehicle, I guess, as a fabric softener. It may be complicated enough that it's not an appropriate vehicle to do a study like that in.

DR. BELSITO: Just pointing them out.

DR. SNYDER: I think we're stuck. I don't think we really know where we're at.

DR. BELSITO: Well, I mean, we do have an EC3 in the LLNA, which is -- gives us a NESIL that's actually higher than the HRIPT on the C18 and the --

DR. LIEBLER: So, that's 245 versus 187?

DR. BELSITO: It's the --

DR. LIEBLER: The NESIL from the LLNA study 3 --

DR. BELSITO: Right.

DR. LIEBLER: -- is 245 mg/cm2, and the NESIL from the study 1 RIPT is 187 mg/cm2 so those numbers are very similar actually.

DR. BELSITO: Right.

DR. LIEBLER: And then we have another number from the study 2, the one --

DR. BELSITO: The quirky study of 25.

DR. LIEBLER: -- the quirky, that's essentially tenfold lower, about tenfold lower. And that's the one I was pointing out as a liquid fabric softener product as the vehicle. And I'm just asking whether or not that could be a source of variability, high background –

DR. BELSITO: Well, there certainly could be a lot of other stuff in a liquid fabric softener that could cause irritation.

DR. BAILEY: Yeah, I mean, I don't think we're going to know what the composition of that will be other than, you know, a fabric softener has multiple ingredients in it. I'm not sure it's characterized anywhere in the studies. Are you familiar, Carol, with (inaudible)? So --

DR. LIEBLER: I'm just raising the possibility that that study may not be a very good basis for flagging amidoamine.

DR. BELSITO: And that's fine and we can say that, you know, we looked at the study and, you know, we felt that the data were -- did not really contribute to our assessment because we didn't know the other ingredients and that potential for irritation from the fabric softener under occlusion is quite high.

DR. SNYDER: So, the fact there was a C18, they got the 187 and the C14, the highest we got was 245, that would be relevant?

DR. LIEBLER: Studies 1 versus 3?

DR. SNYDER: Right.

DR. BELSITO: Mm-hmm.

DR. LIEBLER: So, I think one of the arguments, as I read it in this previous page of the QRA, page 18 of the QRA, is that there's really not a lot of difference between (inaudible).

DR. SNYDER: Once you get the (inaudible).

DR. LIEBLER: Right. Yeah.

DR. BELSITO: So then --

DR. LIEBLER: So, the study 2 might be one -- a reason to wonder about the validity of this QRA, but if we tend to discount study 2, then I'm more comfortable with the conclusions of the QRA, I think.

DR. BELSITO: Okay. So then if you plug all of that in, then the consumer exposure level -- consumer exposure level of less than or equal to 0.45 mg/cm2 of amidoamine. And then what do we do for DMAPA? Before we just simply said it shouldn't be present in product at greater than 100 parts per million. Industry had no problems with that. Do we go back and calculate what it would be in a final if present in the -- what, supplied to industry at 100 parts per million or do we --

DR. SNYDER: So, I have another question (inaudible) later. So when we -- under this concentration of amidoamine in a product, we see CAPB is present in face wash, it says here, at formulated at 20 percent. Is that -- again, getting to this issue of active versus other. I mean, we sometimes list it as 10 percent, 20 percent, we don't specify, and at other times we're specifying 30 percent active. So, how do we know when one is which? So, is that the active or is that just --

DR. EISENMANN: I believe in this case it's the active.

DR. SNYDER: But throughout the report, like on the Hilltop research study on page 20 where you have.03 percent active CAPB testing on 100 human volunteers, started out with CAPB at .6 percent -- and so we don't really specify or maybe we need to have some discussion under the -- way back in the beginning.

DR. BELSITO: Oh, I have it flagged throughout, is this active, is this not?

DR. EISENMANN: Well, in the concentration of use table, in this case, I think I did ask, concentration of active, and I got the activity of the raw material too. So, I think in this case, that's where they got the 20 percent.

DR. BELSITO: Then there's a real question because under -- on your concentration of use you have in parentheses, active, then we have an eye shadow with 3 percent active CAPB, which is significantly higher. We have a lipstick at 3, we have deodorants at 2, those are all going to cause irritation at those levels. I suspect those are percent of the 30 percent. I mean, just look down, I mean, the ones I circled immediately were eye shadow at 3 percent, lipstick at 3 percent, deodorant at 2 percent, a douche at 6 percent. We have data showing that's going to be a vaginal irritant at that concentration. Other at 10 percent, body and hand creams and lotions at 5 percent, I suspect that those are all 5 percent times 30 percent not active.

DR. LIEBLER: So, 5 percent of 30 percent.

DR. BELSITO: Right.

DR. LIEBLER: So, what does the term active mean in this context?

DR. BELSITO: Active is the actual, what is the concentration of cocoamidopropyl betaine.

DR. LIEBLER: In the product, by weight?

DR. SNYDER: On page 40, the (inaudible) say, "The concentration, when expressed as activity, is determined by subtracting the percent of NaCl from the percent total solids," which I didn't understand that.

DR. LIEBLER: Oh, they're just trying to correct for the fact that it's a salt and the salt weighs more.

DR. SNYDER: I don't know, that's why -- if that is it, that's got to be moved up to the use section, because currently don't -- in the use section, we don't address the active versus -- I don't want to say inactive, but the total. That's on page 40 where you had that sentence that talked about, "The concentration, when expressed as activity, is determined by subtracting the percent."

MS. BURNETT: Must be in there -- has to be in there -- it is --

DR. EISENMANN: Where are you finding that 3 percent irritation -- irritating?

MS. BURNETT: It's under the physical and chemical properties.

DR. SNYDER: What page is that?

MS. BURNETT: It's at the top of page 3.

DR. EISENMANN: Thirty percent.

DR. SNYDER: Oh, okay, so you (inaudible).

DR. EISENMANN: No, 7.5 was not irritating on page 11.

MS. BURNETT: In the report --

DR. BELSITO: Up to 50 percent of a 30 percent solution. These are -- page 11.

DR. EISENMANN: A 7.5 percent active solution, no irritation was observed. It's the second complete paragraph.

DR. LIEBLER: Page?

DR. SNYDER: Eleven.

DR. BELSITO: Well, if you look -- I mean, again, I think it has to do with whether it was active or inactive. I'm not sure in all those cases, but I think the most explicit, to my mind, was on page 16, mucus membrane irritation where they did 7.5 percent CAPB, they don't define whether it was active or not, in vaginal irritation, and at 7.5 percent you had diffuse necrosis of the vaginal mucosa 5 of 6 dogs and focal in 1 and then you have it listed here in a douche at up to 6 percent.

DR. EISENMANN: Okay, I'll check that one.

DR. BELSITO: If 7.5 causes that kind of effect, I really doubt you want it at 6 percent which makes me wonder about the others. I mean, to CAPB leave -- I mean, used in an eye shadow at 3 percent, I think that's really pushing the irritation concentration on the eyelid. Same thing for lipstick and, you know, 5 percent in a body and hand cream lotion for detergent. What are you trying to solubilize there with that? I mean, they're just high levels. I mean, they don't make sense to me. I don't think they're active levels, I think they're 5 percent of 30 percent.

DR. LIEBLER: So, I think it probably is necessary in this report to clearly explain this concept of activity in the case of CAPB. It is mentioned, first mentioned, I think, at the top of page three where it says, "Cosmetic-grade CAPB normally is supplied with 35 percent solids. The concentration is described by its activity, which is determined by subtracting the percent sodium chloride from the total percent solids." I think that should go on to say that the active percentages mentioned throughout this report then refer to --

DR. SNYDER: I'd refer to the use data table and say exactly what is being reported there very explicitly because I don't think that that's likely been actively reflected.

DR. BELSITO: Yeah, and I don't think -- I don't think it's necessarily accurate and in terms of irritation, Carol, if you look at the human data, starting on page 18, the last bottom line, which I think is inaccurate because it says, "Irritation studies of formulations containing concentrations of 6 percent active and greater were considered practically nonirritating." I think you mean 6 percent active and less, no? I mean, because otherwise it's like below 6 percent they're irritating, above 6 percent they're not? That sentence doesn't make sense.

But beyond that, the next paragraph -- I mean, almost, you know, negates what you just said because it says in a 1.9 percent active CAPB you tested it in two soap formulations, total irritation score for all subjects was 588 and 581 out of a maximum of 630 and that was -- that's an irritant. I mean, it almost reached the maximum there and that was at 1.9 percent, a 1 percent aqueous dilution of a product formulation containing 6 percent active, so you're at.06 percent, 15 panelists, and that was not irritating. And then in another study where you had 8 percent of a 6.5 active, that was.7, that was also nonirritating.

MS. BURNETT: I think when I wrote the summary I didn't do the calculation for the dilution, so that's probably where I goofed.

DR. BELSITO: Yeah, I think -- I don't know how to approach this. I would -- probably would say table it and very carefully go through and change -- and assure that everything is active in concentrations of use in the studies, and then if, in fact, we do decide tomorrow that our conclusion is going to be for levels of the contaminate in final product, then we have to also decide how we want to express that. You know, is it going to be as micrograms per centimeter squared, which means it's going to vary from product use to product use depending upon how you put it on, which is really the adequate way of doing it? And if that's the case, then we're going to have to calculate out what that would be for DMAPA because at this point we set a limit for DMAPA not in the final -- well, I guess we could set a limit for DMAPA in the final because at 100 parts per million it was negative, so I mean that would be what we would want in the final as well, and then figure out what that converts to in terms of micrograms per centimeter squared from the Angelini study.

So, we're going to -- but I would table this and clean up the document. I think there are just too many loose ends, but with the idea of probably going safe and we probably have all of the information we need to accept a limit. And in the discussion, I think we need to address that quirky study too and I guess Dan's point is it's in a -- we're very happy that the LLNA and the study on the HRIPT on the stearamidopropyl amine were very close, and then we have this one quirky study that was not, but it was done on a fabric softener and we think that what we're seeing was a lot of confounding irritation perhaps due to other ingredients in the fabric softener which, that needs to go in the discussion.

DR. EISENMANN: Put a qualifying on the conclusion, when formulated to be nonirritating too?

DR. BELSITO: That will be in there too, sure, but --

DR. SNYDER: It's the sensitization issue that we're trying to get over the hump.

DR. BELSITO: Right, it's the sensitization.

DR. EISENMANN: Right. But there's also the irritation --

DR. BELSITO: Right. Right.

DR. EISENMANN: -- issue and if you've prevented that then -- because I don't know if somebody could formulate some certain products to not be sensitizing and have --

DR. SNYDER: Irritation issues.

DR. BELSITO: Yeah. Okay. So -- yes?

MR. SILBERGELD: Question for Christina. Top of page 38, high quality CAPB, is that -- does that relate to the purity of the product? I know it's not your conclusion, it's the author's study you site.

MS. BURNETT: I'm sorry, we're at -- on page 38?

MR. SILBERGELD: Page 38 -- very top, first line.

MS. BURNETT: That was probably written word-for- word. So, I can't recall off the top of my head.

DR. BAILEY: So, what you're saying is to table and go through the report to clarify what this activity -- use of the term "activity" means throughout the report, including the tables for use levels.

DR. BELSITO: Yeah, I think it's going to be a lot of work but I think what needs to be done is that each of the studies -- you need to go back to the materials and methods and see what they actually used. If you can't figure out whether it was CAPB at 10 percent or it was 10 percent of a 30 percent, then I think those studies are less meaningful and perhaps should be segregated to the end of any, you know, and put "CAPB of unknown purity" or some heading that alerts us to the fact that we're looking at studies where we're not certain what concentration of CAPB we're looking at as opposed to other studies where they clearly say it was a 2 percent of a 30 percent.

And we know what we're dealing with and when they say that, it shouldn't be talked about 2 percent CAPB it should be, you know, 0.02 times 0.3, so it's, you know, it's a 6 percent CAPB you're looking at so we know exactly, you know, what concentration the cocoamidopropyl betaine you're looking at, and I think that would be, you know, very helpful. I don't think it's going to change anything, I don't think we need any more data, I just think we need to be very clear at what we're stating and I think industry has reported some wrong numbers there and Carol needs to go back and really sit down and say, okay, is this truly 6 percent CAPB you're putting in a vaginal douche or is it 6 percent of what's supplied to you which is really 30 percent.

DR. SNYDER: I think you can capture a lot of that (inaudible) you're setting the stage of how the data is going to be reported and when it's not known just say -- not specified, and then make sure in the intro that you accurately say what's reported in the use table because I think that's a big issue right there.

DR. BELSITO: Right.

DR. BAILEY: And so assuming we can straighten all of this out what you're saying is, safe as used, and -- but with a limit for DMAPA of 100 parts per million. Right?

DR. BELSITO: Mm-hmm.

DR. BAILEY: Okay, now what about the amidoamine?

DR. BELSITO: Well, I think it's, you know, either one -- it depends upon how -- originally what we were going to go for was the concentration of the impurities in the final product, okay? What -- and I think that that's probably the best, you know, way to go and so in that case, you know, when formulated to be nonirritating and when amidoamine is at -- the consumer exposure level that you calculated was 0.45 mg/cm2, based upon your data I think that's fine. So then we just have to go back and calculate what the consumer exposure to

DMAPA would be in terms of mgc2 if it had a limit of 100 parts per million in what was supplied to industry, so we just need to do a little bit more math because -- or, you know, we can go the way we used to go, which we decided we weren't going to which was DMAPA and what supplied 100 parts per million and amidoamine 1.5 percent.

DR. BAILEY: Okay.

DR. BELSITO: I like the impurities in the final product because then, you know, if someone wants to -- I don't know -- suppose if we say "safe as used" the highest used concentration is going to be the 20 percent in the face wash, so -- but then it doesn't necessarily cover the leave- on uses although there would be a highest concentration for leave-on too.

MS. BURNETT: There wasn't -- I think --

DR. EISENMANN: But (inaudible) nonirritating.

MS. BURNETT: The original report did have the qualifications, I thought, for leave-on, rinse-off, so I'm not quite sure.

DR. BELSITO: But that was based on irritation.

MS. BURNETT: Right. Okay.

DR. BELSITO: And we're going to just simply say when formulated not to be irritating.

DR. SNYDER: Yeah, because we did -- we talked about the tenfold difference, 100 parts versus 1000.

DR. BELSITO: Right.

DR. LIEBLER: For the amidoamine and the DMAPA could it be acceptable to state that -- what the weight percent concentration would be in the final product rather than at dosage mg/cm2?

DR. BAILEY: I think that's a more meaningful way to express it.

DR. BELSITO: Which is more meaningful?

DR. BAILEY: To express it in terms of parts per million on a finished product or whatever it happens to be, so that it's easier to, you know, figure out what your target is.

DR. LIEBLER: And then we express it the same way for the DMAPA and the amidoamine, in other words, 1 ppm, the other should probably be ppm?

DR. BAILEY: Well, we need to run the numbers and see what they look like.

DR. BELSITO: Yeah, I mean, I think however we express it needs to be the same.

DR. LIEBLER: Right. Yeah.

DR. BELSITO: The question is, the last time we discussed how we were going to set limits we had -- we used to set limits in what was supplied to industry and then we changed to setting limits to what was in the final consumer product, and so this would be a change back to the old way of --

DR. LIEBLER: No, I was actually suggesting that the ppm concentration limit be --

DR. BELSITO: In the final product.

DR. LIEBLER: -- in the final product, correct.

DR. BELSITO: Okay. Okay.

DR. LIEBLER: But then we just have the same units for both impurities.

DR. BELSITO: Yeah, I think we definitely need that.

DR. LIEBLER: It seems to me it would be just the easiest to interpret and understand.

DR. BELSITO: Okay. So if someone with mathematical skills could do those calculations for presence in final product for DMAPA and for amidoamine after we -- if, in fact, that's what the other group wants to do with this, so.

DR. BAILEY: Using the 0.45 mg/cm2 for the amido --

DR. LIEBLER: For the amidoamine.

DR. BELSITO: Right, 0.45 mg/cm2 for the amidoamine and 100 --

DR. LIEBLER: Parts per million.

DR. BELSITO: -- parts per million for DMAPA. And we'll table it. Carol, you'll re-query industry about that?

DR. EISENMANN: Yeah, I've queried them --

DR. BELSITO: I know.

DR. EISENMANN: -- but I'll query again. And I'll also ask if they have data, if they have (inaudible).

MS. BURNETT: I will go back and check the studies but I do almost guarantee that they're written as provided, so some of those may not be clarified anymore than they are.

DR. BELSITO: Yeah. That's fine, we understand, but what I would like you to do is just --

MS. BURNETT: Just reorganize --

DR. BELSITO: Reorganize and create a subheading of, you know, amidoamine concentration -- actual concentration not verified, and, you know, so we just know, you know, that -- and we may, after we see them and see that we have enough data where the concentration has been, you know, able to be verified, that we don't even want to include those studies where we don't know what is being done. But I think it would be -- you know, it's really easy if, you know, you have under dermal sensitization you see that we have no studies where we really know or, you know, we have 10 where we know and 2 where we don't know, well let's get rid of the 2 where we don't know, but right now it's just really hard.

DR. BAILEY: I assume we're focusing on this table only in those areas where the activity (inaudible) a bunch of these where they did give us activities.

DR. BELSITO: Yes. Yeah, so as you go through it, you know, if we already know then it's just a simple matter of calculating out, you know, what was there and, you know, so for instance on page 19, "daily doses of 0.2 ml of an 8 percent aqueous dilution of a liquid soap formulation containing 6.5 percent active CAPB," what I would like to see is, you know, 0.7 percent, daily doses of 0.7 percent active CAPB or daily doses of 0.2 ml of active 0.7 percent CAPB (8 percent aqueous dilution of)." You know, so I don't have to do -- I don't have to stop reading and do the calculation. This was a study, really, on 0.7 percent. You know, and if all of those -- the one above, 0.1 percent aqueous dilution of 6 percent active -- "0.06 percent active CAPB (1 percent aqueous dilution)." So, you give what was given in the report but you do a little bit more math and tell us what was actually studied.

DR. SNYDER: Then we can being the calculations of parts per million into the discussion.

DR. BELSITO: Right.

DR. LIEBLER: This term "activity" is just confusing.

SPEAKER: It's a terrible word.

DR. LIEBLER: It really is, because you're really talking about --

SPEAKER: (inaudible)

DR. LIEBLER: It's not like you're talking about -- it's not a free base of course, it's zwitterion, the compound we're interested in, but you're talking about the equivalent of the peers zwitterion component of the mixture as opposed to the other salts that accompany the product as it's produced, and if we could come up with a way to simply refer to the weight percent of that material, that would be the most unambiguous and informative way that we could represent it in the report. And I don't know if it's something that could be easily recalculated because I don't know if the activities vary in different --

DR. SNYDER: There's a range (inaudible).

DR. LIEBLER: -- material batches that are provided.

DR. BAILEY: Well, you know, I think we'll take this issue back to our science and support committee too because they bring a lot of experience and perspective on this to help us figure out how to present this, so.

DR. BELSITO: Just -- you know, I think that we have all the data. If we're going to be able to reach a conclusion we're not asking for anything more, we're just asking that the data be presented in a clearer fashion that really helps us, you know, set the restrictions on the impurities. Okey-doke, so we're tabling it and everyone knows what they're doing and no one who's doing it is happy doing it.

DR. MARKS: Okay. So, in the December, I was going to give December 2008, Christina, which you said we tabled it, but that's a year and a half ago. In the March meeting, we added some amidopropyl betaines in March of this year, but it really came down to, again, what's the issue with these contaminants, the DMAPA and amidoamine, and what's the threshold of sensitivity where we could establish a level which we felt comfortable, which would not be sensitizing.

MS. BURNETT: As you saw on the Wave 2, the LLNA study did arrive, I believe.

DR. MARKS: Actually, that's where I got into the -- so the Wave 2, that was electrons sent to us.

MS. BURNETT: Yes.

DR. ANDERSEN: Yes.

DR. MARKS: Could I take a look at that, because, somehow, I missed that. Did you all see the local lymph node assay in Wave 2? Did you, Ron? Yes, okay.

MS. BURNETT: We have it on the laptop here if you'd like to --

DR. MARKS: Well, I'll ask Ron first his reaction, because we had discussed a level for DMAPA. I'll begin there, which, as I recollect, was 100 parts per million.

DR. HILL: Before we even go there, can I take one step back from that?

DR. MARKS: Sure.

DR. HILL: There are a number of ingredients proposed to add here, and the amidoamines are then all different as opposed to cocamido. There's an expanded set of amidoamines that would need to be considered. I mean, we have some idea of what that mixture would be and the ones that are cocamido, but if you expand the list of ingredients, we're off that map.

DR. MARKS: So, you're uncomfortable with this expanded list?

DR. HILL: If we want it to move forward, I am.

DR. MARKS: So --

DR. HILL: DMAPA's in common, and that seems to be the prime culprit, but you can't rule out -- and if you go back and look at the minutes of the meeting from last March, I expressed concerns other than sensitization for that amidoamine component.

DR. MARKS: Right. I was going to ask you, Ron, about you were concerned about mutations, as I recall, and, so, I wanted to be sure that we captured your concerns there, and then I guess is the going back to page 1 and 2 of the present report, which the following ingredients have been added, are there ones in which are simple additions that we could do or would you like a more --

DR. HILL: I think somewhere in here, she's got a fatty acid table, and I think this was the one. So, yes, actually on page 55. So, there were ones that fit within that didn't extrapolate, and there were others that definitely do extrapolate, and if we want to continue to move this thing forward, assuming that we can, then my recommendation would be to consider removing that ones that do extrapolate and then we could press forward. But --

DR. MARKS: Okay, so --

DR. HILL: I think we could press. From my perspective, I think we could press forward. But if the will is to keep them in, then that changes the equation in terms of data for the amidoamine. The DMAPA is in common. That doesn't change. And then we step back to these are impurities, at low concentration, and an ingredient that's not used at very terribly high concentration. So, maybe the concern is not valid, but I always want to know when I say not valid or not particularly worrisome, what I really mean when I say that. What are the parameters?

DR. MARKS: So, if we go on page 1 and 2 on the report, can we go down this list, beginning with the almondamidopropyl betaines, and apricot and avocado, are there ones that you would delete from this? And I get the sense, yes, there are, so, can we -- and then, Ron Shank, I'd like you to comment, too.

DR. HILL: The ones that follow off the map, based on what's in them, are canola -- I'm sorry, I don't know how to say cupuassu, cupuassu. That's a foreign word to me.

DR. MARKS: Right.

DR. HILL: Meadowfoam, mink, olive, soybean.

DR. MARKS: So, that's the -- okay.

DR. HILL: Possibly sunflower, and then wheat germ. Those would all have amidoamine components that would not be in common with the CAPB.

DR. MARKS: Yes.

DR. HILL: And, in some cases, at high concentration and not just small ones, so, that's why I say maybe sunflower is okay because the amounts should be small.

DR. MARKS: Where's the sunflower on here?

DR. HILL: I'm not sure if it is. I'm just looking at the table with fatty acid components.

DR. MARKS: Yes, okay. No, it's not on the list. So, basically, you would have 1, 2, 3, 4, 5, 6, 7 ingredients which you would delete from the list on page 1 and 2 of the report, right at the introduction?

DR. HILL: I would, simply to be within the same parameters of the data that we're getting on the amidoamine sensitivities, including the LLNA, most recently requested, right?

DR. MARKS: Okay. Ron Shank?

DR. SHANK: Okay, I thought these were all natural oils and the fatty acids from that. So, I didn't have a problem with those.

DR. HILL: But without looking at the fatty acids, which I don't have a problem with any of those, I'm looking at the possibility of unknown pieces of information about the amidoamines that are derived from those fatty acids because we don't have any sense that all this happening to those amides in vivo is that they're being chopped back to the fatty acids, and, in fact, I don't think that's what's going on. So, then we have to ask the question, even though they're there as contaminants from production, presumably --

DR. SLAGA: I didn't have any problem with --

DR. HILL: -- what biological -- what's that?

DR. SLAGA: More like Ron, I thought they were all sufficiently related, but --

DR. MARKS: Now, when you say "unknown" amines, are you're saying that in the plural and not just amidoamide?

DR. HILL: Amidoamide is not one compound. Even with the --

DR. MARKS: Right.

DR. HILL: Even with CAPB, we've got an array of amidoamides based on the fatty acids that are present in the original coconut oil. So, when you produce it -- and, actually, it's kind of a flaw in the experiment of doing the sensitization, except that you're just testing mixtures. So, unless, pathologically, there would be some negative synergy, it's really not a big fear.

It's just -- what you know is you X-percent of amidoamine, that actually breaks down further into, based on the percentages of fatty acids that are there in the coconut oil in the first place, we could have as much as those percentages of those particular amides. But then if you expand to these oils that don't include the amides that are there in coconut oil, then any of your sensitization data is not inclusive; you're trying to extrapolate the compounds that have never been tested.

And, similarly, any mutagenicity, any tumor-promoting activity, any of that, you've never evaluated because you haven't expanded to those set of amides. So, you're extrapolating in ways that shouldn't be, particularly because the chain links get longer, there are additional double bonds, there are additional amino fatty acid structures that are not included in that coconut oil, and you have really no data at all. And if they were just entering the skin and being immediately chopped to the fatty acids, you've got no problem, but I don't think that's going on at all.

DR. MARKS: Okay. Ron? Tom?

DR. SHANK: My concern was primarily the skin sensitization and the two impurities. And we have skin sensitization data on humans; 11 studies on normal subjects on various products, and they were all negative for sensitization, every one of them. We have six studies on dermatitic patients, and they were all positive. And I'm wondering if we should base our concern on dermatitic patient data when all the studies on normal patients, normal volunteers, showed no sensitivity.

The second issue, I think, is the two impurities and trying to set a level, a quantitative level, as to what would be a safe exposure. There was a study done on DMAPA in water, and 100 ppm was not sensitizing. But other studies, where the material was given with amidopropyl

betaine or in cosmetic formulations, it was sensitizing as low as 1 part per million. So, since these ingredients are going to be used in formulations, I think it becomes very difficult to determine what level of these impurities would be sensitizing when we have the data on water, using the data on water.

Therefore, my recommendation would be to say that these ingredients are safe for use so long as they're formulated to be non-sensitizing, and I know we've discussed this before, but I don't see any way around this because the sensitizing potential for these two intermediates, these two impurities seems to be highly dependent on the solvent in which they're mixed and applied to the skin. And since we have various formulations, it's very difficult to predict, quantitatively, what level would be safe. I have other comments, but I'll stop at that one.

DR. MARKS: I agree with you, Ron. That's the same way that -- and we'll how the other team reacts to this, but that was what we proposed back in 2008 in December. The idea to formulate so it's not sensitizing at that time, was not well received. We do it for irritation actually quite frequently, but we've never done it for sensitization. I think the issues beyond what you've said, and the reference there is on page 33, I had the same concern, and water under parts per million at DMAPA was safe, but then when you actually mixed it with cocamidopropyl betaine, at least this author, in humans, felt that 0.5 parts per million was the safe limit. So, now, we're way lower, and I don't know if the assay is valid for that. That's one in which Dan had a concern, whether there were valid assays for DMAPA and amidoamines, so, on top of the sensitization issue, and these are actually going to be in these detergents or surfactants that we have assay issue. If we go by the endpoint formulated not to be sensitizing, then we've addressed both of those issues.

Did you get a sense from the local lymph node assay that at least at what limit there, because that's going to come up in the discussion tomorrow, would be a "safe level" with the local lymph node assay for amidoamine? But, again, that doesn't speak to the relevance of what if you put it in cocamidopropyl betaine or these other surfactants?

DR. SHANK: That's right. That in vitro assay, I couldn't translate the data from the in vitro assay to the cosmetic formulations. So, the in vitro assay was interesting, but it didn't help me determine a safe level for any of these impurities as far as non-sensitizing levels in cosmetic formulations.

DR. ANDERSEN: Jim and Ron, my only concern about saying that I don't know that I had any better ability to make that leap, but the risk assessment that was provided by the industry using the quantitative risk assessment approach that Anne Marie Api presented last year, you can fit the LLNA data into that model; you can fit the direct sensitization data into that model, and they presented a -- I don't even remember what NESIL stands for, but their effort --

DR. MARKS: No Observable Effect Sensitization Level.

DR. SHANK: No expected.

DR. MARKS: No expected, okay.

DR. ANSELL: A safety factor was worked into this QSAR, which included accounting for matrix effects. An extra threefold safety factor.

DR. ANDERSEN: As is, yes, the way the model works, it can include that, and they did. So, that's another way to approach it using something shown to be effective for a group of otherwise fairly good sensitizers, namely fragrances. And it was applied to this with a reasonable margin of safety. So, I think that's another way to come at it.

DR. MARKS: Was that in this report with a level for both of these contaminants? Did I overlook that?

DR. ANDERSEN: No.

MS. BURNETT: No.

DR. ANDERSEN: Was it in Wave 2? The risk assessment, I think, was part of the Wave 2 Package.

MS. BURNETT: The --

DR. ANDERSEN: It was the risk assessment, I think, was part of the Wave 2 Package.

DR. MARKS: Okay.

DR. ANDERSEN: It came in very late in the process.

DR. MARKS: Okay. That's why I --

DR. SHANK: (inaudible)

DR. MARKS: Okay, yes. Please?

DR. SHANK: That's for one of the impurities, then are you going to do the same thing for the other one?

DR. ANDERSEN: Okay. I thought for DMAPA, we already had established a threshold level below which you weren't concerned.

DR. SHANK: Well, that --

DR. MARKS: I think that was the Belsito Team. They suggested that.

DR. SHANK: Yes, I'm not very happy with that one.

DR. ANDERSEN: Okay.

DR. SHANK: That one has artifacts in it.

DR. MARKS: So, would you introduce yourself?

DR. GUTELL: Yes. Steve Gutell from Unilever. I'm with the Safety Environments Assurance Center in the U.K. I just wanted to pick up on a point on the local lymph node assay, that we're talking about extrapolating from one system to another, and I wanted to highlight it's a in vivo to in vivo extrapolation that's described in the QRA, the Quantitative Risk Assessment. So, it was just a point of clarification that we've referred to in vitro assays and QSAR models, and this doesn't fall into either of those two descriptions. It is an in vivo test, it's an animal study, and the QRA basically correlates the output from that study with human HRIPT data.

So, the fact that there is a correlation involved is true. So, there is an element of mathematical conversion, but it's in vivo to in vivo, and that's where the safety assessment factors come in. So, it was just a point of clarification. That was all.

DR. MARKS: Okay, so, I see on the front page the suggested limit based on the QRA is 1.5 percent, amidoamine in CAPB. What is the limit? I didn't see the second wave. I apologize. That's why I asked to have clarification this morning with Kevin. What was the conclusion of that?

DR. ANSELL: The suggested limit is 1.5 percent amidoamine in CAPB, which was derived from the 90 the percentile product exposure, a (inaudible) dermal exposure based on the CIR report itself and assuming 100 percent dermal penetration.

DR. MARKS: Do you think a similar Quantitative Risk Assessment could be done with DMAPA? And can you do that with available data or do you need to go back and do a local lymph node assay, again going in vitro or in vivo to in vivo?

DR. HILL: And before you comment, one thing I had scribbled on here is if this is the same risk assessment, it says, "The assumption is that the exposures are rinse-off products," but I wrote, but are they, because, based on use, I wasn't sure that they all were. So, there was a margin of safety threshold based on rinse-off that I'm not sure we ever fully addressed.

DR. SHANK: We have human studies, in addition, that agree with the Quantitative Risk Assessment. So, I have no problem with that. And if you want to use the data, they are already there. But I don't think we need to place a number on safe exposure for the two impurities, and that's what was suggested last time. And I don't want to go there.

DR. MARKS: Okay. So, Ron, you wouldn't like to see in the conclusion safe levels? You still like the idea of safe when formulated not to be sensitizing and then in the discussion, we could elaborate what are either in this case the QRA safe level for amidoamine and discuss the DMAPA with the caveat it was the suggested safe level was much less than 100 parts per million.

DR. ANSELL: Well, the conclusions are always bounded by current applications --

DR. MARKS: Right.

DR. ANSELL: -- and concentrations, and the current concentrations, at least for this material, would be typical because they'll well below the 1.5 percent.

DR. MARKS: Yes, maybe I interpreted the increase data we received differently, but when I looked at the use concentrations on Table 5, page 57, 58, and 59, the only one in which the RIPT would suggest that there was market differences, the RIPT, I believe from page 21, would suggest that for cocamidopropyl betaine at 1.8 percent is the safe level of that product. All the rest, well, the almond, the RIPT was 1 percent and the use is up. We don't have the use concentration. For capryl, it was 1.7 percent, RIPT was okay. Kind of addressing the olive one that you mentioned earlier, Ron, we do have an RIPT of 1 percent, which was okay in that. The olivamidopropyl betaine.

So, I kind of fall back to basically what's happened here is you have the RIPT that shows that there are these safe levels, and that's where you should be, and those are non-sensitizing with a final product. It kind of gets back to what Ron said. And what we, in December 2008, felt the conclusion could be safe formulated not to be sensitizing. Ron Hill, Tom, what do you feel about that conclusion? That's where I arrived independently, Ron.

DR. SLAGA: Good. Well, I like it, too, and I think, however, we will have a lot of debate tomorrow, and it would be nice to know where they're standing right now, too.

DR. MARKS: Okay.

DR. BERGFELD: Did you hear from Ron?

DR. MARKS: Pardon? Yes, Ron Hill?

DR. HILL: I was just going to ask, and nobody but me is concerned about activities other than sensitization for those amidoamine?

DR. MARKS: Well, that was the second point, is whether we were going to also tomorrow recommend that seven ingredients be deleted from the expansion of this report?

DR. HILL: I mean, I'm not sure my concerns are totally resolved for cocamidopropyl betaine, but, anyway, if nobody else is concerned but me, I'll just go on that record, it is already on the record, and let it drop.

DR. MARKS: Oh, no, I think if you have a safety concern, it needs to be discussed tomorrow, and even though it sounds like Ron and Tom weren't concerned about these other fatty acids, you were about the amides or the --

DR. HILL: Right, it's not the fatty acids at all.

DR. MARKS: No.

DR. HILL: It's the amides, which I expect to mostly stay intact in vivo. So, really, we're counting on their presence as a minor impurity and skin penetration shouldn't be huge. That's really what it's resting on, but then we're not capturing the biology of these directly; we're only capturing the biology of the betaines and whatever toxicology is there is only being captured coincidentally as a result of studying the parent compound with the impurities present. We don't have any direct information about the activities that these compounds -- and, I mean, ideally, the impurities should be there at level zero, but it seems not to be the case.

DR. MARKS: Okay. So, although I will not be speaking first, I have a feeling that I won't be seconding the motion from the Belsito Team, but we'll find out tomorrow. So, I will move or at least petition for these ingredients would be safe as long as they're formulated to not sensitize since sensitization is the main concern, and we have difficulty establishing a safe limit with the DMAPA and amidoamine, both on -- unless the valid assay, and we really haven't discussed that these are true, valid assays, and, then secondly, more importantly, that we can't relate the data we have now, transpose that into what we feel are safe limits. And then the editorial, I'm not sure it's editorial, but that I will recommend that we eliminate these ingredients, Ron Hill, that you have concern with, and, Ron, I'll express the concern of the unknown amids or amides, but I'll let you expound upon that tomorrow at the discussion, if that's okay.

DR. HILL: And I guess if the only concern is sensitization, then even making those extrapolations isn't all that problematic to me.

DR. MARKS: Okay.

DR. HILL: I just don't feel 100 percent confident that we know what we need to know about the amidoamine impurities.

DR. MARKS: Okay. So --

DR. HILL: But if it's only sensitization, then making that extrapolation really shouldn't be problematic. I just --

DR. MARKS: Are there any other toxicologic concerns, Ron, you have, other than sensitization with these amides? Ron? Tom? Are there any other concerns you have?

DR. SHANK: No. I had one correction. It wasn't a toxicological concern, but on nitrosation. Before we leave this document, I'd like to make that comment.

DR. HILL: Okay, so, Ron Hill, it seems like sensitization is really the main concern. And since our conclusion would be formulate to not sensitize, then that would address it. So, can we leave those other ingredients in the report then rather than remove them?

DR. MARKS: Yes, okay.

DR. BERGFELD: I think it's worthwhile to put Ron Hill's concerns on the table and then explain, because sensitization is the endpoint that one is looking for, that whatever you've said now in your conclusion, allow them to stay, but I think I'm concerned with those, too, as a non-chemist, adding all of these chemicals that are a little bit unrelated. They're not simple.

DR. HILL: Yes, because at least one would hope that, knowing that that impurity is in there in all of the mutagenicity and tumor-promoting activity studies that we've at least picked up any signals that if they were studied with those in there and all those studies were done, if something was going to be prominently a concern, we would have picked that up. But, as soon as you extrapolate to the ingredients that don't have those potential amidoamines in there, now, we're into the realm of unstudied. They've not even been studied as an impurity present. That is still a concern.

DR. MARKS: Well, Wilma, what I will do is bring that out when we go into the discussion point, and it may end up either in the conclusion or the discussion because sensitivity is the main concern, and at least our conclusion addresses sensitivity, and even though we have some concern about the other amides, that that's been addressed with a conclusion.

DR. HILL: I think if I could prolong the discussion for just a bit, Ron Hill, could you just take a gander at Figure 1, and, Wilma, as well? In the Wave 2 material, Bart Heldreth prepared the stick figures for everything that's listed in this report. And the only thing different as you look at Figure 1 is the R group, and that's huge. That's huge. Because R is not just fatty acids that are there in normally-processed in vivo, it's attached to this dimethylaminopropyl amine. And you wouldn't expect that those amines would be biochemically at all related. They should have a totally different biochemistry than the fatty acids. And we have no capture of what that biochemistry might be. The only information we have on that is that it was present, and it was, again, an array because with that R Group and you look at the fatty acid compositions, the cocamidopropyl betaine, there's an array of fatty acids. And that's captured in one of her tables; it's Table 4. So, you can find out exactly what the fatty acid composition of cocamidopropyl betaine is.

There is no way of knowing, quite frankly, that even though that those amidoamines were produced in the original material that they're consistently present in the same percentages in the finished product. But if we have a total amidoamine impurity of 1.5 percent, it may be 1.5 percent times up to 15 percent for capric, up to 41 to 51 percent for lauric, and like that. So, you would take that 1.5 percent and multiply by those percentages, and, presumably, generate what the maximal level of those individual amidoamines would be there. Okay, and, so, then, those have been studied as impurities, depending on what the material was that was put into those bioassays for mutagenicity, carcinogenicity, whatever, only as impurity. We don't have data for those as primary components in terms –

DR. ANDERSEN: Right.

DR. HILL: -- in terms of what their toxicology is. But they're not fatty acids anymore.

DR. ANDERSEN: But at whatever level, those impurities were present, the stuff is flat-out not needed.

DR. HILL: Yes.

DR. ANDERSEN: Generally, it's not --

DR. HILL: No tumor production.

DR. ANDERSEN: The bacteria (inaudible) is not carcinogenic.

DR. HILL: Yes, all of it.

DR. ANDERSEN: So, if the empirical finding is the absence of an effect where it breaks down, as I understand it, is we have no data on amidoamine or DMAPA in any of these others because nobody has looked.

DR. HILL: Well, DMAPA shouldn't matter because that's the same.

DR. ANDERSEN: Okay.

DR. HILL: If it's there, it's there, it's one single compound.

DR. ANDERSEN: Okay, so, the amidoamine is --

DR. HILL: But if you generate from a different biological oil, so, you have a different array of a fatty acids that extend beyond what's there with the coconut oil, then you now have compounds that have never been studied unless somebody else has studied them separately, and we haven't captured that toxicology in the report at all, and it may not even be out there, or, then again, it might be.

DR. MARKS: Okay. We're back to the discussant points. Ron Shank, you had a comment about the nitrosing?

DR. SHANK: Yes. On page 43, the last paragraph on nitrosation is factually incorrect. These compounds are secondary amines. So, we need to change the wording. The last paragraph, the second sentence starts, "CAPB is a quaternary ammonium cation, and that is not a substrate in N-nitrosation." That's irrelevant. The compound contains a secondary amine nitrogen that may be a substrate in nitrosation. And, therefore, "The expert panel recommended this ingredient should not be included in cosmetic formulations." Somehow, something got --

DR. MARKS: Left out.

DR. SHANK: Changed, in cutting and pasting. These compounds do contain secondary amine nitrogens, which are nitrosatable.

DR. HILL: The impurities do.

DR. SHANK: No, the parent compound.

DR. HILL: No, they don't.

DR. SHANK: Okay, what's that?

DR. HILL: That's an amide. So, are amides nitrosated?

DR. SHANK: Yes, they are.

DR. HILL: They are? So, amides are different than nitrosamine?

DR. SHANK: Yes, that should be nitrosamide. Yes.

DR. HILL: Yes, okay.

DR. MARKS: Thanks, Ron.

DR. SHANK: Sure.

DR. MARKS: We'll capture that as an editorial change.

DR. SHANK: Okay.

DR. MARKS: Christina, you've gotten that? Okay. Any other comments? So, tomorrow, I will discuss it from the point of view formulating these so that they are not sensitizers, and then under the discussant points, we'll obviously explain why DMAPA and amidoamine were not quite ready to a safe level, and then, also, we'll talk about unknown amides in the seven ingredients. Ron, I'll just bring that up as a discussion point, and then, also, your editorial on page 43, Ron.

DR. BELSITO: This initially started as a re-review and at the March meeting we decided to add a number of related amidopropyl betaines and those are listed on pages 1 and 2 of the document, and I won't read all of them. At this meeting the sticking point here was the sensitization potential of contaminants, specifically amidoamine and DMAPA. We had become comfortable of 100 parts per million of DMAPA, but we weren't certain as to the limits for the amidoamine contaminant. Since we last looked at this, industry has provided us with an LLNA and they've also reprovided us with data on HRIPT for several related amine products and essentially I think answered the questions that we had regarding the sensitization and where we could set limits on the contaminants.

Having said all that, when my team was going through all this data, one of the issues became what were we actually being given in terms of sensitization and irritation data? Was it the active or was it a certain percent of the compound as provided which is 30 percent cocamidopropyl betaine? So we had problems when we were looking at all of the data being assured that in fact we were going to be setting safe limits because cocamidopropyl betaine in its own right can be a sensitizer. That was one issue.

The next issue was when we're setting the limits we typically in years past had set limits on the amount of contaminant in the product that was supplied to industry, but in the most recent past we've been setting limits on final product so that the issue became are we going to continue setting limits on the final product or are we going to set limits in what's supplied to industry?

The last issue that arose was as a result of the QRA you look at the dose per unit area and is that the way we wanted to set the limits for both amidoamine and DMAPA or did we want to set limits as parts per million that were perhaps more convenient and understandable to industry?

With those three issues we're grappling with, we would like to go back and see the studies presented as either this was the actual percentage of cocamidopropyl betaine that was tested or in fact we don't know because it just said 2 percent cocamidopropyl betaine and it didn't say whether it was active or not and split those studies where it was not known out to a separate subsection. We were recommending that this document be tabled at this point, that the author go back and separate that out, clarify when it's known what was actually tested or it's not known whether it was 2 percent active or 2 percent of the 30 percent, that we make a decision at least at this meeting how we want to set the limits on the impurities, whether we want to set it in the final product or what's supplied to industry, and finally, how we want to set those limits, as parts per million or
as dose per unit area, so that we're recommending a table at this point, but no additional information. We thought we had all the data we needed. We just need to sort it out a little bit better.

DR. MARKS: We also had a robust discussion about sensitization and also about the ingredient. So if I go on pages 1 and 2 as to what ingredients would be added on and recall since this is reopened that these should be no- brainers. There was concern about what are the unknown amides produced by seven ingredients on pages 1 and 2 as part of the introduction lists the ingredients, canolamidopropyl betaine, cupuassuamidopropyl betaine, meadowfoam, mink, oliva, soya, wheat, all of those we were concerned about what amides might be produced in the metabolism of those.

The second issue was for our team the discussion on trying to arrive at a safe level of these contaminants, the amidoamine and the DMAPA, and the quantitative risk assessment certainly appeared to support the safety of the amidoamine at 1.5 percent. When we relooked at the DMAPA data, yes, at 100 parts per million in water it did not induce sensitivity, but when you added the surfactant agent, the cocamidopropyl betaine, the number of reactions increase significantly and the authors of that paper, I think it's page 33 in the report, had actually recommended 0.5 parts per million, so we again were not quite sure whether we should put that limit on it.

Then in the previous meeting in December there was concern about whether these assays were actually valid for DMAPA and amidoamine and, Dan, I'd like you to comment about that because I believe you were the one who actually raised the issue of assay validity. So we dealt with these since a number of these when you look at the use concentration and there were RIPTs done with these various ingredients at concentrations were similar to the use concentration felt that we propose as we did back in 2008 that this is breaking new ground, but that these ingredients should be formulated in the final product to be nonsensitizing. So that's how we handled the sensitization issue, we left it to the manufacturer to formulate products with these ingredients which were nonsensitizing and obviously would have to have the data to confirm that.

DR. BERGFELD: Dan, do you want to reply?

DR. LIEBLER: Yes. When I raised that question I really was not so much skeptical as just curious as to what the analytical methods were because if the workup induced hydrolysis of the amide you could get artificially high levels of these. The reason I haven't pushed this issue any further is because I think you would have to have either pretty severe conditions or acid or base to cleave these amides and it just doesn't make any sense in any analytical method I could imagine that would be used here so that I decided to let that drop. I don't really have a concern about that at this point.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: I think that kind of conclusion is punting by the panel because we could then just, every ingredient, say once we're assured that it's either not absorbed so there are no internal problems or it's absorbed and there are no significant tox problems say safe when formulated not to be sensitizing or irritating and that could be our conclusion for everything. What

we're asked to do, this is an issue and we're being asked to resolve the issue and I don't think we should punt on it. I think we should resolve it and come up with a level for these contaminants that we feel are safe in cosmetic products.

I think when you're dealing with irritation, that's a different story because it really depends on the pH, it depends on so many other factors what's put into it that in fact that's really not a punt because we acknowledge that there are multiple factors. You can take lactic acid at 12 percent and if you buffer it it's not an irritant. If you reduce it to pH 2 and it's lactic acid then it's going to be irritating. You can't put a concentration range on that, but you can put a concentration range on sensitization and I think we need to do that.

DR. BERGFELD: Dr. Shank?

DR. SHANK: I don't see how we can come to a number, a concentration for these impurities that would be nonsensitizing since the vehicle has such an enormous effect, more than a hundredfold effect, 100 ppm for one in water is nonsensitizing, but 5 ppm is if it's in formulation and how you're going to pick a concentration limit for these two impurities without factoring in the formulation seems to me an insurmountable problem. Therefore, I would support formulating to be nonsensitizing because I don't see how we can come up with the number to limit the concentration of these impurities.

DR. BERGFELD: Dr. Hill?

DR. HILL: Let me just add on the amidoamine that we're not talking about one impurity. Even with the parent cocamidopropyl betaine it's a mixture of fatty acids to the amidoamines that can result are presumably reflective of that array of fatty acids that's present. So if you expand the other ingredients, we similarly have a raise of potential amidoamine impurities. If you were to set limits, what it would boil down to is not only, and you say vehicle, but it's actually including the parent ingredient, so the betaines actually change the sensitization potential of these potential impurities that it would be difficult to carry out the studies with each one of those possible amidoamine impurities in a way that would be able to allow us to set limits on this what's really not one impurity, but an array of impurities in each of these ingredients. So that's where we landed on this dangerous ground, formulated to be nonsensitive, but what we're really doing I think if we did that is put the onus back on the manufacturer to test their formulations in their mode of manufacture.

DR. BERGFELD: Dr. Slaga?

DR. SLAGA: It's hard to dictate what vehicle to use. The only way around it to me is that you go to such low impurity limits that you don't have any potential vehicle effect, but we don't know that right now. That's the problem. We only have this one comparison.

DR. BERGFELD: Dr. Klaassen?

DR. KLAASSEN: I don't have anything to add right now.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: If you look at that study, this is the Angelina that we're talking about, it was 20 patients who had confirmed allergy to both DMAPA and cocamidopropyl betaine so that it's not clear that the increased number of reactions you see when you put DMAPA and cocamidopropyl betaine is due to the fact that they're reacting to DMAPA. It may just be picking up those patients who are also allergic to cocamidopropyl betaine.

DR. SHANK: We have 11 studies on volunteers on healthy skinned individuals, 11 studies on formulations, and every one of them was negative. Eleven negative studies. The only positive sensitization studies were in dermatitic patients. So I think there is a history of use of formulations where there is not a sensitization problem.

DR. BERGFELD: Dr. Liebler, do you have any additions?

DR. LIEBLER: I didn't have a response to what Dr. Shank just said, but I would like to respond to something that Dr. Hill just mentioned about different fatty acid chain lengths contributing to these amides. This is a generic problem we're going to have particularly when we consider ingredients that are derived from these natural product oils where we're always going to have essentially mixtures that will be dominated by one chain length for one product versus another somewhat different chain length for another product. I think that expecting us to be able to evaluate all of the individual ingredients as if they were different is probably unrealistic and unachievable for this panel. So I would propose that we look at it more that these are ingredients which have common average properties across a range of chain lengths and that it's not unreasonable to say that we can arrive at a number based on the test data that are provided to us, that if it's the amidoamine, what we're not talking about is not a pure compound, but with the understanding that we have a series of related compounds of similar chemical and biological properties that average out to be 1.5 percent or whatever the number might turn out to be.

DR. BERGFELD: Dr. Snyder?

DR. SNYDER: I just want to reaffirm that I think that tabling would help us reorganize the data and I'm still unclear and as Don indicated our team was very unclear as to exactly what we're looking at even in the use table. So when we see 6 percent in the use table we don't know if that's 6 percent of a 30 percent active or what. Unless somebody can verify that for us, we couldn't get it verified yesterday, so we're still a little uncertain as to exactly what we're looking at all the way across from the sensitization studies through the report and in the use and concentration table.

DR. MARKS: I think that's fine to table it. I think this discussion was good and robust so that we had an exchange of ideas in terms of how to handle this.

DR. BERGFELD: Are you seconding to table?

DR. MARKS: So I will second to table.

DR. BERGFELD: There is no further discussion on a second for table and only a vote. Calling for the vote to table, please indicate by raising your hands. It will be tabled. Thank you very much. I will ask for any further right now however. Dr. Belsito?

DR. BELSITO: I think it's clear that it's tabled and we've asked the writers to go back and give us either what was the actual concentration tested or in case that it wasn't known to separate that out. We've asked Carol to go back and query industry to make sure that what they're giving us as to percent ranges are active. As Paul was pointing out, there is a vaginal douche use that's reported at 6 percent and when you look at the data for irritation that would be severely necrotic, so we doubt that that in fact is what's being used. It's not 6 percent active, it's 6 percent of 30 percent so that we need clarification on that. But at the end of the day when this comes back to us, it sounds like there is a significant discrepancy in the way the teams want to approach this. Is it worth asking industry to do an LLNA on DMAPA and coming up with a QRA for that one as well?

DR. BAILEY: The LLNA on DMAPA, is it strictly derived from the coconut oil fatty acids, cocamido?

DR. EISENMANN: DMAPA is the starting material for all of them. I don't think it matters.

DR. HILL: But it's a mixture. It's not one compound.

DR. SNYDER: It is one compound.

DR. HILL: Wait a minute. You're talking about DMAPA and I'm on amidoamine. I'm sorry.

DR. MARKS: Don, I like your suggestion. I'd like to see a quantitative risk assessment with DMAPA also and pull that together. I'm not sure that one study would be enough for our team to feel comfortable with it, but again, relooking at this data with the exchange of ideas. I think the other thing that we need to consider in tabling it is those seven ingredients. Are we really going to include those in the final safety assessment?

DR. BERGFELD: Dr. Bailey and then Dr. Hill.

DR. BAILEY: We can take this back to the Science and Support Committee and look at a QRA on it and see what we come up with. I think that we're also comfortable with setting a limit in a finished product. We've been thinking in terms of 100 parts per million for DMAPA and.3 percent for amidoamine. Perhaps to bridge the concern about additional sensitization there could be some language in the discussion that would talk about these cautions, but we're comfortable with the control of setting some kind of limit in the finished product.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: Jim, could you repeat the seven? It was canolamidopropyl betaine, cupuassamidopropyl betaine?

DR. MARKS: So it's the canola, the cup, meadowfoam, mink, oliva, soya and wheat.

DR. BELSITO: Why did you pick those? It's not clear to me.

DR. BERGFELD: Dr. Hill?

DR. HILL: If you look at the fatty acid components of those parent oils, there are significant concentrations of fatty acids in those that are not present in the cocoanut oil. What I was going to add is if there's a desire to add those ingredients back, what is the biochemical mechanism by which sensitization develops, and I will again raise is sensitization the only issue with those impurities? Have we completely captured what is known about amidoamine biology within that array of compounds that can rule out at the levels we're likely to be getting biologically anything like tumor promotion activity?

DR. BELSITO: Some of these reviewed.

DR. BERGFELD: Dr. Liebler?

DR. LIEBLER: I think one of the issues that probably will come up again and again with some of these more exotic oils is uncertainty about their composition, and maybe the data are out there some place and it's known, but it's not data that's easily accessible to the panel at least that I'm aware of. It would be valuable if a set of data or data sets that describe the fatty acid composition of these oils could be made available, if not a data-dredging exercise, it could be perhaps a project for industry.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: I was saying that it seems to me that some of these like mink oil, haven't we reviewed that? So some of these we've reviewed and others, we should know the fatty acid composition of these, so to me I didn't see the need to exclude any.

DR. HILL: Having reviewed the fatty acids is not the same as having reviewed the amidoamines that are produced from it. That's the thing. I don't think we would regenerate those fatty acids, and even if we didn't, I wouldn't be concerned about that. It's the fact that they're converted to the amidoamines that have unknown biochemistry or potentially unknown biochemistry that is of concern to me. At least if you're confined to the compounds that have the fatty acid mixtures consistent with what's in cocamidopropyl betaine, you're presumably captured in the toxicology studies that were done at least by virtue of the presence of the impurities that were there in the study products any signals that might have arisen at the levels that are delivered. But if you extrapolate, you're not necessarily picking any of that up so that that for me raises red flags all over the place as it pertains to what we're saying is safe.

DR. BERGFELD: Christina?

MS. BURNETT: Just to inform the panel, I'm also currently working on a rather large vegetable oil report that includes all of these constituents that you're asking to remove. I have fatty acid profiles on all of them so that in June hopefully you will see the first round of this report.

DR. BERGFELD: Thank you. Don?

DR. BELSITO: I guess I'm still confused as to why you picked these seven. Why olivamido, for instance, and not palmitamido?

DR. HILL: Can you see the tables on pages 54 and that have the fatty acid distributions? What I really did is I looked at what was known to be present in coconut oil and then looked at the other oils and made the conclusions as to what amidoamines might be present based on producing those amidoamines from those other oils and which ones are significantly extrapolating by virtue of significant concentrations of fatty acids that are not present in coconut oil. I arguably could add avocado to that list, but a pretty modest percentage of one fatty acid that even in the amide form didn't seem to worry me that much, but even you could argue that that eighth one could potentially be excluded as well.

DR. BERGFELD: Can we put on hold the exclusion of these particular ingredients until we see the report in June that profiles their fatty acids?

DR. HILL: Yes, but still I would have the discomfort of knowing what is the mechanism by which sensitization arises again and, again, if that's the only concern.

DR. BERGFELD: Dr. Liebler?

DR. LIEBLER: May I ask Dr. Hill to clarify? Are you worried for example about the uncertainty of sensitization different between for example a caproic C6-derived amidoamine versus let's say an oleic or C20?

DR. HILL: Yes.

DR. LIEBLER: That's the point that you're concerned about?

DR. HILL:

DR. LIEBLER: And that the different oil mixtures would have different percentages of those derived ingredients and that would be a problem from your point of view?

DR. HILL: Yes.

DR. BERGFELD: Dr. Slaga?

DR. SLAGA: We may never know the actual mechanism of sensitization, so we can't go on that type of approach without going into something we can deal with.

DR. MARKS: I think we've moved to table it, so we can relook at these seven ingredients then and reconsider that along with determining the level of the amidoamine and the DMAPA and how to deal with the sensitization issue.

DR. BERGFELD: Nicely said, Dr. Marks. We're going to move on. The panel has expressed its many concerns and has some debatable points that need to be clarified at the next meeting. Thank you very much.

Tentative Report

Cocamidopropyl Betaine and Related Amidopropyl Betains as used in Cosmetics

August 30, 2010

The 2010 Cosmetic Ingredient Review Expert Panel members are: Chairman, 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 F. Alan Andersen, Ph.D. This report was prepared by Christina L. Burnett, Scientific Analyst/Writer.

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ABSTRACT

The data on cocamidopropyl betaine (CAPB) and related amidopropyl betaines named in this report are similar in their chemistry, in particular with respect to the presence of 3,3-dimethylaminopropylamine and cocamidopropyl dimethylamine impurities, which are known sensitizers. If these impurities are limited, these ingredients are safe for use in cosmetics.

INTRODUCTION

A safety assessment for cocamidopropyl betaine (CAPB) was published by the Cosmetic Ingredient Review (CIR) in 1991.[1] At that time, the CIR Expert Panel concluded that cocamidopropyl betaine is safe for use in rinse-off cosmetic products at the current levels of use. The concentration of use for products designed to remain on the skin for prolonged periods of time should not exceed 3.0%. The latter is expressed as a 10% dilution of a full-strength cocamidopropyl betaine solution that has an activity of 30%.[1]

Based on new published data that described sensitization in patients from use of rinse-off products, new uses in aerosol products, and a substantial increase in the number of uses, the CIR Expert Panel reopened the final report on CAPB in 2007. The following report is a compilation of new data and data from the original safety assessment on CAPB and related amidopropyl betaines. While few data are available on related amidopropyl betaines, because of chemical similarities to CAPB, the following ingredients have been added to the safety assessment:

- almondamidopropyl betaine,
- apricotamidopropyl betaine,
- avocadamidopropyl betaine,
- babassuamidopropyl betaine,
- behenamidopropyl betaine,
- canolamidopropyl betaine,
- capryl/capramidopropyl betaine,
- coco/oleamidopropyl betaine,
- coco/sunfloweramidopropyl betaine,
- cupuassuamidopropyl betaine,
- isostearamidopropyl betaine,
- lauramidopropyl betaine,
- meadowfoamamidopropyl betaine,

- milkamidopropyl betaine,
- minkamidopropyl betaine,
- myristamidopropyl betaine,
- oatamidopropyl betaine,
- oleamidopropyl betaine,
- olivamidopropyl betaine,
- palmamidopropyl betaine,
- palmitamidopropyl betaine,
- palm kernelamidopropyl betaine,
- ricinoleamidopropyl betaine,
- sesamidopropyl betaine,
- shea butteramidopropyl betaine,
- soyamidopropyl betaine,
- stearamidopropyl betaine,
- tallowamidopropyl betaine,
- undecyleneamidopropyl betaine, and
- wheat germamidopropyl betaine.

CHEMISTRY

Definition and Structure

The general structure of amidopropyl betaines is as shown in Figure 1, where RCO- represents the fatty acids derived from various oils.[2] For example, RCO- represents the fatty acids derived from coconut oil in CAPB (CAS No. 61789-40-0). Table 1 presents the definitions and structures of CAPB and related amidopropyl betaine ingredients.

Technical names for CAPB and its related amidopropyl betaines are found in Table 2. There are numerous trade names and trade name mixtures containing CAPB and its related amidopropyl betaines.[2]

Physical and Chemical Properties

CAPB is a clear, pale yellow liquid of medium viscosity (300-600 cps) with a slight fatty odor.[3;4] CAPB has a boiling point of 230°F, a specific gravity of 1.04 relative to water, and no flash point.[5] CAPB is soluble in water, ethanol, and isopropanol and insoluble in mineral oil.[3]

CAPB is supplied as a solution in water and with sodium chloride (see Table 3). The concentration of CAPB in such

supplied material is described by its activity.[6] The concentration of cosmetic-grade CAPB ("active" concentration) is what is left in the supplied solution after water (62% - 66%) and sodium chloride (4.6% - 5.6%) have been accounted for, which is ~30% of supplied solution. In this report, unless a concentration has been reported as being "active", a concentration of CAPB in solution will be calculated since it is unclear in some cases which is the true concentration that was tested.

Commercial grades containing concentrations of CAPB greater than 30% may contain solvents, such as propylene glycol. Although most commercial grades contain sodium chloride, low-salt products also are available. The concentration of sodium chloride in cosmetic grade CAPB ranges from 4.0% to 6.0%. Cosmetic grade CAPB may also contain a maximum of 3.0% glycerol.[1]

The fatty acid compositions of the oils that are components of the additional amidopropyl betaines described in this report are presented in Table 4.

Method of Manufacture

Figure 2 depicts the formation of CAPB through reacting coconut oil fatty acids (coconut oil or hydrolyzed, glyceryl-free coconut acid) with 3,3-dimethylaminopropylamine (DMAPA), which yields cocamidopropyl dimethylamine (amidoamine or dimethylaminopropyl cococamide). The amidoamine, a tertiary amine, is then reacted with sodium monochloroacetate to produce CAPB. In Figure 2, R represents the coconut fatty acid chain that varies between C-8 and C-18.[1;3;7-10]

Supplier information provided to the Personal Care Products Council [formerly, the Cosmetic, Toiletry, Fragrance Association (CTFA)] indicated that babassuamidopropyl betaine, coco/sunfloweramidopropyl betaine, cupuassuamidopropyl betaine, isostearamidopropyl betaine, lauramidopropyl betaine, meadowfoamamidopropyl betaine, oleamidopropyl betaine, ricinoleamidopropyl betaine, and wheat germamidopropyl betaine are manufactured in the same manner as CAPB.[11] Manufacturing data on the remaining amidopropyl betaines were not provided.

In cupuassuamidopropyl betaine, the intermediate is cupuassuamidopropyl dimethylamine, which can be found at a maximum level of 0.2% in final product. The DMAPA level in final cupuassuamidopropyl betaine product is 0.05%. In meadowfoamamidopropyl betaine, the intermediate is meadowfoamamidopropyl dimethylamine (MF-DMAPA), which can be found at less than 0.5% in final product. The manufacturing process for meadowfoamamidopropyl betaine exhausts DMAPA. The levels of DMAPA and amidoamine were reported to be below 0.0002% (the detection limit) and < 0.5%, respectively, in babassuamidopropyl betaine, coco/sunfloweramidopropyl betaine, isostearamidopropyl betaine, lauramidopropyl betaine, oleamidopropyl betaine, ricinoleamidopropyl betaine, and wheat germamidopropyl betaine.[11]

Impurities

No *N*-nitroso compounds were detected in samples of commercially supplied CAPB.[12] CAPB samples with and without internal standards of *N*-nitroso compounds were analyzed using gas chromatography with a thermal energy analyzer (TEA). CAPB has a secondary amido group that is susceptible to *N*-nitrosation to an *N*-nitrosamide. Although a highly sensitive analytical method failed to detect traces of volatile *N*-nitrosamines in samples of commercial CAPB, this result does not exclude the possibility that in the presence of *N*-nitrosating agents CAPB gives rise to reactive and unstable nitrosamides. The TEA method does not detect nitrosamides.[13]

Coconut oil impurities may be present in CAPB, depending on the degree of refining to which the coconut oil is subjected, including free fatty acids and low concentrations of sterols, tocopherol, squalene, and lactones. Concentrations of pigments, phosphatides, gums, and other nonglyceride substances are usually low in coconut oil in contrast to other vegetable oils.[14]

Impurities associated with CAPB are the reactants and intermediates from production and include amidoamine, sodium monochloroacetate, and DMAPA.[7;9;10] Depending on the manufacturer, residual amidoamine and DMAPA can range from 0.3% to 3.0% and 0.0003% to 0.02%, respectively.[9]

In 2007, the Personal Care Products Council surveyed suppliers regarding the levels of DMAPA and amidoamine in CAPB. The limit of detection for DMAPA is 100 ppm in some analytical methods, but some methods may detect this impurity at concentrations as low as 2.5 ppm. Several companies reported DMAPA below the 100 ppm detection limit, with one supplier reporting a DMAPA below the limit of detection of 0.0002%. The survey found levels of amidoamine ranged from 0.5% to 5%, with 0.5% the typical value and 1.5% the suggested maximum level. The variability of the amidoamine levels may be due to differences in analytical methods.[11;15]

Meadowfoam seed oil has been reported to have a typical value of <1 ppm for the heavy metals iron, copper, lead, mercury, cadmium, selenium, and chromium. The maximum value is 10 ppm.[16]

USE

Cosmetic

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), CAPB is used in a total of 2743 products (Table 5).[17] A use concentration survey conducted by the Personal Care Products Council showed CAPB use at concentrations ranging from 0.005% to

11%.{Personal Care Products Council, 2010 197 /id;Personal Care Products Council, 2008 196 /id}

The VCRP also reported uses of babassuamidopropyl betaine, capryl/capramidopropyl betaine, coco/oleamidopropyl betaine, lauramidopropyl betaine, oatamidopropyl betaine, olivamidopropyl betaine, soyamidopropyl betaine, and undecylenamidopropy betaine, with the highest total of uses reported for lauramidopropyl betaine at 187.[17] Concentration of use ranges were reported for almondamidopropyl betaine, babassuamidopropyl betaine, capryl/capramidopropyl betaine, lauramidopropyl betaine, myristamidopropyl betaine, oatamidopropyl betaine, palm kernelamidopropyl betaine, shea butteramidopropyl betaine, soyamidopropyl betaine, and undecylenamidopropyl betaine, with the highest concentration of use reported for lauramidopropyl betaine at 13%.{Personal Care Products Council, 2010 197 /id} For complete information on these ingredients, see Table 5. No uses or concentrations of uses were reported for the remaining amidopropyl betaine ingredients listed in this report.

CAPB is primarily used as a pseudoamphoteric surfactant in hair shampoos.[1] Gottschalck and Bailey described the current uses of cocamidopropyl betaine as: antistatic agent; hair conditioning agent; skin conditioning agent - miscellaneous; surfactant-cleansing agent; surfactant-foam booster; and viscosity increasing agent-aqueous.[2]

CAPB is used in hair sprays and other spray products, and effects on the lungs that may be induced by aerosolized products containing this ingredient are of concern.

Jensen and O'Brien reviewed the potential adverse effects of inhaled aerosols, which depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.[20] The aerosol properties associated with the location of deposition in the respiratory system are particle size and density. The parameter most closely associated with this regional deposition is the aerodynamic diameter, $\mathbf{d_a}$, defined as the diameter of a sphere of unit density possessing the same terminal setting velocity as the particle in question. These authors reported a mean aerodynamic diameter of 4.25 $\pm$ 1.5 μm for respirable particles that could result in lung exposure.[20]

Bower reported diameters of anhydrous hair spray particles of 60 - 80 μm and pump hair sprays with particle diameters of $\geq$80 μm.[21] Johnsen reported that the mean particle diameter is around 38 μm in a typical aerosol spray.[22] In practice, he stated that aerosols should have at least 99% of particle diameters in the 10 - 110 μm range.

CAPB was not included among the substances listed as prohibited, restricted, or provisionally allowed in the use of cosmetic products marketed in Japan.[23;24] In addition, CAPB was not restricted from use in any way under the rules governing cosmetic products in the European Union.[25]

Non-Cosmetic

CAPB is used in household cleaning products, including laundry detergents, hand dishwashing liquids, and hard surface cleaners.[26]

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

No studies were found on the absorption, distribution, metabolism, and excretion of CAPB. It is unclear whether the amide bond of CAPB can be hydrolyzed to yield the fatty acids and 3-aminopropylbetaine. No metabolism data are available on the latter compound.

Antibacterial/Antimycotic Activity

A 30% active CAPB solution was tested for antibacterial and antimycotic activity using the agar cup plate method.[27] Zones of inhibition were measured for the bacteria and molds around agar cups containing 0.2 ml of the ingredient, which had been diluted with distilled water to 0.5% activity. No inhibition against *Escherichia coli* or *Pseudomonas aeruginosa* was observed. Bacteriostatic activity was detected in cultures of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis*. Fungicidal activity was observed in cultures of *Candida albicans*, *Trichophyton mentagrophytes*, and *Pityrosporum ovale*.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

CAPB (30% -35.61% active) is not a potent acute oral toxicant in mice or rats, with LD_{50} values greater than 1 g/kg. Clinical signs observed at 2 g/kg and greater included decreased motor activity, abnormal body posture, coordination disturbance, lethargy, piloerection, increased salivation, ataxia, cyanosis, diarrhea, decreased body temperature, nasal hemorrhaging, and wetness around hindquarters, with severity increasing with dosage.

A full-strength CAPB solution, 30% active, was administered by gastric intubation to groups of 10 CFR mice of the Carworth strain weighing 18 to 21 g. Mice were observed for 7 days following the administration. An oral LD_{50} of 6.45 ml/kg within 95% confidence range from 5.66 to 7.35 ml/kg was calculated.[28]

Undiluted CAPB, 30% active, with a pH of 5.5, was administered by gavage to groups of 10 (5 female, 5 male)

Wistar rats.[29] Dosage groups were 5.00, 6.30, 7.94, and 10.00 ml/kg. The rats were observed for 14 days. The oral LD_{50} was 7.45 ml/kg, with a range of 6.48 to 8.57 ml/kg. Rats in all dosage groups had decreased motor activity, abnormal body posture, coordination disturbance, cyanosis, diarrhea, and decreased body temperature beginning approximately 20 min after dosage and persisting for 24 h. Surviving rats in all groups had body weight gains of 36 to 45 g and were normal in appearance and behavior. Redness of the stomach and intestinal mucous membrane was observed at necropsy.

A full-strength solution of CAPB, 30% active, was administered by gavage to groups of 5 albino rats at single doses of 2.0, 4.0, 5.0, 6.3, 8.0, and 16.0 g/kg and the rats were observed for 14 days. [30] Sluggishness, nasal hemorrhaging, diarrhea, and wetness around the hindquarters were observed, increasing in severity with dosage. The oral LD_{50} for this full-strength, 30% active CAPB solution was estimated at 4.9 g/kg, with a 95% confidence limit of 3.7 to 6.5 g/kg.

A full-strength solution of CAPB, 30% active, was administered by gavage to groups of 10 (5 female, 5 male) Sprague-Dawley rats at single doses of 2.0, 2.71, 3.68, 5.0, or 6.78 g/kg and the rats were observed for 15 days.[31] At necropsy, a blood-like, viscous liquid was found in the intestines. Surviving rats gained an average between 20 and 130 g by day 15. Diarrhea was observed in rats of all treatment groups, and decreased motor activity was observed in rats of all treatment groups, except at the lowest dose. Dried blood around the nose and salivation were observed in male rats of the 5.0 g/kg dosage groups. The acute oral LD_{50} for this full-strength CAPB, 30% active, was 4.91 g/kg within 95% confidence limits of 4.19 to 5.91 g/kg.

The American Chemistry Council summarized an acute oral toxicity study on 35.61% active CAPB.[32] Fasted Sprague-Dawley rats (5 female, 5 male; 220-294 g) received a single, oral dose via gavage of undiluted test material. The rats were weighed before dosing and at study termination, and they were observed frequently from the day of dosing and for 14 days. Animals that died during the study underwent gross necropsy. All of the female rats died on day 2 of the study. Prior to death, the females exhibited salivation, diarrhea, ataxia, and/or decreased activity. Male rats exhibited similar clinical signs on day 1 (day of dosing) and day 2, but had recovered by day 3. Necropsy data were not reported. The acute oral LD_{50} for 35.61% active CAPB was >1.8 g/kg for male rats.

CAPB (31% active) was orally administered to male and female CD rats (5/sex; 110-150 g) at 5.0 g/kg body weight via gavage. Animals were observed daily until 14 days after dosing and were killed on day 15. Individual body weights were recorded on days 1, 8, and 15. Macroscopic postmortem examinations performed. Clinical signs of toxicity included piloerection, increased salivation, hunched posture, and diarrhea. Animals recovered by day 4. Slightly low body weight

gains were recorded for 4 males and 3 females on day 8, but all animals achieved expected weight gains by day 15. No abnormalities were observed at necropsy. The acute oral LD_{50} was greater than 5.0 g/kg.[32]

In another acute oral toxicity study reported by the American Chemistry Council, fasted Wistar rats (5 rats per dose, sexes combined; 200-300g) received a single oral gavage dose of CAPB (30% aq.) at levels of 4.0, 8.0, 10.0, 12.5, 16.0, or 32.0 g/kg.[32] The rats were observed daily for 2 weeks after dosing. No postmortem or histopathology examinations were performed. Clinical signs included slight diarrhea and unkempt coats in the 4.0 g/kg dose group, and lethargy, diarrhea, nasal hemorrhage, and unkempt coats was observed in the 8.0 g/kg and above dose groups, with severity increasing proportionately. The acute oral LD_{50} was 8.55 g/kg. *(Concentration tested was either 30% active CAPB solution or 9% active CAPB, we are not certain which).*

Acute Dermal Toxicity

Dermal application of CAPB (31% active) produced local erythema, but no other toxicity.

The American Chemistry Council summarized an acute dermal toxicity study of CAPB (31% active) using male and female CD rats (5/sex; 200-232 g).[32] The animals received 2.0 g/kg body weight on the clipped surface of the dorsolumbar region. The treated area was occluded. After 24 h, the dressings were removed and the treated area was washed with warm water and blotted dry. The treated areas were examined daily for 14 days for signs of dermal irritation. The rats were weighed on days 1, 8, and 15. At day 15, the rats were killed and necropsied. No deaths occurred and no clinical signs of systemic toxicity were observed. No abnormalities were observed at necropsy. Slight or well-defined erythema was observed on day 2, with well-defined erythema persisting in 3 males and all females on day 3 and completely resolving by day 6. Slough or hyperkeratinization affected 6 rats on days 4 and 5 only. The acute lethal dermal dose of CAPB (31% active) was greater than 2.0 g/kg.

Short-Term Oral Toxicity

CAPB was tested from 0.1 g/kg to 1.0 g/kg in two oral short-term rat studies. One study reported mortality, but not in a dose-dependent manner. In another study, a NOEL and LOEL for CAPB was reported to be 0.5 g/kg/day and 1 g/kg/day in rats, respectively. Both studies reported treatment-induced lesions in the nonglandular portion of the stomach likely due to the irritant effect of CAPB.

Bailey conducted a short-term oral study using male and female Sprague-Dawley rats (8/sex/group) and a full strength (30.6% active) solution CAPB.[33] Three dose groups (100, 500, and 1000 mg/kg body weight) were treated daily by

gavage for at least 28 days. A control group of 16 animals received deionized water. Rats dying during the study and those killed on completion of dosing were necropsied, and tissues were collected for histopathological evaluation.

Mortality was increased in the treated groups as compared to controls, but mortality did not follow a dose-response relationship. The principal necropsy finding in the rats that died was congestion noted in several tissues, with additional alterations in the lungs of some rats. The death of a high-dose female was ascribed to a dosing accident. It was considered possible that the one death of a male of the low-dose group and one female of the mid-dose group could be attributed to dosing accidents. The other deaths were related to compound administration. This conclusion was supported by the observation that deaths occurred later (3-4 weeks of study in the mid-dose group, as compared to the high-dose groups: deaths at 1-2 weeks of study). However, doubling of the dose of compound (from 500 to 1000 mg/kg) did not increase mortality, so a dose-response relationship with the mortality was not evident.

Lesions (subacute inflammation and epithelial hyperplasia) of the nonglandular portion of the stomach were suggestive of irritation by CAPB. Lesions were found in 1 of 5 stomachs examined from the high-dose males and in all 7 from high-dose females. The loss of 3 males during the first 2 weeks of dosing prevented adequate evaluation of the response of male rats to the compound. Both males and females of the 100 mg/kg dose group were comparable to concurrent controls.

The American Chemistry Council summarized a 28-day short-term oral toxicity of CAPB (concentration not stated) in Sprague-Dawley rats.[32] Male and female rats received 0, 250, 500, and 1000 mg/kg body weight of the test material once daily via oral gavage on 5 consecutive days per week. The number distribution of the rats per group was not described.

No treatment-related deaths or decreases in feed or water consumption were observed over the course of the study. Hematological evaluations, clinical chemistry, ophthalmic examinations, and absolute and relative organ weights also did not find any treatment-related effects. Head protrusion at the beginning of week 3 and salivation at the beginning of week 4 were observed in the 1000 mg/kg dose group. Compound-related edema of the mucosa of the non-glandular stomach was observed at macroscopic examination in the 1000 mg/kg dose group, which disappeared in the rats in the recovery group. Microscopic examination of the rats in the 1000 mg/kg dose group found acanthosis of the gastric mucosa, inflammatory edema of the submucosa, and multiple ulcerations. Effects were greater in the females than the males. These effects were considered to be the result of the irritating properties of CAPB and not of systemic toxicity, especially since the 1000 mg/kg recovery animals has complete and regular regeneration of the non-glandular mucosa. No other treatment-related effects were observed in the organs. The study concluded that the NOEL was 500 mg/kg/day and the LOEL was 1000 mg/kg/day for exposure to CAPB in this rat study.

Subchronic Oral Toxicity

The American Chemistry Council summarized a subchronic oral toxicity study of CAPB (concentration not stated) on Crl:CF(SD)BR Sprague-Dawley rats.[32] Groups of 10 male (115-174 g) and 10 female (97-174 g) rats received either 0, 250, 500, or 1000 mg/kg/day CAPB in distilled water by one daily dose via oral gavage at a dose volume of 10 ml/kg/day for 92 days. Clinical signs were recorded daily and body weight and feed consumption were recorded once weekly. Ophthalmic examinations were performed on the control and 1000 mg/kg/day dose groups prior to dosing and to all groups during the final week of treatment. Blood and urine samples were collected from all rats during the final week of treatment. Complete necropsy was performed on surviving rats at study termination. Histopathology was performed on select tissues from the rats in the control group and the 1000 mg/kg/day dose group. Because treatment-related histopathological changes were observed in the stomachs of the 1000 mg/kg/day, stomachs from the 250 and 500 mg/kg/day groups also were examined microscopically.

No treatment-related deaths or effects were observed during the course of the study for either sex. Necropsy revealed stomach ulcers at the fundic and cardiac regions in 1 male and 1 female in the high-dose group. Microscopic evaluations found non-glandular gastritis in 6 male and 3 female rats in the 1000 mg/kg/day group, and in 2 male and 2 female rats in the 500 mg/kg/day group. This effect was not observed in the 250 mg/kg/day dose group. No other treatment-related effects were observed. The study concluded that the NOEL for this subchronic study of CAPB in rats was 250 mg/kg/day.[32]

Dermal Irritation

In rabbits, CAPB was not considered to be a dermal irritant at concentrations up to 50% in most studies. One study of a full-strength CAPB solution (30% active) found the ingredient to be a mild primary irritant with a PII of 0.5.

The available data on skin irritation studies are summarized in Table 6.

A full-strength CAPB solution, 30% active, was tested for skin irritation using 6 albino rabbits.[34] The pH of the solution was not stated. Volumes of 0.5 ml of the CAPB solution were applied to intact and abraded sites on the backs using occlusive patches. After 4 h, sites were rinsed and scored. Treatment sites also were evaluated 24 and 48 h following the application. Slight to well-defined erythema (scores of 1 - 2 on scale of 0 - 4) was observed at intact and abraded sites. No edema was observed. The mean primary irritation index (PII, scale 0 - 8) was 0.5, and the CAPB solution was considered a mild primary irritant.

A 7.5% active solution of CAPB was applied topically to intact and abraded sites on the clipped backs of 3 albino

rabbits.[35] The pH was not stated. Treatment sites received applications of 0.5 ml CAPB and were covered with occlusive patches for 24 h. Sites were scored for irritation at patch removal and 48 h later. No irritation was observed.

A 10% active solution of CAPB with a pH of 6.1 was tested for skin irritation using 1 albino rabbit.[36] A 0.5 ml volume of the CAPB sample was applied under occlusive patch to intact and abraded skin sites. After scoring 24 and 72 h later, a PII of 0.25 was calculated (non-irritating).

CAPB (0.5 ml of a 10% active solution) was applied to intact and abraded skin of 6 NZW rabbits for 24 h under occlusive patches.[37] The CAPB sample was a clear liquid with a pH of 4.5. Slight erythema (score of 1) was observed at 2 intact sites (one after 24 h and the other after 72 h). Four abraded sites had very slight erythema after 24 h, which subsided at 2 sites 48 h later. No edema was observed. The PII for the 10% active CAPB sample was 0.3; the sample was considered a nonirritant.

A full-strength CAPB solution, 30% active, was tested for skin irritation using 6 adult New Zealand White (NZW) rabbits weighing 2 to 4 kg.[38] Volumes of 0.5 ml of the undiluted CAPB (pH not stated) were applied to intact and abraded sites on the clipped backs of the rabbits. Sites were covered by occlusive patches for 24 h. Scoring was done 1/2 h after patch removal and 72 h later. Scores for intact and abraded sites were similar. With a mean PII of 3.75 (scale of 0-8), CAPB was moderately irritating. CAPB was corrosive to the skin of rabbits because eschar formation was observed at both sites in all rabbits after 72 h.

A 15% active solution of CAPB was tested for skin irritation using 3 male albino rabbits.[39] A volume of 0.5 ml was applied under 24-h occlusive patch to intact and abraded sites on the clipped abdomen of each rabbit. The pH of the test material was not stated. Sites were scored 24 and 72 h after CAPB application. Well-defined erythema (score of 2) was observed at intact and abraded sites after 24 and 72 h. Slight edema (score of 2, max = 4) was observed at both sites after 24 h. Edema was barely evident after 72 h. With a PII of 3.50, CAPB was not considered a primary skin irritant (for comparison, a primary skin irritant would have been expected to have a PII $\geq$ 5).

Leberco Laboratories tested the irritation potential of 25% CAPB (a dilution of 1 part plus 1 part [v/v] in distilled water of 50% CAPB) on 3 albino rabbits.[40] The test material was applied at a volume of 0.5 ml to intact and abraded sites on clipped backs. The sites were patched (nonocclusive) for 24 h and evaluated for irritation at 24 h and 72 h post-treatment. No erythema, eschar, or edema was observed on intact or abraded skin. The authors concluded that 50% CAPB was not a primary skin irritant in this study. *(Concentration tested was either 25% active CAPB solution or 7.5% active CAPB, we are not certain which).*

Dermal Sensitization

No delayed contact hypersensitivity was observed in guinea pig studies of 0.5% and 0.75% CAPB; however, a guinea pig maximization/Draize study of CAPB at 0.1% and 0.15% was positive for sensitization. A LLNA study was positive for sensitization to CAPB.

Delayed contact hypersensitivity of 15 male Pirbright white guinea pigs (400 $\pm$ 50 g) to a commercial 10% active sample of CAPB was examined using a maximization test .[41] Test animals were administered 0.1 ml of a 50% aqueous solution of Freund's complete adjuvant at the first pair of sites on the clipped, dorso-scapular region, 0.1 ml of 0.5% (v/v) dilution of the CAPB (0.05% active CAPB) sample in sterile isotonic saline at the second pair of sites, and 0.1 ml of 0.5% (v/v) dilution of the CAPB (0.05% active CAPB) sample in a 1:1 mixture of isotonic saline and Freund's complete adjuvant at the third pair of sites. One week following the injections, a single occlusive 48-h induction patch of 60% (v/v) dilution of the CAPB (6% active CAPB) sample in distilled water was applied to the same shaved interscapular area. Five control animals received intradermal injections and induction patches without the CAPB solution. All animals received a single occlusive 24-h challenge patch of 10% (v/v) dilution of the CAPB (1% active CAPB) sample in distilled water on the left flank 2 weeks after the induction.

Well-defined irritation was observed at all sites receiving intradermal injections of Freund's adjuvant. Temporary slight irritation was observed following injections of the 0.5% CAPB sample dilution in all test animals. Topical application of the 60% CAPB sample dilution resulted in slight dermal reactions. The barely perceptible erythema observed on the skin of 2 test animals after 24 h was considered unrelated to CAPB treatment, but was attributed to reactions to the elastic adhesive bandages used for site occlusion. With the exception of slight reactions to the bandages, no reactions were observed in controls throughout the 72-h observation period. No evidence of delayed contact hypersensitivity was found.

A formulation containing 0.75% active CAPB was tested in a delayed contact hypersensitivity test. Closed patches containing 0.4 ml of the test solution were applied to the shaved area on the left shoulder of 20 albino guinea pigs.[42] After 6 h, the patch was removed, and the area was rinsed with warm water. This procedure was repeated at the same site for the following 2 weeks. The animals were left untreated for 2 weeks before the primary challenge test, which used 1.875% CAPB (a 2.5% solution of the 0.75% active CAPB) applied to a freshly clipped skin site not previously treated, for 6 h. Responses were graded after 24 and 48 h. There was no evidence of sensitization following the exposure to the 3 dermal treatments or challenge dose.

A full-strength, 30% active CAPB sample was tested for skin sensitization using a maximization test and a modified Draize test.[43] Albino guinea pigs (20 animals) received intradermal injections of (1) Freund's complete adjuvant alone, (2)

0.1% aqueous dilution of the CAPB sample (0.03% active CAPB), and (3) 0.1% aqueous dilution of the CAPB sample (0.03% active CAPB) plus the adjuvant. One week later, a topical 48-h occlusive induction patch containing the 10% aqueous dilution of the CAPB sample (3% active CAPB) was applied. Animals in the control group received intradermal injections and topical application of water alone. After 3 weeks, single 24-h occlusive patches were applied to the clipped flanks of all animals. A 10% aqueous dilution of the CAPB sample (3% active CAPB) was applied to the left flank, and water was applied to the right. The lesions at necropsy were erythema and edema in 8 of the 20 test animals after the challenge application. Microscopic findings included epidermal acanthosis, inter- and intracellular edema, and massive infiltration of the superficial layers of the dermis with lymphocytes, monocytes, and a few eosinophils with a tendency to invade the epidermis in 2 of the animals. Less prominent microscopic lesions of acanthosis, mild intracellular edema and a moderate lymphomononuclear infiltrate in the superficial dermis were found in 4 additional animals. Slight acanthosis was observed in the remaining 2 animals.

This same laboratory also tested 0.15% active CAPB using the same assay. Slight erythema and edema were observed macroscopically in 6 of the 20 test animals. Slight acanthosis was observed microscopically. Control animals in the maximization and modified Draize tests had no dermatitis-type clinical or histological alterations. A few controls had moderate acanthosis with edema and vasodilation in the subjacent papillary layer of the dermis. The investigators concluded that the commercially supplied CAPB is capable of producing a delayed-type contact sensitization.

Basketter et al. reported that CAPB was positive for sensitization in a local lymph node assay (LLNA). The EC_3 value was not reported.[44]

Ocular Irritation

CAPB (2.3% active and greater) was mild to moderately irritating to rabbit eyes in the majority of these ocular studies.

The available data on ocular irritation studies are summarized in Table 7.

In a Draize test for ocular irritation, 2 groups of 3 albino rabbits received 0.1 ml instillations of 4.5% active solution of CAPB into the conjunctival sac of one eye.[45] Treated eyes of one group were rinsed. Slight conjunctival erythema and chemosis were noted in all treated, unrinsed eyes by day 2 following instillation and subsided by day 7. Slight conjunctival irritation was observed in 2 of 3 treated, rinsed eyes on the first 2 days of observation. There was no corneal involvement or iris congestion.

CAPB (30% active) was instilled (0.1 ml) into the conjunctival sac of one of the eyes of 3 albino rabbits using the

Draize method.[46] Diffuse corneal opacity was observed by day 3 following instillation. Slight iritis was observed by day 4. Mild conjunctival erythema, chemosis, and discharge were noted from day 1.

Three albino rabbits received a 0.1 ml instillation of a 6% active CAPB solution into the conjunctival sac of the right eye.[47] Mild conjunctival erythema and slight discharge were observed in all treated eyes for the first 2 days after instillation, clearing by the third day.

Six NZW rabbits (body weight range 2.4-2.6 kg) received an instillation of 0.1 ml of 7.5% active CAPB with a pH of 8.3 into the conjunctival sac of the left eye.[48] Mild to moderate conjunctival irritation was observed in all treated eyes after 24 h. The treated eye of 1 rabbit had moderate corneal opacity after the second day. These alterations disappeared by the sixth day after instillation.

One rabbit receiving a 0.1 ml administration of a 10% active CAPB solution (pH 6.1) had Draize scores of 28 after day 1, 25 after day 2, 30 after day 3, 14 after day 4, and 7 after day 7 of the observation period.[36]

A full-strength sample of CAPB (30% active) was tested for ocular irritation using 9 NZW rabbits.[49] A volume of 0.1 ml was instilled into the conjunctival sac of one eye of each rabbit. Mean eye irritation scores for treated, unrinsed eyes were 32.5 $\pm$ 4.4 after 24 h, 31.7 $\pm$ 3.3 after 48 h, 41.7 $\pm$ 11.7 after 72 h, and 27.2 $\pm$ 11.4 after 7 days (scale 0 - 110). Corneal opacity, slight iritis, and conjunctival irritation and necrosis were noted in treated, unrinsed eyes. Under these conditions, the sample was considered corrosive. Minimal irritation (mean score = 10.0 $\pm$ 2.0 after 24 h), subsiding after 48 h, was noted in treated eyes that had been rinsed.

An instillation of 0.1 ml of a sample of 10% active CAPB was made into the conjunctival sac of one of the eyes of 9 NZW rabbits.[50] Mean eye irritation scores for treated, unrinsed eyes were 25.7 $\pm$ 8.3 after 24 h, 16.7 $\pm$ 10.9 after 48 h, and 9.3 $\pm$ 11.4 after 72 h. No irritation was observed on day 7. Treated, rinsed eyes had a mean score of 2.0 $\pm$ 2.0 after 24 h, returning to normal after 48 h. The CAPB sample was considered moderately irritating to treated, unrinsed eyes and practically nonirritating to treated, rinsed eyes under these conditions.

In 2 ocular irritation studies by Hazelton Laboratories, 0.1 ml of either 5% or 10% CAPB was instilled into the left eye of groups of 6 NZW rabbits.[51;52] CAPB was not an ocular irritant in the 5% group (Draize score = 4.90), but was considered moderately irritating in the 10% group (Draize score = 27.3).

In a Draize test for ocular irritation, two 3.0% active CAPB samples were instilled into the conjunctival sac of 6 albino rabbits.[53] Scores for corneal irritation were 0 for the first 2 observation days, 1.66 for the third and fourth days, and 4.16 on the seventh day (max score = 80) for one of the CAPB samples. No corneal irritation was observed in eyes treated

with the other sample. Both samples produced iritis by the first day (scores of 8.33 and 5, respectively, on a scale of 0 to 10), which decreased in severity by the seventh day (scores of 4.16 and 0, respectively). Both samples produced conjunctival irritation (scores of 15.37 and 14.33, respectively, on a scale of 0 to 20), which decreased in severity by the seventh day (scores of 6 and 0, respectively).

A 3.0% active CAPB sample was tested for ocular irritation using 6 male albino rabbits.[54;55] The average ocular index was 41.6 (max = 110) 24 h after instillation of 0.1 ml of the sample. The sample was considered an ocular irritant.

A volume of 0.1 ml of a liquid soap formulation containing 2.3% active CAPB was instilled into the conjunctival sac of each of 9 NZW rabbits.[56] An average irritation score of 18.7 (max 110) was calculated for unrinsed eyes, which compared with 20.0 for rinsed eyes. Irritation was observed primarily in the iris and conjunctiva. Under both sets of conditions, the liquid soap formulation was considered moderately irritating.

Another liquid formulation containing 2.3% active CAPB was tested for ocular irritation using 9 NZW rabbits.[57] The maximum average irritation score for the 6 treated, unrinsed eyes was 1.7 (max 110). Slight conjunctival erythema and chemosis were observed in 1 rabbit 2 days after treatment and in the eye of another for the entire 7-day observation period. Slight discharge also was observed in the treated eye of the latter from 72 h to 7 days following treatment. The formulation was considered minimally irritating to treated, unrinsed eyes of rabbits. The maximum average irritation score for the 3 treated, rinsed eyes was 3.3. Mild conjunctival erythema and chemosis were observed in all tested eyes 1 to 2 days following the instillation. The formulation was considered mildly irritating to treated, rinsed eyes of rabbits.

A liquid soap formulation containing 6.5% active CAPB was tested for ocular irritation by instilling 0.1 ml into the conjunctival sac of one eye of each of 4 NZW rabbits, followed by rinsing.[58] Mean corneal irritation scores were 13.8 after 1 h, 18.8 after 24 h, 11.3 after 48 h, 5 after 72 h, and 1.3 after 7 days (max 80). Mean iridial irritation scores were 3.8 after 1 h and 24 h, decreasing to zero after 7 days. Mean conjunctival irritation scores were 11 after 1 h, 7.5 after 24 h, 4 after 48 h, 3.5 after 72 h, and 2 after 7 days. No irritation was observed 14 days after the instillation. With a total mean irritation score of 30.0 (max. total = 110.0), the formulation was considered moderately irritating.

A single 0.1 ml dose of a product formulation containing 6.0% active CAPB was instilled into the conjunctival sac of each of 6 albino rabbits in a Draize test.[1] Conjunctival irritation (mean score of 4; max = 20) was observed in all treated eyes on the first day following instillation, decreasing in severity on the second day. No corneal irritation or iritis was observed.

Mucous Membrane Irritation

Two soap formulations containing 7.5% CAPB were tested for vaginal irritation potential in Beagle dogs (7-10 months old; 8.2-10 kg). The formulations were tested in 3 dogs each. Prior to treatment and again before termination, sampling for hematology, clinical chemistry, and urinalysis occurred. A volume of 20 ml of the test material was administered into the vagina via a syringe once a day for 15 days (weekdays only). Vaginas and vulvas were examined 6 hours prior to and after each daily treatment. The dogs were killed at study end for gross necropsy. Tissue samples were taken from the liver, kidney and vulva/vagina and examined. Blood was in the urine of 5/6 dogs. Gross necropsy revealed discoloration of the lining of the vagina in 5/6 dogs. Diffuse necrosis of vaginal mucosa occurred in 5/6 dogs and focal vaginal necrosis occurred in 1 dog (this dog was in estrus). There was corresponding inflammatory cell infiltration (mainly neutrophils), and often a fibrinopurulent membrane adherent to the injured surface. It was concluded that lesions were the result of test material application. Morphologic changes in the liver and kidneys in all dogs were not considered significant and were within normal parameters.[59;60] *(Concentration tested was either 7.5% active CAPB solution or 2.25% active CAPB, we are not certain which).*

GENOTOXICITY

CAPB was not genotoxic in bacterial assays, a mouse lymphoma assay, or a mouse micronucleus test.

Bacterial Assays

A commercial sample of CAPB (31.0% active) was tested using *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, both with and without metabolic activation. The concentrations of CAPB solution tested were 0.004, 0.02, 0.1, 0.2, and 0.4 µl/plate. CAPB is toxic above 0.3 µl/plate. The test material did not cause a significant increase in mutation frequency in any of the strains tested with or without metabolic activation.[61]

CAPB (30% active) was tested using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, with and without metabolic activation. Eight concentrations between 0.001 and 0.300 µl/plate were used, based on CAPB solubility. CAPB did not produce an increase in mutation frequency, with or without metabolic activation.[62]

In a study summarized by the American Chemistry Council, CAPB (28.5-30.5% active) was tested using *S. typhimurium* strains TA98, TA1535, TA1537, and TA1538, both with and without metabolic activation at 0, 50, 150, 500, 1500, or 5000 µg/plate.[32] Positive controls were N-ethyl-N'-nitro-N-nitrosoguanidine (for TA100 and TA1535), 9-

aminoacridine (for TA1537), 4-nitro-o-phenylenediamine (for TA1538), 4-nitroquinoline-1-oxide (for TA98), and 2-aminoanthracene (in all strains with metabolic activation only). Cytotoxicity was observed at 150 µl/plate and above. CAPB in this assay was found to be non-mutagenic.

The American Chemistry Council also summarized the findings of a CAPB (concentration not stated) mutagenicity assay using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, with and without metabolic activation.[32] The test material was tested at 1, 4, 16, 64, or 256 µg/plate without S-9 activation and at 4, 16, 64, 256, and 1024 µg/plate with S-9 activation. CAPB did not increase the mutation frequency, with or without metabolic activation.

Mammalian Cell Assays

The mutagenic potential of a 30.9% active sample of CAPB was tested in a L5178Y TK $\pm$ mouse lymphoma assay with and without metabolic activation. The test substance was solubilized in water and diluted for testing at concentrations of 0.001, 0.01, 0.1, 1.0, 10, and 100 µl/ml. None of the treated cultures had a significant increase in mutation frequency over the average mutant frequency of the solvent controls.[63]

Animal Assays

The American Chemistry Council summarized a mouse micronucleus test that studied CAPB (concentration not stated).[32] Groups of 5 male and 5 female OF1 mice received two doses of either 0.02 or 0.2 g/kg of the test material in sterile distilled water via intraperitoneal injection (dose volume 10 g/kg) at 24-h intervals. Negative and positive controls received sterile distilled water and cyclophosphamide, respectively. The rats were killed 6 h after the second administration of the test material and bone marrow slides were prepared. One thousand polychromatic erythrocytes (PCEs) per animal were studied for the presence of micronuclei. In both dose groups, the number of micronucleated PCEs was not increased when compared to the negative control. The positive control group yielded expected results. CAPB was not a mutagen under the conditions of this study.

CARCINOGENICITY

An aqueous preparation of a non-oxidative hair dye formulation containing an unspecified grade of CAPB at a concentration of 0.09% active CAPB was tested for carcinogenicity using groups of 60 male and female random-bred Swiss Webster mice from the Eppley colony.[64] The formulation also contained 5% propylene glycol, 4% benzyl alcohol, 0.6%

Kelzan (xanthan gum), 0.9% lactic acid, 0.04% fragrance, and less than 0.1% each of the disperse brown, red, yellow, and blue dyes. A dose of 0.05 ml per mouse was applied 3 times weekly for 20 months to interscapular skin that was clipped free of hair and shaved. Mortality, behavior, and physical appearance of the mice were observed daily. Dermal changes in particular were noted. Body weights were recorded weekly. Ten males and 10 females from each group were killed at 9 months for a hematological study, urinalysis, and necropsy. At termination, all mice were necropsied, and the tissues were examined microscopically. No adverse effects were noted on average body weight gains, survival, hematological or urinalysis values in any group. Varying degrees of chronic inflammation of the skin were seen in all groups, including controls. Other lesions occurred, but were considered unrelated to hair dye treatment. The incidence of neoplasms in treated animals did not differ significantly from control groups.

CLINICAL ASSESSMENT OF SAFETY

Fisher's Contact Dermatitis recommended that patch testing with CAPB should be performed at a concentration of 1% aq.[65] Care was advised for patch test readings since mild false-positive irritant reactions may occur.

de Groot, in a review of contact allergy literature, stated that CAPB in rinse-off products such as shampoo, shower gel, bath foam, and liquid soap was linked to cosmetic allergy.[7] Because patch testing for sensitization with these products may result in both false-positive and false-negative reactions, the author suggested that CAPB should be tested separately. The author also suggested that CAPB should be included in the hairdresser's series and the cosmetic series with the knowledge that commercial concentration of CAPB (1% in water, possibly 0.3% active) is a marginal irritant and not all positive patch test reactions indicate contact allergy to CAPB.

Another review of contact allergy literature by Mowad described CAPB as "contact allergen of the year" for 2004.[10] Because impurities in CAPB may be responsible for allergic reactions, the author advised patch testing for amidoamine and DMAPA along with CAPB. The author further suggested that patients that test positive to amidoamine or DMAPA should be advised to avoid products that contain CAPB.

Dermal Irritation

Irritation studies of formulations containing active CAPB up to 6.5% before dilution were considered practically nonirritating. A study of 1.0% wheat germamidopropyl betaine had a PII of 0.35.

Cocamidopropyl Betaine

In a study of cumulative irritation, 0.3 ml of 2 soap formulations were applied to skin sites on the backs of 10 panelists using occlusive patches.[66] Each formulation contained 1.9% active CAPB. Daily 23 h patches were applied for 21 consecutive days. The total irritation scores for all subjects for all 21 applications of the 2 formulations were 588 and 581 (max. 630). The average irritation times for the formulations were 1.48 and 1.69 days, and the median irritation time was 2 days.

CAPB at 0.06% (1.0% aq. dilution of a product formulation containing 6.0% active CAPB) was tested for skin irritation using a single insult occlusive patch test and 19 panelists.[1] Fifteen panelists had no irritation and a ± score was recorded for 4 panelists. The formulation was considered practically nonirritating.

Daily doses of 0.2 ml of 0.52% CAPB (an 8% aq. dilution of a liquid soap formulation containing 6.5% active CAPB) were applied via occlusive patches to the forearms of 12 human subjects for 5 days.[1] An erythema score of 0.48 (scale 0-4) was calculated.

Wheat Germamidopropyl Betaine

The irritation potential of 0.005% active wheat germamidopropyl betaine (a 0.5% aqueous solution of 1.0% wheat germamidopropyl betaine in a body polisher) was evaluated against a control shower gel in a single 24 h insult patch test. Twenty subjects completed the study. Two panelists had a ± score and 4 panelists had a + score and the primary irritation index (PII) was calculated at 0.25. The control substance elicited a ± score in 4 panelists, a + score in 2 panelists, and a ++ score in 2 panelists, yielding a PII of 0.35. The authors concluded that the test material containing 1.0% wheat germamidopropyl betaine was milder than the reference control.[67]

Dermal Sensitization

CAPB at 6% active in cleansing cloths was not a sensitizer in a repeat patch test, nor was it a sensitizer in similar studies at lower active concentrations in formulations. Patch tests of patients with contact dermatitis indicate that CAPB can be a contact sensitizer, but sensitization may be dependent on the purity of the CAPB in personal care products. Formulations containing almondamidopropyl betaine, olivamidopropyl betaine, capryl/capramidopropyl betaine, lauramidopropyl betaine, and shea butteramidopropyl betaine were not considered contact sensitizers in maximization studies on human volunteers. Dose per unit area calculations have been performed by CIR when adequate data are available. The available data on clinical sensitization studies are summarized in Table 8.

Cocamidopropyl Betaine

A repeated open application procedure was performed with 1.872% CAPB (a 10% w/v aqueous dilution of a

shampoo containing 18.72% active CAPB), using 88 human volunteers to determine skin sensitization *[Estimated dose/unit area = 1.7 x 10^4 µg/cm^2]*. The disk was removed after 10 minutes. Induction applications were made 3x a week for 3 weeks. Challenge patch strips were applied simultaneously to both the induction arm and the alternate arm, positioned between the shoulder and elbow, 18 days after the last induction application. The areas were scored 24, 48, and 72 h following the removal of the patch after a 6-h period. The same procedures were performed with another test substance containing an identical concentration of CAPB. No sensitization was seen in any of the 88 subjects exposed to either of the test materials.[68]

Another study was performed with a 0.93% active aqueous solution of CAPB *[Estimated dose/unit area = 7.7 x 10^2 µg/cm^2]*.[69] Ninety-three volunteers completed the study. Induction applications were made to the same site unless reactions became so strong that a first or second adjacent site had to be used for complete induction, and the sites were scored following a 48-h period. An alternate site was used for the challenge test and was scored after 48 and 96 h. Ten subjects had slight responses to the test material. These responses were attributed to primary irritation, rather than sensitization, during both the induction and challenge tests.

In a similar study by Hill Top Research, Inc., a formulation containing 0.3% active CAPB was tested on 100 human volunteers.[70] The study had started out with CAPB at 0.6%, but due to several incidences of mild to moderate skin irritation early in the induction phase, the concentration was diluted *[Estimated dose/unit area = 2.5 x 10^1 µg/cm^2 at 0.3%]*. No evidence of sensitization was observed in the formulation at 0.3% active CAPB.

CAPB was studied using 141 human subjects. All applications contained a concentration of 1.5% active CAPB in distilled water, until a protocol modification changed the concentration to 3.0% active CAPB. Subjects who began the study a week earlier received 2 applications at a concentration of 1.5%, and all other applications of the test material at a concentration of 3.0% *[Estimated dose/unit area = 5.8 µg/cm^2 at 1.5%, 1.2 x 10^1 µg/cm^2 at 3%]*. Induction applications were made to the same, previously untreated site on the back 3 times per week for 3 successive weeks. Patches were removed after 24 h. Following a 10 to 15 day non-treatment period, the challenge application was applied to a previously untreated site for 24 h, and the site was scored 24 and 72 h after patch removal. No responses were observed during either the induction or challenge tests.[71]

Clinical Research Laboratories, Inc. performed a RIPT study on 6% active CAPB in cleansing cloths in 2 groups of subjects (In phase I, 104 subjects completed the study. In phase II, 106 subjects completed the study).{Clinical Research Laboratories, 2002 224 /id}{Clinical Research Laboratories, 2002 225 /id} The test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material was cut to a ½ inch square and applied to the upper back under a semi-occluded

patch for 24 h. There were a total of 9 induction patches. Induction sites were scored for irritation. Following a 2-week rest period, challenge patches were applied to a virgin site on the back. After 24 h, the patches were removed and evaluated for dermal reactions. The test sites were scored again at 48 and 72 h. No reactions were observed in either group of subjects. It was concluded that 6% active CAPB in cleansing cloths did not demonstrate a potential for eliciting dermal irritation or sensitization.

In a study by KGL, Inc., 0.018% active CAPB (a 0.5% aqueous dilution of a facial cleanser containing 3.6% active CAPB) was tested on 27 volunteers to determine skin sensitization.{KGL, 2007 223 /id} In the induction phase, the volunteers were pre-treated with 0.05 ml of 0.25% aqueous SLS under an occluded 15 mm Webril disc for 24 h on the upper outer arm, volar forearm, or back. After 24 h, the SLS patch was removed and 0.05 ml of the test material was applied to the same site and occluded. The induction patch was left in place for 48 h and the site was scored for irritation. *[Estimated dose/unit area = 4.0 x 10^1 µg/cm^2]*. If no irritation was present, the SLS patch followed by the test material patch procedure was repeated for a total of 5 induction exposures. If irritation developed at any time during the induction phase, the SLS treatment patch was eliminated and only the test material was reapplied after a 24 h rest period. Following a 10-day rest period, the subjects received 0.05 ml of 5% SLS for 1 h prior to receiving the challenge patch of the test material to the opposite side of the body. The challenge patch was occluded and left in place for 48 h. After patch removal, the site was scored 15-30 minutes later and again at 24 h. No reactions were observed during the induction or challenge phases of this maximization study. It was concluded that 0.018% active CAPB in a facial cleanser was not likely to cause contact sensitivity reactions under normal use conditions.

de Groot et al. studied 2 groups of patients for CAPB allergy.[72] The first group consisted of 781 patients that were patch tested with the European standard series, hairdresser's series, cosmetics series, and with other relevant allergens, including the patients' personal care products, and 1% aq. CAPB from February 1991 to June 1994. Most of the patients in this group were suspected of having occupational contact dermatitis (217 patients were hairdressers). The second group was studied in approximately the same time period, and consisted of 102 patients suspected of having cosmetic dermatitis. The patients were patch tested with 1% aq. CAPB along with the cosmetic screening series. In both groups, relevance was only declared if the patients used products with CAPB and if their dermatitis cleared upon cessation of use of these products. In the first test group, 56 patients (7.2%) had positive reactions to CAPB, and of these, 17 were classified as definite and all used shampoos and/or shower gels that contained CAPB. Eight of the 17 were hairdressers and had experienced dermatitis on their hands. In the second test group, only 3 patients (3%) had a positive reaction to CAPB. The patients had been using

shower gels, shampoos, and/or body lotions containing CAPB.[72] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Armstrong et al. patch tested patients with suspected contact dermatitis (from January 1991 to September 1998) with a standard series that included 1% aq. Tegobetaine L7 (from 1991-1994) or 1% aq. CAPB (from 1995-1998). The authors noted that the latter had significantly lower intermediate and reactant impurities.[73] Of the 10,798 patients tested, 29 (0.27%) had a positive reaction to CAPB (24 reactions to Tegobetaine L7). Twenty-three of the 29 cases were deemed relevant and had reported dermatitis to the face, neck, hands, or widespread areas. The authors suggested that higher purity CAPB was linked to a diminished frequency of CAPB sensitization. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

In a double-blind randomized controlled study by Shaffer et al. to evaluate allergenicity to coconut oil derivatives, 10 control subjects and 12 subjects with previously diagnosed allergy to CAPB were patch tested with 11 coconut-derived surfactants, coconut oil, and lauric acid.[74] Patch testing was performed in random order according to standardized procedures with readings at 48 and 96 hours. Three of the 12 subjects had doubtful reactions to CAPB in the patch test and 1 control subject had a doubtful reaction to CAPB. The authors suggested that doubtful reactions to CAPB represent irritant reactions and not allergic reactions.

Almondamidopropyl Betaine and Olivamidopropyl Betaine

The irritation/sensitization potential of 1% active almondamidopropyl betaine and 1% active olivamidopropyl betaine in a body cleanser was evaluated in a repeat insult patch test of 103 subjects *[Estimated dose/unit area = 1.0 x 10^3 $\mu g/cm^2$]*. After the induction phase (3x per week for 3 weeks) and a 2 week rest period, the subjects received a single challenge patch. No reactions were observed. It was concluded that a body cleanser containing 1% almondamidopropyl betaine and 1% olivamidopropyl betaine was not a primary sensitizer or irritant to the skin.[75]

Capryl/Capramidopropyl Betaine

KGL, Inc. evaluated the contact-sensitizing potential of a mousse (concentrate) containing 1.72% active capryl/capramidopropyl betaine in a maximization study.[76] Twenty-six adult volunteers completed the study. During the induction phase, ~0.05 ml of aqueous SLS (0.25%) was applied to a test sites on the upper outer arm, volar forearm, or the back of each subject. After 24 h, the SLS patch was removed and 0.05 ml of the test material was applied to the same site and

occluded *[Estimated dose/unit area = 3.8 x 10^2 µg/cm^2]*. The induction patch was left in place for 48 h (72 h if placed over a weekend). After patch removal, the site was examined for irritation. If no irritation was observed, the sequence of patching with SLS followed by patching with the test material was repeated for a total of 5 induction exposures. If irritation was observed during the induction phase, the SLS patch step was eliminated for that subject and only the test material was applied.

At the end of the induction period, the volunteers had a 10-day rest period followed by a single challenge application of the test material to a new skin site. Prior to challenge, the test site was patched with ~0.05 ml of 5% SLS under occlusion for 1 h. After 1 h, the SLS patch was removed and the site was patched with 0.05 ml of the test material under occlusion for 48 h. After 48 h, the patch was removed and graded on a scale of 0 (not sensitized) to 3 (strong sensitization; large vesiculo-bullous reaction) 1 h and 24 h after removal. No adverse or unexpected reactions occurred, and no incidences of contact allergy were recorded. The study concluded that the mousse (concentrate) containing 1.72% capryl/capramidopropyl betaine did not have a detectable contact-sensitizing potential and was not likely to cause contact sensitivity reactions under normal use conditions.[76]

Lauramidopropyl Betaine

Consumer Product Testing Company performed a repeated insult patch test on a shampoo with 0.042% lauramidopropyl betaine (test material was prepared as a 1% dilution in distilled water of 4.2% active lauramidopropyl betaine).[77] *[Estimated dose/unit area = 2.3 x 10^2 µg/cm^2]*. There were in 51 subjects. A total of 9 applications were made during the induction phase. Following a 2-week rest period, a challenge patch was applied to a virgin test site on the back. After 24 h, the patch was removed and the site was scored 24 and 72 h post-application. No reactions were observed in any of the subjects during the induction or challenge phases of this study. The study concluded that the shampoo containing 4.2% lauramidopropyl betaine, diluted to 1%, did not indicate a potential or dermal irritation or allergic contact sensitization.

In another human repeated insult patch test, the potential of a body cleanser with 0.03955% lauramidopropyl betaine (a 1% dilution of 3.955% active lauramidopropyl betaine) to cause dermal irritation and sensitization was studied.[78] One hundred-nine subjects completed the study. Prior to patch application, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test solution was applied to the upper back and remained in direct skin contact for 24 h. The induction period was comprised of a total of 9 applications on the same site. The sites were graded for dermal irritation 24 h after patch removal. Following a 2-week rest period, a challenge patch was applied to a virgin test site on the back. After 24 h, the patch was removed and evaluated for dermal reactions. The sites were re-evaluated at 48 and 72 h. Several subjects

had barely perceptible erythema reactions were observed on one or two days of induction phase of the study. No incidences of dermal reaction were recorded during the challenge phase. The study concluded that the body cleanser with 3.955% lauramidopropyl betaine, diluted to 1%, did not demonstrate a potential for eliciting dermal irritation or sensitization.

A maximization study to evaluate the contact-sensitizing potential of a shower gel containing 14% active lauramidopropyl betaine was conducted by KGL, Inc.[79] Twenty-five adult volunteers completed the study. The study was conducted in the same manner as the capryl/capramdiopropyl betaine maximization study described above with the exception that ~0.1 ml of aqueous SLS (0.25%) and 0.1 ml of the test material *[Estimated dose/unit area = 6.2 x 10^3 µg/cm^2]*were used during the induction and challenge phases. No adverse or unexpected reactions occurred, and no incidences of contact allergy were recorded. The study concluded that the shower gel containing 14% lauramidopropyl betaine did not have a detectable contact-sensitizing potential and was not likely to cause contact sensitivity reactions under normal use conditions.

Shea Butteramidopropyl Betaine

In a human repeated insult patch test, the potential of a body scrub containing 0.04% shea butteramidopropyl betaine (a 1% w/v dilution of 4.0% active shea butteramidopropyl betaine) to cause dermal irritation and sensitization was studied.[78] One hundred-one subjects completed the study. The study followed standard RIPT methodology with a total of 9 induction applications of 24 h in length and a single challenge application following a 2-week rest period. No adverse events were reported and no incidences of dermal reaction were recorded during the challenge phase. The study concluded that the body scrub with 4.0% shea butteramidopropyl betaine, diluted to 1%, was not sensitizing.

A maximization study to evaluate the contact-sensitizing potential of a body wash containing 0.54% active shea butteramidopropyl betaine was conducted by KGL, Inc. *[Estimated dose/unit area = 120 µg/cm^2].* [80] Twenty-five adult volunteers completed this RIPT study. The study was conducted in the same manner as the capryl/capramdiopropyl betaine study described above with the exception that the patches were made only to the upper outer arm. No adverse or unexpected reactions occurred, and no incidences of contact allergy were recorded. The study concluded that the body wash containing 0.54% shea butteramidopropyl betaine did not have a detectable contact-sensitizing potential and was not likely to cause contact sensitivity reactions under normal use conditions.

Provocative Studies

In 706 patients studied for skin allergy, 93 (83 women and 10 men) were provisionally diagnosed with cosmetic contact dermatitis. Four of the 93 had positive reactions to CAPB 1% aq. Two subjects had scalp itch and erythema on the forehead, ears, and neck following use of shampoos with CAPB. The other two subjects had eczema on the face and/or neck

following use of face cleansers that contained CAPB.{Vilaplana J, 1990 119 /id} *(Concentration tested was either 1% CAPB or 0.3% active CAPB).*

Fowler studied 210 patients clinically suspected of having allergic contact dermatitis to cosmetics and toiletries.[82] Patch testing with CAPB (1% aq.) in addition to the North American Contact Dermatitis Group (NACDG) series (70 allergens total) was performed. Twelve of the subjects (5.7%) had positive reaction to CAPB in the patch test. Positive reactions were also observed for formaldehyde or formaldehyde releasers, neomycin, and nickel. All but two of the subjects had initially reported with head and neck dermatitis. The remaining 2 subjects had hand dermatitis. Of the 12 subjects, 7 were determined definitely relevant when the reported dermatitis cleared after cessation of use of products with CAPB.[82] Specific case reports for two of the subjects are detailed in the case reports section. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Photosensitization

An investigation of the potential of a 3.0% active aqueous solution of CAPB to induce contact photoallergy was tested using 30 human subjects. The 11 subjects who had mild to moderate erythemic responses at the irradiated sites during the induction testing were those that received both UVA and 2 MED of UVB irradiation. These responses were expected from the UVB exposure. CAPB was not a photosensitizer in this study.[71]

Case Reports

Numerous case studies of allergic contact dermatitis reported positive patch tests to CAPB at concentration as low as 0.5%.

Van Haute and Dooms-Goossens reported cases of contact dermatitis from CAPB in two women.[83] The first was a 44-year-old with acute eczematous lesions with erythema, edema, and vesiculation on the backs and palms of her hands. She also had a slightly red, itchy scalp. Symptoms occurred after the patient used a shampoo with chestnut leaf extract. Patch testing performed prior to this case of dermatitis found the patient reacted positively to p-phenylenediamine, benzocaine, wool alcohols, parabens, chinoform, perfumes, nickel sulphate, and cobalt chloride. Patch testing with the shampoo and its individual components showed a ++ reaction to the shampoo at 2% aq., two ++ reactions to parahydroxybenzoic acid esters (each 5% pet.), and one +++ reaction to CAPB (2% aq.). The reaction to the shampoo's perfume was negative. The dermatitis cleared when the patient changed shampoos. *(Concentration tested was either 2% active CAPB or 0.6% active CAPB, we do not know which).*

The second case involved a 22-year-old with a red, swollen face with weeping eczematous lesions and red, oozing and crusted acute lesions on the scalp and shoulders. Two days prior to the allergic reaction, the patient had washed her hair with a new shampoo. A patch test and tests with the shampoo, its individual components, and any topical agents and cosmetics used by the patient were performed. A +++ reaction to the shampoo (in both an open test, as is, and a patch test at 2% aq.), a ++ reaction to CAPB (2% aq.), and a ++ reaction to sodium lauryl ether sulfate (SLES, 2% aq) were observed. Again, the dermatitis cleared when the patient changed shampoos. In this case report, 3 control patients were patch tested with the shampoos and had negative reactions.[83] *(Concentration tested was either 2% active CAPB or 0.6% active CAPB, we do not know which).*

In Andersen et al., 2 patients presented with itchy, burning papules on the scalp after use of a shampoo.[84] The symptoms disappeared after the patients discontinued use of the shampoo, but reappeared 1 day after renewed use. One of the patients had face, neck, and hand dermatitis that was related to shampooing. The patients were tested with the International Contact Dermatitis Research Group (ICDRG) standard and the shampoo (1% in water). Both patients had ++ reactions to the shampoo and the remaining tests were negative. The patients were then tested with the shampoo's individual ingredients. Positive reactions occurred with TEA-PEG-3 cocamide sulfate (1% aq.) and CAPB (1% aq.). Twenty consecutive eczema patients were patch tested as a control. All tested negative to the 2 ingredients (10% aq.). *(Concentrations tested were either 1% active CAPB or 0.3% active CAPB and 10% active CAPB or 3% active CAPB, respectively, but we do not know for certain).*

Sertoli et al. reported on a 30-year-old female, with no history of atopy, with dermatitis of the eyelids for 2 years.[85] The patient had been a contact lens-wearer for 12 years and had recently substituted her regular brand of lens cleaning solution for another that contained 1.8% Tegobetaine L7, 0.02% benzalkonium chloride, and 0.2% EDTA. The patient was patch tested with the Italian Research Group on Contact and Environmental Dermatitis (GIRDCA) standard series, the previous contact lens cleanser and its components (including merthiolate 1% pet. and EDTA 1% pet.), the new lens cleaning solution and components (Tegobetaine L7, 1% and 2% in both water and pet. and benzalkonium chloride 0.1% aq.), chlorhexidine digluconate 0.5% pet., CAPB 2% aq., Tego 103 G (1% aq.), and Tego 103 S (1% aq.). Merthiolate and CAPB both elicited positive results as did both cleaning solutions. Forty volunteer controls were negative for CAPB. The authors concluded that the patient's dermatitis was initially caused by sensitization to merthiolate and then developed chronic dermatitis after sensitization to CAPB. *(Concentration tested was either 2% active CAPB or 0.6% active CAPB, we do not know which).*

Two cases of eyelid dermatitis were reported by Cameli et al.[86] Both patients (females, ages 23 and 35) wore hard contact lenses and were using a cleaning solution that contained 1.8% CAPB, 0.02% benzalkonium chloride, and 0.2% ethylenediaminetetraacetic acid. The patients were patch tested with the GIRDCA standard, the cleaning solution, and the individual components of the solution. Both tested positive to the solution and CAPB (1% pet. and aq.). The dermatitis cleared when the patients changed lens cleaning solutions. *(Concentration tested was either 1.8% active CAPB or 0.54% active CAPB, we do not know which).*

Ross and White reported on a 60-year-old woman with a 2 month history of eyelid eczema and no personal or family history of atopy.[87] The patient was patch tested with the European standard series, facial allergen series, and the patient's cosmetics. An oil-free eye makeup remover used by the patient produced a ++ reaction at 2 days and a + reaction at 4 days. An open test on the forearm caused diffuse erythema after 2 days. The ingredients of the eye makeup remover were examined individually in a patch test and a + reaction to CAPB (1% aq.) was observed. Fifty control subjects patched with CAPB 1% aq. had negative results. The patient's eczema cleared after discontinuing use of the eye makeup remover. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Four women were reported with allergic dermatitis to the scalp and/or hands from use of shampoos (2 women had dermatitis to the hands only and were hairdressers). Patch testing was performed with the ICDRG and German Contact Dermatitis Group standards. All 4 patients had a + reaction to CAPB (1% aq.) at the 72 hour observation. The patients also tested positive to glyceryl monothioglycolate, nickel sulfate, p-phenylenediamine, ammonium persulfate, and hydroquinone.[88] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Taniguchi et al. reported on a 22-year-old male hairdresser with erythematous swelling lesions on the hands, fingers, and forearms.[89] The patient had no personal or family history of atopy. Patch testing with the European standards series, hairdressing series, and shampoos used by the patient revealed a ++ reaction at day 3 in 2 shampoos. CAPB (1% aq.) was an ingredient in one of the shampoos. A second patch test revealed ++ reactions to CAPB at 0.5% and 1% in water. A control patching of 15 subjects with CAPB (1% aq.) revealed no positive reactions. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Six cases of allergic contact dermatitis to CAPB (body region not specified) were reported by Peter and Hoting.[90] Three of the cases were identified using a commercially available patch test (CAPB 1% aq.) and the other 3 were determined through testing the individual ingredients in both leave-on and rinse-off products used by the patients that yielded a positive patch test (CAPB at product concentration diluted with water; shampoo = 0.1 to 0.2% active CAPB). One of the patients also

had a positive reaction to cocamido monoethanolamine.

Brand and Delaney reported on a 50-year-old woman with a 10 month history of severe scalp dermatitis that was initially diagnosed as seborrheic dermatitis.[91] Contact dermatitis was suspected after the rash spread to the neck, trunk, and limbs. The patient was patch tested with the extended European series, Chemotechnique cosmetic series, and the patient's shampoos in accordance with the ICDRG. A strong allergic reaction was observed to Kathon CG, CAPB (1% aq.), and to most of the shampoos. The dermatitis cleared with cessation of shampoo use and with applied topical steroid. Recurrence was observed when the shampoo was used again. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

As part of Fowler's clinical study on allergic contact dermatitis, a case of a 30-year-old woman was reported with a 4 month history of dermatitis on the hands and forearms.[82] The patient was employed as a nursing assistant responsible for bathing patients daily. Patch testing produced positive reactions to CAPB and to several preservatives and fragrances. CAPB and the fragrances were found in the bath gels used at the patient's place of employment. The dermatitis cleared when the employer switched bath gel products.

In part of a clinical study of patients with allergic contact dermatitis, Fowler reported the case of a 48-year-old woman with a 3 year history of progressive eyelid dermatitis.[82] The patient did not wear contact lenses or use eye makeup. Patch testing revealed positive reactions to CAPB, neomycin, gentamicin, and nickel. The patient's shampoo contained CAPB. The dermatitis cleared 3 weeks after discontinuing the shampoo.

A 47-year-old female hairdresser was reported with a 4 year history of itchy erythema on the hands.[92] The patient had no family or personal history of atopy. Patch testing was performed using the European standard series, hairdressing series, and shampoos (1% aq. and 5% aq.) and hair dyes (5%) used by the patient. At the day 4 observation, a ++/+++ reaction was noted for CAPB. The patient also had a +++ reaction to the hair dye, ++ reactions to p-phenylenediamine dihydrochloride, quinoline, benzocaine, colophony, and 3-aminophenol, and a + reaction to the shampoo (5% aq.) at the day 4 observation.

Mowad reported on a 75-year-old male with a patchy rash on the trunk.[93] The patient reported minimal relief from topical steroids. The patient was patch tested with a standard of 51 allergens. A positive reaction was observed for CAPB, which was found in a shampoo used by the patient. The rash cleared after the patient discontinued using the shampoo.

Agar and Freeman reported a two-year history of progressively worsening cheilitis in a 10-year-old girl.[94] Signs included persistent dryness, episodic swelling, burning, stinging, cracking, and bleeding. No personal history of atopy other

than a sibling with minor asthma. Prior to the onset of the symptoms, the patient had switched to a toothpaste and mouthwash combination that contained triclosan, chlorhexidine digluconate, and CAPB. Temporary relief of the symptoms occurred with steroid treatment. The patient was patch tested with the European standard battery, the toothpaste series, and chlorhexidine digluconate 0.5%. The product was not tested as a false-positive result was anticipated. A 2+ reaction was observed to CAPB and all other patch tests were negative. The cheilitis resolved within a few weeks of discontinued use of the toothpaste.

STUDIES WITH AMIDOAMINE, DMAPA AND RELATED AMINES

Animal Studies

Amidoamine was considered a moderate contact sensitizer in 2 guinea pig maximization studies and had skin sensitizing activity in an LLNA study, while DMAPA in various vehicles had SI values indicating it was a contact sensitizer in an LLNA study. Stearamidopropyl dimethylamine elicited contact hypersensitization in one guinea pig in a maximization study, but was considered a potential sensitizer in a LLNA study. Dose per unit area calculations were performed by CIR when adequate data were available.

Hill Top Research, Inc. performed a delayed contact hypersensitivity study of stearamidopropyl dimethylamine in guinea pigs.[95] A pre-induction primary irritation study was conducted to determine the concentration for the induction phase of the study. Twenty Hartley outbred guinea pigs were treated with 1.0% w/v stearamidopropyl dimethylamine in 80% ethanol/20% distilled water. The test material was applied for 6 h at a dose volume of 0.3 ml using 25 mm diameter occluded Hill Top chambers on clipped, intact skin on the left shoulder *[Estimated dose/unit area = 6.1 x 10^1 µg/cm^2]*. The exposure sites were rinsed after removal of chambers and re-exposed once a week for a total of 3 exposures. A control group of 10 guinea pigs received the vehicle alone. After a 2-week rest period, the animals received primary challenge patches of 0.25% w/v stearamidopropyl dimethylamine in acetone on naïve skin *[Estimated dose/unit area = 1.5 x 10^1 µg/cm^2]*. One guinea pig had delayed contact hypersensitivity to the test material. The control animals had no reactions. A rechallenge was conducted in 6 guinea pigs 13 days after the primary challenge with 0.25%, 0.125%, and 0.0625% w/v stearamidopropyl dimethylamine. An additional 5 animals were used as controls. One guinea pig had a positive response to the test material at 0.25%. No other reactions were observed.

Palmityl/stearylamidopropyl dimethylamine at a concentration of 25% active in 8.95% phosphoric acid and 66.05%

water was studied for delayed contact hypersensitivity using albino Dunkin/Hartley guinea pigs.[96] A preliminary irritation test was conducted to determine the maximum concentration for the induction and challenge phases of the study. In the induction phase, 10 male and 10 female animals received 0.4 ml of test material on a 4 cm^2 patch on the clipped skin of the left shoulder for a period of 6 h. *[Estimated dose/unit area = 2.5 x 10^4 µg/cm^2].*The patches were occluded. An additional 5 male and 5 female animals were left untreated as the control. A total of 3 induction patches were applied, once weekly, for 3 weeks. Following a 2-week rest period, all animals received primary challenge patches of 0.4 ml of test material on the right flank for 6 h. The test sites were scored at 24 and 48 h post-application. All but 3 of the 20 guinea pigs had patchy to severe erythema at the 24 and 48 h observation periods. Four control animals had slight to moderate patchy erythema during the observation periods. Rechallenges were conducted on 0.25% active and 0.5% active palmityl/stearylamidopropyl dimethylamine. No sensitization was observed with the 0.25% active material, but 0.5% active material elicited reactions in sensitized animals. The study concluded that palmityl/stearylamidopropyl dimethylamine had the potential to cause delayed contact hypersensitivity in guinea pigs.

Two guinea pig maximization studies to assess the skin sensitization potential of amidoamine were provided by the Personal Care Products Council.[96] [97979797979797979797]In the first study, preliminary tests determined the maximum concentrations of intradermal injections, topical induction, and challenge applications. Ten albino Dunkin/Hartley guinea pigs (6 females and 4 males) received two 0.1 ml injections of 50% Freund's complete adjuvant at the first pair of sites, two 0.1 ml injections of 0.1% amidoamine at the second pair of sites, and two 0.1 ml injections of amidoamine in DOBS/saline vehicle and Freund's complete adjuvant (50/50 ratio) to yield a final concentration of 0.1% amidoamine at the third pair of sites. One week following the injections, a single occlusive 48-h induction patch (2 x 4 cm) of 0.2-0.3 ml amidoamine 5% in acetone/PEG400 vehicle was applied to the same shaved area. Four male control animals received intradermal injections and induction patches using only the vehicles. Two weeks after the induction patch, all animals received a single occlusive 24-h challenge patch (8 mm diameter patch in a Finn chamber) saturated with 0.5% amidoamine in acetone/PEG 400 on a clipped and shaved flank. The treatment sites were examined 24 and 48 h after patch removal. Two more challenges were made 1 and 2 weeks after the first challenge. Reactions were scored on a scale of 0 (no reaction) to 3 (severe erythema and edema).

At the first challenge, 7 animals had a reaction score of $\geq$ 0.5 at 24 h after the removal of the patch. After 48 h, 6 animals had a reaction $\geq$ 0.5. Three out of 10 animals had a reaction score of 2. At the second challenge, 7 guinea pigs had a score of $\geq$ 0.5 at 24 h after patch removal. These scores were consistent at the 48 h reading. Five out of 10 animals had a reaction score of 2. At the third challenge, all 10 guinea pigs had a score $\geq$ 1 at 24 h after patch removal. These score

remained largely consistent at the 48 h reading. Eight of the 10 animals had a reaction score of 2. The study concluded that amidoamine was a moderate sensitizer.[97]

The second maximization study was conducted in the same manner as the first with the only changes being that 0.025% amidoamine was used in the intradermal injections instead of 0.1%, 1% amidoamine was used in the topical induction, only 2 challenges were made, and 4 female guinea pigs were used as controls.

At the first challenge, 3 animals had a reaction score of $\geq$ 1 at both the 24 and 48 h readings, with one of the animals scoring a 2. At the second challenge, 3 animals had a reaction score of $\geq$ 1 at 24 and 48 h readings, although 1 animal had no reaction at 48 that had one at 24 h while another that had no reaction at 24 h had one at 48 h. The study concluded that amidoamine was a moderate sensitizer.[97]

Wright et al. reported on the results of an LLNA study performed on 4 chemicals that are recognized human contact allergens, including DMAPA (99.0+% pure).[98] The chemicals were tested in 7 different vehicles: acetone, olive oil [4:1], dimethylsulfoxide, methethylketone, dimethyl formamide, propylene glycol, and 50:50 and 90:10 mixtures of ethanol and water. Groups of 4 female CBA/Ca mice were exposed topically on the dorsum of both ears to 25 µL of 0.5%, 1.0%, 2.5%, 5.0%, or 10.0% of the test material, or to an equal volume of the appropriate vehicle alone, daily for 3 consecutive days. Five days after the initial topical treatment, all animals were injected intravenously with 20 µCi of [^{3}H] methyl thymidine. Approximately 5 h after injection, the animals were killed and the auricular lymph nodes were excised. Single cell suspensions were prepared from pooled lymph nodes, with the cells precipitated by trichloroacetic acid (TCA), and radioactivity measured by liquid scintillation. The stimulation indices (SI) were calculated, and at 10.0% DMAPA ranged from 2.2 in propylene glycol to 15.7 in dimethyl formamide. The estimated concentrations for a SI of 3 (EC_3) ranged from 1.7% (in dimethyl formamide) to >10% (in propylene glycol).

An LLNA study was performed using stearamidopropyl dimethylamine (TEGO AMID S 18).[99] A certificate of analysis reported that the DMAPA level conformed to the $\leq$ 20 ppm limit, the amine value was 150.8 mg KOH/g (limit range = 148.0-152.0 mg KOH/g), and the melting point was 68.0°C (limit range 66.0-69.0°C).[100] CBA/Ca female mice were divided into 5 groups of 4 and received 0.1%, 0.5%, 1%, 2.5%, or 5% (w/v) of the test material in ethanol/water (7/3, v/v) on the dorsum of each ear lobe (25 µl per ear, diameter ~ 8 mm) once daily for 3 consecutive days. A control group of 4 mice was treated with the vehicle only. The positive control group received α-hexylcinnamaldehyde in acetone:olive oil (4:1, v/v). The mice were treated with [^{3}H] methyl thymidine, killed, and the lymph nodes were prepared in the manner as described in the previous study.

No deaths occurred during the treatment period in any dose group. No clinical signs of toxicity were observed during treatment in the control group or in the 0.1% and 0.5% dose groups. Slight to moderate ear erythema was observed after the second or third application at both dosing sites in all mice in the 1%, 2.5%, and the 5% dose groups. This persisted for 2 days in the 1% dose group and until treatment end in the 2.5% and 5% dose groups. Body weight development was not affected in any of the animals. The SI were1.4, 2.1, 2.1, 5.8, and 3.9 for the 0.1%, 0.5%, 1%, 2.5%, and 5% dose groups, respectively. The EC_3 was calculated at 1.4%. The positive control group had expected results and validated the study. The study concluded that steramidopropyl dimethylamine (TEGO AMID S 18) was a potential skin sensitizer in this LLNA test.[99]

Calvert Laboratories, Inc. performed an LLNA study using amidoamine.[101] In the preliminary study, groups of 2 female CBA/J mice received 0.1%, 0.5%, 1%, 2.5%, or 5% (w/v) of the test material in ethanol/water (7:3, v/v) on the dorsal surface of both ears once per day for 3consecutive days. Mice were observed for erythema and edema for 6 days. Animals in the 5% dose group had very slight erythema on edema on days 3-5, and very slight erythema and slight edema on day 6. No reactions were observed.

In the main study, groups of 5 mice received 0%, 0.1%, 0.5%, 1%, 2.5%, or 5% of the test material in the same vehicle as the preliminary study mice. An additional 5 mice received the positive control, 35% hexylcinnamaldehyde. The mice were treated on the dorsal surface of both ears once daily for 3 days. On day 6, the mice were injected i.v. with 20 μCi of ^{3}H-thymidine. Five hours later, the mice were killed and the draining auricular lymph nodes were removed, processed and assessed for lymphocyte proliferation. No mortality or adverse effects were observed throughout the study. Very slight erythema was observed on day 3 and very slight erythema and edema were observed on days 4-6 of the 2.5% dose group. In the 5% dose group, 4 of the 5 mice treated had very slight erythema and very slight edema on day 2. On days 3-6, mice in this dose group had well defined erythema and slight edema. The SI were 1.8, 1.0, 3.1, 24.5, and 60.6 for the 0.1%, 0.5%, 1%, 2.5%, or 5% dose groups, respectively. The EC_3 for amidoamine was calculated at 0.98%. The positive control group had expected results and validated the study. This LLNA study concluded that amidoamine has skin sensitizing activity.

Human Studies

Studies of stearamidopropyl dimethylamine and stearyl/palmitylamidopropyl dimethylamine in human subjects indicated no significant sensitization potential of these compounds. Patch testing of CAPB, DMAPA, and amidoamine elicited positive reactions to DMAPA at concentrations as low as 0.05% and to amidoamine at concentration as low as 0.1%. A clear determination of which compound is responsible for CAPB sensitization has not been made, but in some studies, the role of CAPB alone causing allergic dermatitis could not be ruled out. The mechanism of sensitization is not completely

understood, but some studies suggest cross-reactivity of DMAPA and amidoamine causes CAPB sensitization. Dose per unit area calculations have been performed by CIR when adequate data are available.

Hill Top Research, Inc. performed an investigation of the potential of stearamidopropyl dimethylamine to induce skin sensitization in 112 human subjects. [102] Applications contained a concentration of 0.25% w/v of the test material in undiluted mineral oil. Induction applications of 0.3 ml were made to the same site with a Webril patch for a total of 9 applications. Challenge applications were made to naïve alternate sites. Frequent incidences of slight to moderate irritation, including erythema, some edema, papules, glazing, and cracking, were observed during the induction period, but were considered transient. Five subjects had a reaction of Grade 1 or greater during the challenge phase. The responses to stearamidopropyl dimethylamine were indicative of primary irritation rather than contact sensitization.

In a study by Inveresk Research International, the sensitization potential of a 4% aqueous liquid fabric softener formulation containing 0.5% stearyl/palmitylamidopropyl dimethylamine was investigated using 77 subjects.[103] During the induction phase, the test material was applied at a dose volume of 0.5 ml with a ¾ inch square Webril pad to the dorsal surface of the upper arm. [Estimated dose/unit area = 688.71 $\mu g/cm^2$]. Patches were applied for a duration of 24 h, 9 times over a period of 3 weeks. The test material caused some degree of irritation in most volunteers. After a rest period of 2 weeks, the subjects received challenge patches with the same concentration of test material on both arms. Patch sites were graded 48 and 96 h after patching. Eight subjects reacted at challenge, and 7 submitted to rechallenge with 4% and 0.4% aqueous formulations. No reactions indicative of sensitization occurred at rechallenge. The test formulation containing stearyl/palmitylamidopropyl dimethylamine had no significant sensitization potential.

Foti et al. patch tested 285 consecutive dermatitis patients with the European standard series supplemented with oleamidopropyl dimethylamine (0.5% aq.), CAPB (1% aq.), and DMAPA (1% aq.).[104] The standard patching technique was employed and test sites were scored on days 2, 3, 4, and 7. Twenty-three patients (8%) had allergic responses to DMAPA, 14 patients (4.9%) had allergic responses to DMAPA and oleamidopropyl dimethylamine, and 8 patients (2.8%) had allergic responses to all three of the supplemental chemicals. Analyses by TLC of the oleamidopropyl dimethyl amine sample revealed contamination by DMAPA (6 ppm or 0.12% of the sample) and indicates that the allergic responses in the last group were not due to cross-reaction. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

In a 2-year study by Pigatto et al., 1190 eczema patients were patch tested with 1% aq. CAPB using standard technique and grading according to the European Contact Dermatitis Group (ECDG).[105] From this patch test, 17 patients

were diagnosed with allergic contact dermatitis to CAPB. Relevance was established with an additional positive patch test of 2+ or more to at least one personal care product containing CAPB used by the patients. Fifteen patients were further tested with CAPB 0.01%, 0.5%, 1% (from 2 different manufactures), and 2% in water; and DMAPA at 0.05%, 0.1%, and 1% in petrolatum; and, if possible, the patients' reported cosmetics diluted in water at 1:10, 1:100, and 1:1000.

In 12 patients tested with their own personal cosmetics, 9 had positive reactions to at least one dilution and 5 had irritant reactions. All except 3 patients, who were not tested, had 2 or 3+ reaction to DMAPA at concentrations as low as 0.05%. Only one patient had a positive reaction to CAPB. The presence of DMAPA was investigated via thin-layer chromatography in the personal cosmetics of 4 of the patients that had positive reactions. These positive reactions from DMAPA suggest that the positive reaction to CAPB-containing products was likely due to a certain concentration of DMAPA that was an impurity . DMAPA was measured in the products at 50 - 150 ppm. The concentration of DMAPA was also measured in the 2 CAPB types: one had a concentration of DMAPA at 200 ppm and DMAPA was below detection level (level not reported) in the other type. The authors stated that the sensitizing agent in CAPB allergy is DMAPA, although their findings did not exclude the role of CAPB itself from causing allergic dermatitis.[105] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

A study of sensitization to commercially available CAPB in patients with dermatitis was performed by Angelini et al.[106] Twelve hundred consecutive patients with dermatitis of various types were patch tested with the European standard series and CAPB 1% aq. (30% active ingredient). Some of the patients that had allergic or irritant reactions to CAPB were then patch tested with the chemicals that were intermediates or reactants in the synthesis of CAPB (amidoamine, DMAPA, and monochloroacetic acid) along with a sample of CAPB of greater purity and Tego 103 G 1% aq.

Positive allergic reactions to CAPB were observed in 46 subjects (3.8%) while irritant reactions were recorded in 15 subjects (1.25%). Of these 46 subjects, 30 had positive reactions to DMAPA 1% aq. In these 30 subjects, 3 and 16 were positive to the purer grade of CAPB 0.5% aq. and CAPB 1% aq., respectively. Patients with irritant reactions had negative reactions to the synthesis materials and to the purer grade of CAPB. No allergic or irritant reactions to DMAPA were observed in 50 healthy controls. No positive reactions to amidoamine 0.05% were observed. The authors concluded that the results suggested that DMAPA impurity was responsible for CAPB allergy.[106] *(Concentrations tested were either 0.5% active CAPB or 0.15% active CAPB and 1% active CAPB or 0.3% active CAPB, respectively, but we do not know for certain).*

A further study by Angelini et al. was performed to determine if CAPB or an impurity of CAPB was responsible for cases of contact dermatitis.[107] In this study, thin-layer chromatography was employed to analyze a sample of CAPB (Tego

Betaine F 30% solution) and isolate and identify unknown impurities other than DMAPA, chloroacetic acid, and amidoamine found in the CAPB solution. An infrared spectrum analysis was used to confirm the presence of the sodium salt of N, N-dimethyl-propylene-diaminotriacetic acid.

Upon identifying the impurity, 30 patients with a history of contact allergy to 1% aq. CAPB and 1% DMAPA were patch tested with pure CAPB and a blend containing sodium chloride and N, N-dimethyl-propylene-diaminotriacetic acid (both at 1%). None of the subjects reacted to any of the chemicals. The authors suggested that pure CAPB, chloroacetic acid, amidoamine, and N, N-dimethyl-propylene-diaminotriacetic acid were not the components responsible for CAPB sensitivity and the involvement of DMAPA cannot be ruled out.[107] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

In another study by Angelini et al., DMAPA was tested at varying concentrations with other tensioactive chemicals to determine if they enhanced sensitivity to DMAPA.[108] Thirty-four subjects with confirmed contact allergy to 1% aq. DMAPA were patch tested with DMAPA in water, DMAPA in a SLES 2% aq. solution, and DMAPA in a polysorbate 20 2% aq. solution, all in decreasing concentrations from 0.1% to 0.00005%. The subjects were also patch tested with CAPB and a series of 10 substances chemically related to DMAPA. Test sites were occluded for 2 days and the sites were measured for reactions on days 2, 3, 4, and 7.

Eighteen subjects had positive reaction to DMAPA in water at 0.1%. No positive reactions were noted for DMAPA in water at 0.01% to 0.00005% . Positive reactions were observed in DMAPA in SLES, with 27 subjects positive at the highest concentration, 10 subjects positive at 0.01%, 5 subjects positive at 0.005%, and 1 subject positive at 0.0001%. Positive reactions were also observed in DMAPA in polysorbate 20 in 21 subjects at 0.1% and 4 subjects at 0.01%. Patch tests for the chemically related structures were positive in 28 subjects for N,N-dimethyl-2-ethylenediamine 1% aq., 12 subjects for cocamidopropylamine oxide 1% aq. (35% active material), and 18 subjects for CAPB 1% aq. (30% active material). No other reactions occurred. The authors concluded that tensioactives such as SLES and polysorbate 20 may enhance the risk of sensitization to DMAPA at low concentrations. They also concluded that the primary amine and the tertiary amine groups (dimethyl-substituted) are the sensitizing chemical structures in DMAPA and related molecules when they are separated by 2 or 3 carbon atoms.[108]

In another study by Angelini et al., 20 patients (ages 17-51 years, 13 females and 7 males) with confirmed contact allergy to DMAPA (1% aq.) and CAPB (1% aq.) were tested.[109] All the patients had an intolerance to detergents and shampoos and none were sensitized through an occupation. The patients were patch tested using serial dilutions of DMAPA

(100 ppm) in surfactant solutions (1% or 2% w/w surfacatants) that included purified CAPB (DMAPA < 1 ppm), SLES, polysorbate 20 (Tween 20), lauryl polyglucoside (APG), SLES/CAPB 3:1 (w/w), and APG/CAPB 3:2 (w/w). The test sites were scored on days 2, 3, 4 and 7. *(Concentration tested was either 1%active CAPB or 0.3% active CAPB, we do not know which).*

Positive reactions were observed in serial dilutions of DMAPA in 1% CAPB at 1 ppm and higher (1 reaction each to 1 ppm and 5 ppm DMAPA, 3 reactions to 10 ppm DMAPA, and 4 reactions to 50 ppm DMAPA). Similar positive observations were made in serial dilutions of DMAPA in 1% SLES/CAPB 3:1. No positive reactions were observed when DMAPA (100 ppm) was tested in water, but 7 positive reactions were recorded when the material was tested in 2% CAPB. A greater number of reactions were observed when 100 ppm DMAPA was mixed with 2% SLES/CAPB (5 reactions) than when mixed with 2% APG/CAPB (2 reactions). The authors noted that CAPB and SLES/CAPB 3:1 act as carriers for DMAPA when applied under occlusion at 1%, and that surface activity in more concentrated surfactant solutions may be responsible for allergic reactions by DMAPA. The authors concluded that the concentration limit for DMAPA in 1% CAPB or 1% SLES/CAPB 3:1 should be 0.5 ppm (corresponding to 15 ppm and 60 ppm, respectively) and that betaine should be blended with non-ionic surfactants to reduce allergy risks.[109] *(Concentrations tested were either 1% active CAPB or 0.3% active CAPB and 2% active CAPB or 0.6% active CAPB, but we do not know for certain).*

Uter studied 80 subjects (mainly hairdressers) with dermatitis from 1996 to 1999.[110] During this period the subjects were patch tested with the hairdresser's series supplemented with DMAPA (1% pet. and 1% aq.). The hairdresser's series contained CAPB (1% aq.) that had a maximum residual DMAPA of <15 ppm. Of the 80 subjects, 6 had + to +++ reactions to CAPB, but none of the six had reactions to DMAPA. A housewife with scalp and neck dermatitis had a + reaction to DMAPA 1% aq. and a +? reaction to DMAPA 1% pet. This subject had no positive reaction to CAPB. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

McFadden et al. studied 7 subjects that had relevant dermatitis to CAPB.[111] The dermatitis occurred after use of liquid soaps, and in one case an eye make-up remover, that contained CAPB. Four of the 7 subjects were patch tested with partially purified CAPB (1% aq.) containing <0.5% cocamidopropylamine and to 0.1% and 0.01% cocamidopropylamine. The patch sites were read at day 2 and day 4 after the initial patching. One subject had a positive reaction that appeared only to cocamidopropylamine. Another had a reaction only to CAPB; however irritancy could not be ruled out since the subject's patch sites were only read on day 2. The other 2 patients had positive reactions to cocamidopropylamine and CAPB. Control subjects had negative patch results.

Six out of the 7 original subjects with dermatitis were patched tested with DMAPA along with controls on normal and tape stripped skin at 0 ppm to 10,000 ppm. The subjects were also tested with DMAPA in the presence of 0.2% aq., sodium lauryl sulfate (SLS), or in the presence of 1.0% pure CAPB (<0.3% cocamidopropylamine, <10 ppm DMAPA). The patch sites were again read on day 2 and day 4 after the patch applications. One of the 6 subjects reacted to DMAPA on normal and tape-stripped skin at concentrations >1000 ppm. Three of the 6 subjects reacted to DMAPA in the presence of SLS (one at 10,000 ppm, one at 1000 to 10,000 ppm, and one at 100 to 10,000 ppm). None of the subjects reacted to the 1.0% pure CAPB. The authors concluded that the sensitization experienced by the subjects to the CAPB products was likely due to the residual intermediates from the CAPB production, with reaction to cocamidopropylamine more likely than DMAPA.[111] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

The impurities DMAPA and amidoamine in CAPB were further analyzed for sensitization potential in 10 subjects with CAPB allergy.[112] The subjects that had all tested positive to CAPB 1% aq. (Firma type) were patch tested with CAPB 1% aq. (Chemotechnique type), DMAPA 1% aq., and purified amidoamine at 0.5%, 0.25%, and 0.1% aq. All the subjects had ++ reactions to DMAPA at 1% and purified amidoamine at 0.5%. Most subjects also had ++ reactions to purified amidoamine at 0.25% and the remaining had + reactions to this concentration. Four patients had positive reactions (++) to the purified amidoamine at 0.1%. No reactions were observed to the CAPB from Chemotechnique, which was suggested to have a higher purity by the authors. Control patches in 20 volunteers were negative for amidoamine. The authors concluded that cross-reactivity between DMAPA and amidoamine causes CAPB allergy. They also suggested that DMAPA is the true sensitizing material and amidoamine aids in the trans-epidermal penetration of DMAPA. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Brey and Fowler performed a retrospective study of patients that had positive patch test results to 1.0% aq. CAPB and/or 1.0% amidoamine in the year 2001.[113] Reactions to other allergens were also recorded. Out of 957 patients patch tested in 2001, 49 had positive reactions to CAPB, amidoamine, or both. Follow-up evaluation in 35 patients was performed to establish relevance of reactions to CAPB and amidoamine with use of products containing these chemicals. Fifteen patients (42.9%) reacted to CAPB, 12 patients (34.3%) reacted to amidoamine, and 8 patients (22.8%) reacted to both. Of the 35 patients, 29 (83%) could identify products containing CAPB at home. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Fowler et al. performed a retrospective study of patients with CAPB and/or amidoamine contact allergy in 2001.[114] Out of 975 patients, 15 had a positive patch test reaction to 1.0% CAPB only, 25 had a positive patch test reaction to 0.1%

amidoamine only, and 18 had positive reactions to both (58 patients total). Definite and probable relevance (known exposure to CAPB) was determined in 16 patients that tested positive for amidoamine and in 16 that tested positive for CAPB. This study also evaluated formaldehyde allergy. Of the 58 patients, 12.7% were also allergic to formaldehyde. This was compared to the 10.1% of the total 975 patients that had formaldehyde allergy. The authors suggested that there is no significant relationship between CAPB or amidoamine allergy and formaldehyde allergy. *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

The NACDG evaluated 4913 patients for allergic contact dermatitis with an extended screening series of 65 allergens from January 1, 2001 to December 31, 2002. CAPB (1% aq.) and the by-product of CAPB production, amidoamine (0.1% aq.), were both included in this screening series. Positive results for CAPB were observed in 2.8% of the patients while 2.3% were positive for amidoamine. The relevance of the CAPB and amidoamine reactions (present and past) was 90.9% and 85%, respectively.[115] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

In a study by Li to determine the sensitization rate of CAPB in China and to analyze the relationship between CAPB and DMAPA, 429 patients (105 male, 324 female; 9-81 years old) with suspected contact allergy were patch tested with 1% aq. CAPB (purified) and 1% aq. DMAPA.[116] The patients were also tested with the European standard series.

Of the 429 subjects tested, 9 had irritant reactions, 12 had questionable reactions, and 42 had + reactions to CAPB. No reactions to CAPB greater than ++ were observed. Also of the 429 patients, 76 were diagnosed with cosmetic allergic contact dermatitis. Twenty-seven of these subjects and 15 (out of 353) of the subjects with cosmetic allergic contact dermatitis had positive reactions to CAPB ($P<0.05$). Only 25 of the former and none of the latter had relevant reactions. Ten of the 429 patients had positive reactions to DMAPA, 8 of which were considered relevant. Six of the 10 patients also had positive reactions to CAPB. Because the subjects of this study had positive reactions to both CAPB (purified) and DMAPA, the authors recommended that patch tests in cases of suspected cosmetic allergic contact dermatitis contain both CAPB and DMAPA.[116] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Provocative Use Studies

Provocative use studies identified DMAPA and amidoamine as potential causative agents in CAPB sensitivity, although these studies could not rule out that CAPB alone was not an allergen.

A provocative use study of products containing CAPB was performed by Fowler et al.[117] Ten subjects were identified through positive reactions to 1% aq. CAPB in routine patch testing. Ten control subjects negative to CAPB were

also enrolled. The provocative use test was divided into 3 phases, with 3 different test products (shampoo, liquid hand soap, and body wash) used in each phase. The products were specially formulated with CAPB-F grade (active level of CAPB in shampoo was 5.0%; active level in hand soap and body wash was 5.2%). Phase I was a forearm wash test with the shampoo diluted to 10% in tap water. If no allergic reaction occurred in Phase I, subjects then entered Phase II of the study: daily use of shampoo as hair cleanser. Subjects proceeded to Phase III of the study if no allergic reactions to the shampoo occurred. In Phase III, the subjects used the shampoo, body wash, and hand soap for 3 weeks.

At least 2 months after the product use tests, the subjects were patch tested with CAPB grades F and S (both 1% aq.), DMAPA (0.1% pet), amidoamine (0.1% aq.), sodium monochloroacetate (0.1% aq.), a proprietary mixture of preservatives for CAPB, and other potential allergens (perfumes and preservatives) that were in the test product formulations. Control subjects were only patched with 1% CAPB.

Three subjects completed the product use phases without experiencing an allergic reaction. Seven subjects had erythema, scaling, and pruritus on the arms, face, and/or neck in either Phase I or II of the study. One subject that experienced a positive reaction in the first phase was asked to repeat the forearm use test with the CAPB-containing shampoo on the left arm and with a CAPB-absent shampoo on the right arm. The subject experienced a positive reaction on both arms, which was likely caused by the preservatives in the shampoo products (as shown through patch testing). In Phase III, 3 subjects had scalp, face, and/or neck and body dermatitis.

Patch testing was performed in 9 of the 10 subjects, with 6 subjects reacting to 0.1% amidoamine. Five of these 6 subjects had positive reactions during the product use phases. Two subjects had reactions to the CAPB-F grade with preservative, 3 had reactions to CAPB-F grade without preservative, one reacted to the CAPB-S grade, and one reacted to the proprietary preservative mixture. Two subjects had questionable reactions to DMAPA. No other adverse reactions were noted in the subjects. *(Concentration used for patch testing was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

A follow-up patch test with 7 of the subjects was performed using purified CAPB (containing only 1 ppm amidoamine), CAPB-F grade (with approximately 3000 ppm amidoamine), and 2 concentrations of amidoamine (0.1% and 0.01% aq.). Two subjects had questionable reactions to the purified CAPB while there were 3 positive reactions to the CAPB-F grade, 4 positive reactions to the higher concentration of amidoamine, and 2 positive reactions to the lower concentration of amidoamine. The authors concluded that the impurity amidoamine may be the causative allergen in CAPB sensitivity and they recommend that cosmetics and personal care products should be formulated to minimize contamination

with this impurity. In addition, the authors could not rule out the possibility that CAPB alone was not an allergen to pre-sensitized individuals.[117] These results were also reported in Hunter and Fowler.[118]

Another provocative use test was conducted by Fartasch et al.[119] Subjects with eczema were tested for CAPB allergy while undergoing patch testing for the standard allergen series. Out of 1063 patients, 13 were identified with a positive patch reaction; however, relevance could only be established in 4 of the subjects. Another 6 patients were referred to the study for eczematous eruptions of the scalp and/or hand dermatitis and had positive 1% aq. CAPB patch test reactions. Twenty volunteers served as controls for the study.

The product use study consisted of 3 phases. In Phase I, a 0.1 ml test sample of shower gel containing CAPB (25% dilution; DMAPA below 1 ppm) was applied, lathered for 1 minute, and rinsed on the subjects' forearms twice daily for 7 days. The second phase of the study consisted of patch testing in order to differentiate irritant reactions from allergic reactions and to reconfirm sensitivity to CAPB and DMAPA. The subjects were patch tested with 0.1%, 0.3%, and 1.0% dilutions of CKKB (Tegobetaine CKKB5; 1.1 ppm DMAPA) and DMAPA, respectively. Patch sites were read on days 2, 3, and 4 following application. Subjects that had no allergic reactions in Phase I participated in Phase III. In this phase, the subjects used the shower gel as they would in normal daily hygiene practices for 4 weeks.

No skin irritation was observed in Phase I of the study. One subject with a history of atopic dermatitis was removed from the study due to a flare. Another subject had an immediate "wheal like reaction" on days 3 and 6 that cleared within minutes. This subject continued the forearm test an extra week and had no further effect. In Phase II, one control had an irritating reaction to 1% CAPB. In the study group, 5 out of the 10 subjects had a positive reaction to 1% CAPB and another 3 had marginal and/or irritant reactions. One subject had a positive reaction to DMAPA but had no clear reaction to CAPB. Another subject that had a positive reaction to CAPB had a doubtful reaction to 1% DMAPA. Eight subjects did not react to DMAPA. Only 7 subjects participated in Phase III of the study (the other 2 were not available), and no adverse reactions were observed in these subjects. The authors concluded that CAPB as tested may be used safely in individuals with CAPB sensitivity.[119] *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

Case Reports

Several case studies of allergic contact dermatitis reported positive patch tests to amidoamine and DMAPA, with one study reporting DMAPA elicited reaction at concentrations of 0.1% and greater.

Speight et al. reported on two case studies of occupational allergic contact dermatitis.[120] In the first case study, a 50-year-old man who worked in a chemical factory (which produced amines) developed a red itchy face. The reaction cleared

after treatment with topical corticosteriods and a week away from work. The patient had 4 more episodes over 6 months with swelling and spreading to the neck, shoulders, arms and hands. Patching testing with the European series yielded a + reaction only to ethylenediamine. Further patch testing with other amines, including DMAPA, produced a positive reaction (++) to DMAPA. Patch testing with serial dilutions of DMAPA revealed a ++ reaction at 1%, a ?+ reaction at 0.1%, and negative reactions for 0.01% and 0.001%. Twenty controls had negative reactions when patch tested with 0.1% and 1% DMAPA. DMAPA was being utilized at the factory where the patient worked to make CAPB. The dermatitis signs improved but did not completely clear when the patient was moved to another part of the plant to work.

In the second case study, a 54-year-old man who worked with DMAPA and CAPB developed an itchy red scaly face and right palm that cleared over 2 weeks. However, the patient had 6 more episodes over the next year. The dermatitis was resolved after the patient avoided contact with DMAPA. Patch testing with the chemicals used at the chemical factory yielded a ++ reaction only to DMAPA (1% pet.) on day 3 of site scoring.[120]

A 34-year-old woman employed as an assistant nurse developed dermatitis on both hands without earlier skin symptoms. The dermatitis would clear during periods of leave from work, but would reappear as soon as the patient resumed work. The patient was patch tested with the standard series, an antimicrobial series, and a cosmetics series. This testing only yielded a positive reaction to nickel. Initially, the hand dermatitis was considered to be occupational irritant contact dermatitis. The patient was forced to leave her career because of the condition and experienced occasional relapses afterward. Four years later, the patient was patched tested with the European standard series (minus nickel sulfate), an antimicrobial series, and a cosmetics series which included CAPB, oleamidopropyl dimethylamine, DMAPA, and coconut diethanolamide. Only DMAPA (>99% purity, 1% pet.) elicited a positive reaction with + readings on days 2 and 3 and a ++ reading on day 4. The authors stated that the dermatitis was from occupational exposure to shampoos and hand cleansers that may have contained DMAPA as a contaminant.[121]

A 37-year-old woman with no history of atopic or seborrheic dermatitis was reported by Fowler to have a 5 month history of eyelid dermatitis.[122] A family physician had instructed the patient to apply baby shampoo to the eyelids daily to treat an infection of the eyelids. Patch testing revealed a + reaction to CAPB and a ++ reaction to amidoamine (concentrations tested not reported. CAPB was present in the baby shampoo used by the patient. The dermatitis cleared after discontinuing use of the product.

A 39-year-old woman with personal history of eczema and asthma reported with a 6 month history of persistent dermatitis of the face and eyelids.[9] The patient complained of a burning sensation, pruritus, erythema, and occasional

swelling of the eyelids. The dermatitis would worsen when the patient's hair contacted her face. Patch testing using the NACDG standard series; the preservatives, vehicles and cosmetics series; and the patient's facial creams was conducted. Concentrations of the materials tested were not reported. On day 4, the patient reacted positively to nickel sulfate (++), gold sodium thiosulfate (++), cobalt chloride (+), tosylamide formaldehyde resin (+), CAPB (+), amidoamine (+), DMAPA (+), and oleamidopropyl dimethylamine (+). The patient did not have a positive reaction to cocamide diethanolamide.

Hervella et al. reported on 3 cases of allergic contact dermatitis.[123] The patients (a 58-year-old housewife, a 36-year-old male office worker, and a 24-year-old hairdresser) underwent patch testing with several test types including the standard series, the cosmetics series, the hairdresser's series, and with their own personal care products. All 3 patients tested positive to DMAPA (reactions ranged from + to ++ on day 7), but were negative for CAPB. After the initial patch testing, the patients were further tested with serial dilutions of 1% aq. DMAPA and 1% aq. CAPB (concentrations tested were 0.1%, 0.2%, 0.5%, and 1% for each). The first patient had a +/- reaction to 1% CAPB only. The other patients had no reactions to CAPB at any concentration. Allergic response were noted in all 3 patients to DMAPA at concentrations of 0.2% and higher (+/- to + at 0.2%, +/- to ++ at 0.5%, and + to +++ to 1%). *(Concentration tested was either 1% active CAPB or 0.3% active CAPB, we do not know which).*

A 42-year-old female reported with a 4 month history of severe recalcitrant eyelid dermatitis.[124] The patient's condition did not improve after use of all eye makeup was discontinued. The patient presented with bilateral periorbital and postauricular erythema, and a biopsy found spongiotic dermatitis. Patch testing using a modified NACDG standard series and a comprehensive cosmetic series was conducted. On day 4, the patient had + reaction to 1% aqueous DMAPA, a + reaction to neomycin, and a +++ reaction to bacitracin. There were no reactions to CAPB or amidoamine.

Quantitative Risk Assessment

Based on several of the animal and human studies described above for amidoamine, the Personal Care Products Council Task Force found that CAPB containing up to a maximum of 1.5% amidoamine in personal care products was safe. {Personal Care Products Council Task Force, 2010 220 /id} This latest finding includes the LLNA study of amidoamine up to 5% that is described above. An earlier weight-of-evidence no expected sensitization induction level (WoE NESIL) review for DMAPA by the Council's task force was performed on the Wright et al. LLNA study and found the value to be 425 $\mu g/cm^2$.{Personal Care Products Council Task Force, 2008 221 /id}

SUMMARY

Cocamidopropyl betaine (CAPB) is a zwitterionic ammonium compound containing a moiety of either a saturated or

unsaturated fatty acid ranging in length from 6 to 18 carbons in amide linkage with aminopropyl betaine. The source of these fatty acids, predominately lauric acid, is coconut oil. Other related ingredients are amidopropyl betaines with attached fatty acid moieties unique to the source, e.g., sesame oil for sesamidopropyl betaine.

Cosmetic grade CAPB, an aqueous solution, normally contains 35% solids. The NaCl content of these solids ranges from 4.5 to 5.6%. The concentration, when expressed as activity, is determined by subtracting the percent NaCl from the percent total solids. Because of uncertainty in whether concentrations given are "active" or dilutions of an active cosmetic grade material, in some cases the actual concentration of CAPB or other tested material is not known. No N-nitroso compounds were detected in samples of commercially supplied CAPB analyzed by gas chromatography-thermal energy analysis.

CAPB is used primarily as an amphoteric surfactant in shampoos, conditioners, and other cleansing preparations. It was listed as an ingredient in 2460 cosmetic formulations voluntarily reported to FDA. Reported use concentrations range from 0.2 to 25%.

The oral LD_{50} of full-strength commercial samples of 30% active CAPB was 4.91 g/kg in CFR mice and 7.45 ml/kg in Wistar rats. Another study of 30% active CAPB in Wistar rats found the acute oral LD_{50} to be 8.55 g/kg. The oral LD_{50} of 30% active CAPB in albino rats of an unspecified strain was 4.9 g/kg. The acute oral LD_{50} for 35.61% active CAPB was >1.8 g/kg for male Sprague-Dawley rats. All female rats in this study died before study end. The acute oral LD_{50} was greater than 5.0 g/kg and the acute lethal dermal dose was greater than 2.0 g/kg in studies of CAPB (31% active) with CD rats.

In a 28-day short-term study in which groups of 8 male and female animals received 0, 100, 500, or 1000 mg/kg of 30% active CAPB, treatment induced lesions were produced in the nonglandular portion of the stomach in the high-dose groups. Both males and females of the low-dose (100 mg/kg) group were comparable to concurrent controls.

In another 28-day oral toxicity study, rats received 0, 250, 500, or 1000 mg/kg CAPB. In the 1000 mg/kg dose group, compound-related edema of the mucosa of the non-glandular stomach was observed at macroscopic examination and acanthosis of the mucosa, inflammatory edema of the submucosa, and multiple ulcerations were observed during microscopic examination. These effects were thought to be the result of the irritating properties of CAPB and not of systemic toxicity. The NOEL and LOEL for this study were 500 mg/kg/day and 1000 mg/kg/day, respectively.

A subchronic oral toxicity study of CAPB rats that received 0, 250, 500, or 1000 mg/kg/day CAPB concluded that the NOEL was 250 mg/kg/day. Forestomach gastritis was observed in rats in the 500 and 1000 mg/kg/day dose groups.

Topical administration of varying commercial grades of CAPB (7.5 - 30% activity) in single insult occlusive patch

tests involving rabbits resulted in PIIs ranging from 0 to 3.75 (maximum score = 8). Slight edema was observed with CAPB with a 10% activity, but not with CAPB with a 7.5% activity.

No evidence of delayed contact hypersensitivity was found in Pirbright white guinea pigs topically administered solutions of 10% active CAPB in a Magnusson-Kligman maximization test. Microscopic changes in the treated skin of albino guinea pigs indicated slight delayed-type contact sensitization by a 3.0% active CAPB solution in a maximization test and modified Draize test.

Maximum mean irritation scores for eyes of rabbits treated with 30% active CAPB and left unrinsed ranged from 26 to 42 (maximum score = 110). Score for rinsed eyes ranged from 2 to 10. Irritation was observed primarily in the conjunctivae of treated eyes. At 4.5% active CAPB, there was slight conjunctival irritation in unrinsed eyes and very slight irritation in rinsed eyes. Scores for product formulations containing 2.2 to 6.3% active CAPB ranged from 4 to 30 in unrinsed, treated eyes of rabbits and were 3.3 and 20.0 in rinsed, treated eyes of rabbits.

The mutagenic potential of 30.9% and 31.0% active CAPB formulations was tested in the *Salmonella*/mammalian microsome mutagenicity assay and the L5178Y TK +/- mouse lymphoma assay. CAPB was nonmutagenic in these assays. CAPB was not mutagenic to the *S. typhimurium* indicator organisms in Ames *Salmonella*/microsome reverse mutation assays and in a mouse micronucleus assay.

In a single insult occlusive patch test of a 1.0% aq. dilution of a product formulation containing 6.3% active CAPB, no skin irritation was observed in 15 of 19 human subjects; 4 of the subjects had slight irritation. Slight erythema was observed after occlusive patching of 12 subjects with an 8% aq. dilution of a soap formulation containing 2.0% active CAPB daily for 5 days. Two soap formulations containing 2.25% active CAPB were considered primary irritants after a 21-day consecutive occlusive patch study.

An additional study investigated the potential of a 3.0% active solution of CAPB to induce contact photoallergy. There was no response to the challenge tests except for those exposed to both UVA and UVB radiation, who had mild to moderate erythemic responses that were not uncommon and were said to have resulted from the sunburn derived from UVB exposure.

CAPB was not a skin sensitizer at 1% in a study of 100 volunteers or in another study at 1.5% in 141 volunteers. Clinical sensitization studies and case studies show that persons already sensitized to CAPB react to concentrations of 1.0% of the material in water. Several case reports have found patients reporting contact allergy to multiple types of personal care products, including shampoos, contact lens solutions, eye makeup remover, bath gels, and toothpaste. Researchers have

included the CAPB impurities, DMAPA and amidoamine, in the scope of sensitization and case studies and have found that one or both of the impurities may be the responsible agent for contact allergy to CAPB.

DISCUSSION

While very few toxicity studies were identified specifically in the published literature for the additional amidopropyl betaines that were added to this safety assessment, there is no reason to expect these ingredients to differ in toxicity from CAPB. The amidopropyl betaines appear to be manufactured in the same manner as CAPB, with the difference only being in the fatty acid composition of the base compound. Some of these fatty acid compounds have already been reviewed by the CIR Expert Panel and have been found safe for use in cosmetic ingredients. Therefore, the Expert Panel determined that the toxicity data on CAPB could be extrapolated to include:

- almondamidopropyl betaine,
- apricotamidopropyl betaine,
- avocadamidopropyl betaine,
- abassuamidopropyl betaine,
- behenamidopropyl betaine,
- canolamidopropyl betaine,
- capryl/capramidopropyl betaine,
- coco/oleamidopropyl betaine,
- coco/sunfloweramidopropyl betaine,
- cupuassuaidopropyl betaine,
- isostearmidopropyl betaine,
- lauramidopropyl betaine,
- meadowfoamamidopropyl betaine,
- milkamidopropyl betaine,
- minkamidopropyl betaine,
- myristamidopropyl betaine,
- oatamidopropyl betaine,

- oleamidopropyl betaine,
- olivamidopropyl betaine,
- palmamidopropyl betaine,
- palmitamidopropyl betaine,
- palm kernelamiodpropyl betaine,
- ricinoleamidopropyl betaine,
- sesamidopropyl betaine,
- shea butteramidopropyl betaine,
- soyamidopropyl betaine,
- stearamidopropyl betaine,
- tallowamidopropyl betaine,
- undecyleneamidopropyl betaine, and
- wheat germamidopropyl betaine.

The Expert Panel recognized that use concentration data are not available for all ingredients in this group and that some ingredients in this group are not in current use. The Panel considered that the use concentrations for the ingredients that are in use are not likely to be different from the use concentration for CAPB. In reviewing studies involving CAPB and related ingredients, often the %active material in the test material was clearly stated, but in other cases, it was not clear if the test material was active material or a dilution of active material. Because the difference, at most, would be a factor of 3, the uncertainty was factored into the review process.

The CIR Expert Panel considered that the available acute, short-term, and subchronic animal toxicity studies were supportive of the safety of CAPB. In vitro genotoxicity studies supported the absence of mutagenic activity. The Expert Panel noted the absence of reproductive and developmental toxicity and absorption data, but also noted that CAPB does not produce systemic toxicity in a 92-day oral toxicity study in rats. Because these ingredients are very large molecular weight structures and water soluble, the Expert Panel considered that they would not be readily absorbed into the skin.

In the absence of inhalation toxicity data, the Expert Panel determined that CAPB can be used safely in hair sprays, because the ingredient particle size is not respirable. The Expert Panel reasoned that the particle size of aerosol hair sprays (~38 μm) and pump hair sprays (>80 μm) is large compared to respirable particulate sizes (≤10 μm).

In past ingredient safety assessments, the CIR Expert Panel has expressed concern over N-nitrosation reactions in ingredients containing amine groups. CAPB, and the other betaine ingredients in this assessment, contain secondary amides that may serve as substrates for N-nitrosation. Additionally, these ingredients may contain secondary amine inpurities which may serve as substrates for N-nitrosation. Therefore, the Expert Panel recommended that this ingredient should not be included in cosmetic formulations containing N-nitrosating agents.

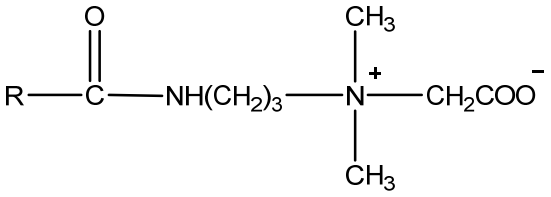
CAPB, while not a significant dermal irritant, can be a dermal sensitizer. The Panel considered that there is adequate demonstration that this sensitization is primarily the result of the presence of two impurities in CAPB: 3-dimethylaminopropylamine (DMAPA) and cocamidopropyl dimethylamine (amidoamine). The Panel considered establishing limits for levels of these impurities. Available data demonstrated that DMAPA was non-sensitizing at a concentration up to 100 ppm (0.01%); a quantitative risk assessment of amidoamine calculated that amidoamine was non-sensitizing at a concentration us to 1.5%. The Expert Panel recommended that an empirical approach in which the content of DMAPA and amidoamine are minimized in cosmetic formulations containing CAPB and related chemicals.

CONCLUSION

(Version 1) The CIR Expert Panel concluded that CAPB and its related aminopropyl betaines are safe in cosmetics as long as they are formulated to be non-sensitizing.

(Version 2) The CIR Expert Panel concluded that CAPB and its related aminopropyl betaines are safe in cosmetics as long as the impurity DMAPA is limited to 100 ppm and the impurity amidoamine is limited to 1.5%.

Figure 1. Amidopropyl Betaine



RCO- represents a fatty acid derived from various oils.

Figure 2. Reaction process of cocamidopropyl betaine.

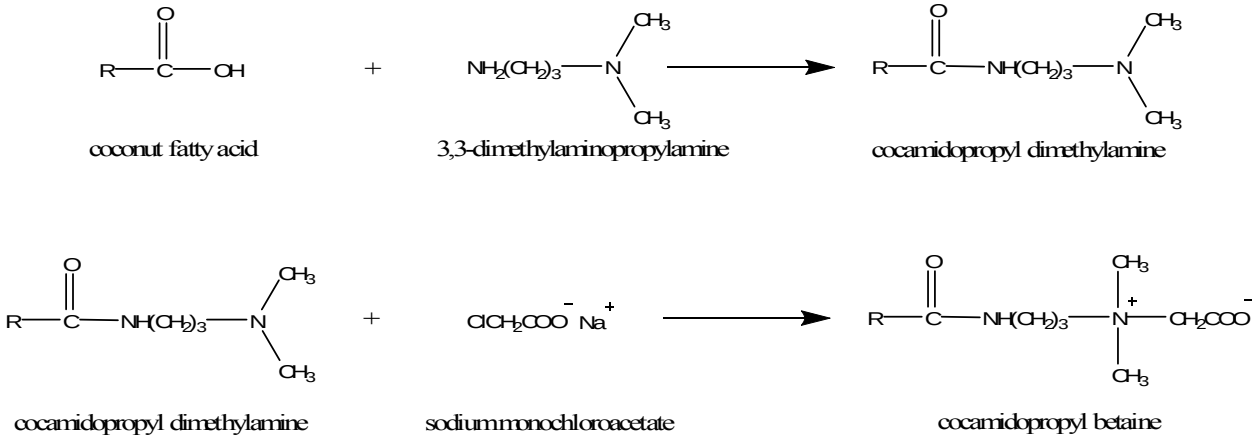


Table 1. Definitions, structures, and functions for CAPB and related amidopropyl betaine ingredients.[2]

Ingredient	Definition	Function	Related CIR Reviews and Conclusions
Cocamidopropyl Betaine (CAS Nos. 61789-40-0; 83138-08-3; 86438-79-1)	The zwitterion (inner salt) that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from coconut oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Coconut Oil & Acid 1986, Safe; 2008 Safe
Almondamidopropyl Betaine (CAS No. not found)	The zwitterion (inner salt) that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from almond oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Almond Oil 1983, Safe; 2005, Not Reopened
Apricotamidopropyl Betaine (CAS No. 133934-08-4)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from Prunus Armeniaca (Apricot) Kernel Oil (q.v.).	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Avocadamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from Persea Gratissima (Avocado) Oil (q.v.).	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Avocado Oil 1980, Safe; 2003, Not reopened.
Babassuamidopropyl Betaine (CAS No. 147170-44-3)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from Orbignya Oleifera (Babassu) Oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Behenamidopropyl Betaine (CAS No. 84082-44-0)	The zwitterion that conforms generally to the structure in Figure 1.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Canolamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from canola oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Capryl/Capramidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from caprylic and capric acids.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Coco/Oleamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from Coconut Oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Oleic Acid 1987, Safe; 2006, Not reopened Coconut Oil & Acid 1986, Safe; 2008 Safe
Coco/Sunfloweramidopropyl Betaine (CAS No. 147170-44-3)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived	Slip Modifiers; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Surfactants - Solubilizing Agents; Viscosity Increasing Agents -	NA

Table 1. Definitions, structures, and functions for CAPB and related amidopropyl betaine ingredients.[2]

Ingredient	Definition	Function	Related CIR Reviews and Conclusions
	from a blend of Coconut and Sunflower Seed Oils.	Aqueous	
Cupuassamidopropyl Betaine (CAS No. 657350-94-2)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from the pulp of the cupuassu tree (Theobroma grandiflorum).	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Isostearamidopropyl Betaine (CAS No. 63566-37-0)	The zwitterion that conforms generally to the structure in Figure 1.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Isostearic Acid 1983, Safe; 2005, Not Reopened.
Lauramidopropyl Betaine (CAS Nos. 4292-10-8; 86438-78-0)	The zwitterion that conforms generally to the structure in Figure 1.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Lauric Acid 1987, Safe; 2006, Not Reopened
Meadowfoamamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from meadowfoam seed oil.	Humectants; Skin Protectants	None
Milkamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from milk.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Minkamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from mink oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Mink Oil 2005, Safe
Myristamidopropyl Betaine (CAS No. 59272-84-3)	The zwitterion that conforms generally to the structure in Figure 1.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Myristic Acid 1987, Safe; 2006, Not Reopened; Currently under reivew with the Myristates Group.
Oatamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from Avena Sativa (Oat) Kernel Oil (q.v.).	Antistatic Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Oleamidopropyl Betaine	The zwitterion that conforms generally	Antistatic Agents; Hair Conditioning Agents;	Oleic Acid 1987, Safe; 2006, Not Reopened

Table 1. Definitions, structures, and functions for CAPB and related amidopropyl betaine ingredients.[2]

Ingredient	Definition	Function	Related CIR Reviews and Conclusions
(CAS No. 25054-76-6)	to the structure in Figure 1.	Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	
Olivamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from olive oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Palmamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from palm oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Palm Oil 2000, Safe.
Palmitamidopropyl Betaine (CAS No. 32954-43-1)	The zwitterion that conforms generally to the structure in Figure 1.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Palmitic Acid 1987, Safe; 2006, Not Reopened
Palm Kernelamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from palm kernel oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Palm Kernel Oil 2000, Safe
Ricinoleamidopropyl Betaine (CAS No. 71850-81-2)	The zwitterion that conforms generally to the structure in Figure 1.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Ricinoleic Acid 2005, Safe
Sesamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from sesame oil.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Sesame Seed Oil 1993, Safe; Currently under review.
Shea Butteramdiopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from Butyrospermum Parkii (Shea Butter).	Surfactants - Cleansing Agents; Surfactants - Foam Boosters	None
Soyamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from soy.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Stearamidopropyl Betaine	The zwitterion that conforms generally	Antistatic Agents; Hair Conditioning Agents;	Stearic Acid 1987, Safe; 2006, Not

Table 1. Definitions, structures, and functions for CAPB and related amidopropyl betaine ingredients.[2]

Ingredient	Definition	Function	Related CIR Reviews and Conclusions
(CAS No. 6179-44-8)	to the structure in Figure 1.	Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Reopened
Tallowamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from tallow.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Tallow 1990, Safe; 2006, Not Reopened
Undecyleneamidopropyl Betaine (CAS No. not found)	The zwitterion that conforms generally to the structure in Figure 1.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	None
Wheat Germamidopropyl Betaine (CAS No. 133934-09-5)	The zwitterion that conforms generally to the structure in Figure 1, where RCO- represents the fatty acids derived from wheat germ.	Antistatic Agents; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents; Surfactants - Foam Boosters; Viscosity Increasing Agents - Aqueous	Wheat Germ Oil 1980, Safe; 2003, Not Reopened.

Table 2. Technical names for CAPB and related amidopropyl betaines.[2]

Ingredient	Technical/Other Names
Cocamidopropyl Betaine	CADG N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxococonut)Amino]-1-Propanaminium Hydroxide, Inner Salt Cocamido Betaine Cocamidopropyl Dimethyl Glycine Cocoyl Amide Propylbetaine Cocoyl Amide Propyldimethyl Glycine Cocoyl Amide Propyldimethyl Glycine Solution 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethy-3-[(1-Oxococonut)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Cocoamidopropyl)Dimethyl, Hydroxides, Inner Salts
Almondamidopropyl Betaine	Almond Amide Propylbetaine Almondamidopropyl Dimethyl Glycine N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoalmond)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoalmond)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Almondamidopropyl) Dimethyl, Hydroxide, Inner Salt
Apricotamidopropyl Betaine	Apricot Amide Propylbetaine Apricotamidopropyl Dimethyl Glycine N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoapricot)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, 3-Amino-N-(Carboxymethyl)-N,N-Dimethyl-, N-Apricot-Oil Acyl Derivs., Hydroxides, Inner Salts 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoapricot)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Apricotamidopropyl) Dimethyl, Hydroxide, Inner Salt
Avocadamidopropyl Betaine	Avocado Amide Propylbetaine Avocadoamidopropyl Dimethyl Glycine N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoavocado)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoavocado)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Avocadoamidopropyl) Dimethyl, Hydroxide, Inner Salt
Babassuamidopropyl Betaine	Babassu Amide Propylbetaine Babassuamidopropyl Dimethyl Glycine N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxobabassu)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxobabassu)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Babassuamidopropyl) Dimethyl, Hydroxide, Inner Salt
Behenamidopropyl Betaine	Behenamide Propylbetaine Behenamidopropyl Dimethyl Glycine 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxobehenyl)Amino]-, Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxodocosanyl)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Behenamidopropyl) Dimethyl, Hydroxide, Inner Salt
Canolamidopropyl Betaine	None found.
Capryl/Capramidopropyl Betaine	None found.
Coco/Oleamidopropyl Betaine	None found.
Coco/Sunfloweramidopropyl Betaine	1-Propanaminium, 3-Amino-N-(Carboxymethyl)-N,N-Dimethyl-, N-(C8-18 and C18-Unsatd. Acyl) Derivs., Hydroxides, Inner Salts
Cupuassuamidopropyl Betaine	1-Propanaminium, 3-Amino-N-(Carboxymethyl)-N,N-Dimethyl- N-(Theobroma Grandiflorum Acyl) Derivs.

Table 2. Technical names for CAPB and related amidopropyl betaines

Isostearamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoisooctadecyl)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoisooctadecyl)Amino]-, Hydroxide, Inner Salt
Lauramidopropyl Betaine	Ammonium, (carboxymethyl)(3-lauramidopropyl)diemthyl-, Hydroxide, Inner Salt N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxododecyl)Amino]-1-Propanaminium Hydroxide, Inner Salt N-(Dodecylamidopropyl)-N,N-diemthylammonium Betaine Glycine, (3-lauramidopropyl)Diemthylbetaine Lauroyl Amide Propyldimethyl Glycine Solution 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxododecyl)Amino]-, Hydroxide, Inner Salt
Meadowfoamamidopropyl Betaine	None found.
Milkamidopropyl Betaine	None found.
Minkamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxomink)Amino]-1-Propanaminium Hydroxide, Inner Salt Mink Amide Propylbetaine Minkamidopropyl Dimethyl Glycine 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxomink)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Minkamidopropyl) Dimethyl, Hydroxide, Inner Salt
Myristamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxotetradecyl)Amino]-1-Propanaminium Hydroxide, Inner Salt Myristamidopropyl Dimethyl Glycine 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxotetradecyl)Amino]-, Hydroxide, Inner Salt
Oatamidopropyl Betaine	None found.
Oleamidopropyl Betaine	Ammonium, (Carboxymethyl)Dimethyl(3-Oleamidopropyl)-, Hydroxide, Inner Salt N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxooctadecenyl)Amino]-1-Propanaminium Hydroxide, Inner Salt Oleamidopropyl Dimethyl Glycine 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxooctadecenyl)Amino]-, Hydroxide, Inner Salt
Olivamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoolive)Amino]-1-Propanaminium Hydroxide, Inner Salt Olivamidopropyl Dimethyl Glycine Olive Amide Propylbetaine 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoolive)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Oliveamidopopyl) Dimethyl, Hydroxide, Inner Salt
Palmamidopropyl Betaine	None found.
Palmitamidopropyl Betaine	Ammonium, (Carboxymethyl)Dimethyl(3-Palmitamidopropyl)-, Hydroxide, Inner Salt N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxohexadecyl)Amino]-1-Propanaminium Hydroxide, Inner Salt pendecamaine (INN) 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxohexadecyl)Amino]-, Hydroxide, Inner Salt
Palm Kernelamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxopalm Kernel)Amino]-1-Propanaminium Hydroxide, Inner Salt Palm Kernel Amide Propylbetaine Palm Kernelamidopropyl Dimethyl Glycine

Table 2. Technical names for CAPB and related amidopropyl betaines

	Palm Kernel Oil Amide Propyl Dimethyl Glycine Solution 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxopalm Kernel)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Palm Kernelamidopropyl) Dimethyl, Hydroxide, Inner Salt
Ricinoleamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoricinoleyl)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoricinoleyl)Amino]-, Hydroxide, Inner Salt Propyl Betaine Ricinoleate Amide Solution Ricinoleamidopropyl Dimethyl Glycine
Sesamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxosesame)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxosesame)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Sesameamidopropyl) Dimethyl, Hydroxide, Inner Salt Sesame Amide Propylbetaine Sesamidopropyl Dimethyl Glycine
Shea Butteramidopropyl Betaine	None found.
Soyamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxosoy)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxosoy)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Soyamidopropyl) Dimethyl, Hydroxide, Inner Salt Soy Amide Propylbetaine Soyamidopropyl Dimethyl Glycine
Stearamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxooctadecyl)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxooctadecyl)Amino]-, Hydroxide, Inner Salt Stearoyl Amide Propyl Dimethyl Glycine
Tallowamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxotallow)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxotallow)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Tallowamidopropyl)Dimethyl, Hydroxides, Inner Salts
Undecylenamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoundecylenyl)Amino]-1-Propanaminium Hydroxide, Inner Salt 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxoundecylenyl)Amino]-, Hydroxide, Inner Salt Quaternary Ammonium Compounds, (Carboxymethyl)(3-Undecylenamidopropyl) Dimethyl, Hydroxide, Inner Salt Undecylenamide Propylbetaine Undecylenamidopropyl Dimethyl Glycine
Wheat Germamidopropyl Betaine	N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxowheat Germ Alkyl)Amino]-1-Propanaminium Hydroxides, Inner Salts 1-Propanaminium, 3-Amino-N-(Carboxymethyl)-N,N-Dimethyl-, N-Wheat-Oil Acyl Derivs, Hydroxides, Inner Salts 1-Propanaminium, N-(Carboxymethyl)-N,N-Dimethyl-3-[(1-Oxowheat Germ)Amino]-, Hydroxide, Inner Salt

Table 3. Composition, chemical, and physical characteristics of batches of cosmetic grade CAPB.[5]

Color	Clear pale yellow liquid
Odor	Faint
pH	4.6 - 5.6
Water content	62% - 66%
NaCl	4.6% - 5.6%
Active materials (100 - H_2O - NaCl, %)	29.5% - 32.5%
Alkalinity	0.725 - 0.825 M_{eq}/g
Boiling point	230°F
Specific gravity	1.04
Solubility at 25°C	
Water	2 g/10 ml
Alcohol	2 g/10 ml
Fatty Acids	
C_8	5.6% - 6.0%
C_{10}	5.4% - 5.7%
C_{12}	53.1% - 53.2%
C_{14}	16.1% - 17.4%
C_{16}	8.1% - 8.3%
C_{18}	10.0% - 10.2%

Table 4. Fatty acid compositions of the oil components of amidopropyl betaines (%).[16;127-131]

Fatty Acids	Coconut	Almond	Apricot	Avocado	Babassu	Canola	Cupuassu	Meadowfoam Seed
Caproic (C6)	0.008 - 1.2							
Caprylic (C8)	3.4 - 15				4 - 8			
Capric (C10)	3.2 - 15				4 - 8			
Lauric (C12)	41 - 51.3				44 - 47			
Myristic (C14)	13 - 23				15 - 20			
Palmitic (C16)	4.2 - 18	5.5 - 6.5	small quantities	13 - 17	6 - 9	2.8 - 3	5.8	
Stearic (C18)	1.6 - 4.7	2 - 3			3 - 5	1.3	38.3	
Oleic (C18:1)	3.4 - 12	70 - 77		67 - 72	10 - 12	57.1 - 57.4	42.8	
Oleic/Linoleic			90 - 93					
Linoleic (C18:2)	0.9 - 3.7	17 - 20		10 - 12	1 - 3	20.1 - 22.1		
Arachidic (C20)	1.03						4.8	
Palmitoleic (C16:1)				3 - 5.1				
Linolenic (C18:3)						10.8 - 12.5	8.3	
Eicosenoic (C20:1)						2.5 - 3.1		52 - 77*
Erucic (C22:1)						1 - 3.3		8 - 29*
C22:2								7 - 20*

* Natural Plant Products, Inc reports the fatty acid composition of meadowfoam seed oil to be 58-64% C20:1 (Δ5), 3-6% C22:1 (Δ5), 10-14% C22:1 (Δ13), and 15-21% C22:2 (Δ5Δ13).

Table 4. Fatty acid compositions of the oil components of amidopropyl betaines (%) (continued).[16;127-131]

Fatty Acids	Mink Crude	Olive	Palm	Palm Kernel	Sesame	Shea	Soybean	Sunflower	Tallow	Wheat Germ
Caprylic (C8)				3 - 4%						
Capric (C10)				3 - 7%						
Lauric (C12)	0.1			46 - 52%						
Myristic (C14)	3.5		1 - 6	15 - 17%					3 - 6	
Myristoleic (C14:1)	0.9									
Pentadecanoic (C15)	0.1									
Palmitic (C16)	17.2	7.5 - 20	32 - 47	6 - 9%	7 - 10.9%	5 - 9		5.2 - 7.2	24 - 32	11-16
Heptadecanoic (C17)	0.4									
Heptacdecanoic (C17:1)	0.5									
Stearic (C18)	2.5	0.5 - 3.5	1 - 9	1 - 3%	3.4 - 6%	30 - 41		2.7 - 6.5	20 - 25	1 - 6
Oleic (C18:1)	40.9	53 - 86	39 - 53	13 - 19%	32.7 - 53.9%	45 - 50	11.5 - 60	14.7 - 35	37 - 43	8 - 30
Linoleic (C18:2)	15.0	3.5 - 20	2 - 11	0.5 - 2%	37 - 59%	4 - 5	25 - 63.1	51.5 - 73.5	2 - 3	44 - 65
Arachidic (C20)					0.3 - 8%			0.3 - 1		
Palmitoleic (C16:1)	17.0	0.3 - 3.5								
Linolenic (C18:3)	0.6	0 - 1.5					2.9 - 12.1	0.01 - 0.3		4 - 10
Eicosenoic acid (C20:1)										
Eicolenoic (C20:1)	0.6									
Other							12 - 13.5 (unknown saturated acids)			0 - 1.2 (C20-C22 saturated acids)
Cholesterol, arachidonic acid, elaidic acid, and vaccenic acid									Small quantities	

Table 5. Current cosmetic product uses and concentrations for cocamidopropyl betaine and its related amidopropyl betaines.

Product Category	2010 uses (total number of products in category){Food and Drug Administration, 2010 173 /id}	2010 concentrations of use (active){Personal Care Products Council, 2010 197 /id} (%)	Activity(s) of Raw Material[19] (%)
	Cocamidopropyl Betaine		
Baby products			
Shampoos	41 (57)	2 - 4	30
Other	65 (149)	3 - 6[1]	30
Bath products			
Oils, tablets, and salts	7 (338)	0.06 - 7	30
Soaps and detergents	826 (1781)	0.5 - 10	28, 30, 32, 34
Bubble baths	81 (176)	0.2 - 6	8, 10, 30
Capsules	- (4)	0.9	30
Other	92 (227)	3 - 6	6, 28, 30, 35
Eye makeup			
Eye shadow	- (1343)	3	35
Eye makeup remover	7 (133)	0.005	1
Mascara	1 (528)	-	-
Fragrance products			
Colognes and toilet waters	1 (1426)	-	-
Other	7 (641)	-	-
Noncoloring hair care products			
Conditioners	24 (1313)	2 - 4	28, 30, 35
Sprays/aerosol fixatives	13 (321)	0.2	36
Straighteners	13 (181)	0.4 - 9	30, 35, 36
Permanent waves	- (75)	2 - 9	30, 35
Rinses	4 (34)	9	30
Shampoos	818 (1487)	3 - 9	13, 28, 30, 35, 36, 38
Tonics, dressings, etc.	48 (1321)	0.2 - 5	28, 30, 35
Wave sets	3 (60)	-	-
Other	36 (838)	-	-
Hair coloring products			
Dyes and colors	392 (2382)	0.6 - 6	30
Tints	2 (6)	6	30
Rinses	- (40)	1 - 6	4, 30
Shampoos	27 (36)	-	-
Color sprays	- (7)	6	30
Lighteners with color	2 (22)	6	30
Bleaches	- (147)	6	30
Other	3 (168)	0.6 - 3	30
Nail care products			
Other	1 (137)	0.8	39
Oral hygiene products			
Dentifrices	- (60)	0.6 - 6	NA
Personal hygiene products			
Deodorants (underarm)	- (623)	2	31
Douches	4 (13)	3.8	30
Other	422 (925)	2 - 10[2]	30, 32, 35, 36
Shaving products			
Shaving cream	4 (128)	0.03 - 9	30, 35
Shaving soap	1 (10)	9	30
Other	8 (126)	11	32
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	272 (1528)	1 - 7	28, 30, 31, 35, 38, 40
Face and neck creams, lotions, powder and sprays	6 (1652)	-	-
Body and hand creams, lotions, powder and sprays	11 (1875)	0.4 - 3	35
Foot powders and sprays	3 (46)	4	30
Moisturizers	9 (2750)	-	-
Night creams, lotions, powder and sprays	1 (386)	-	-
Paste masks/mud packs	6 (462)	0.2	35
Other	26 (1446)	-	-

Product Category	2010 uses (total number of products in category){Food and Drug Administration, 2010 173 /id}	2010 concentrations of use (active){Personal Care Products Council, 2010 197 /id} (%)	Activity(s) of Raw Material[19] (%)
Total uses/ranges for Cocamidopropyl Betaine	**3287**	**0.005 - 11**	**1 – 40**
	Almondamidopropyl Betaine		
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	- (1528)	3	-
Total uses/ranges for Almondamidopropyl Betaine	-	**3**	-
	Babassuamidopropyl Betaine		
Noncoloring hair care products			
Conditioners	1 (1313)	0.9	-
Shampoos	14 (1487)	4	-
Hair coloring products			
Shampoos	1 (36)	-	-
Bath products			
Soaps and detergents	4 (1781)	2	-
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	5 (1528)	0.9	-
Total uses/ranges for Babassuamidopropyl Betaine	**25**	**0.9 - 4**	-
	Capryl/Capramidopropyl Betaine		
Noncoloring hair care products			
Conditioners	1 (1313)		-
Shampoos	- (1487)	0.3	-
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	1 (1528)	-	-
Moisturizers	1 (2750)	-	-
Suntan products			
Suntan gels, creams and liquids	- (106)	2	-
Other	- (61)	2	-
Total uses/ranges for Capryl/Capramidopropyl Betaine	**3**	**0.3 - 2**	-
	Coco/Oleamidopropyl Betaine		
Bath products			
Soaps and detergents	3 (1781)	-	-
Noncoloring hair care products			
Shampoos	1 (1487)	-	-
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	2 (1528)	-	-
Body and hand creams, lotions, powder, and sprays	3 (1875)	-	-
Other	1 (1446)	-	-
Total uses/ranges for Coco/Oleamidopropyl Betaine	**10**	-	-
	Lauramidopropyl Betaine		
Bath products			
Soaps and detergents	63 (1781)	2 - 8	-
Bubble baths	5 (176)	4 - 5	-
Other	7 (227)	3 - 8	-
Fragrance products			

Product Category	2010 uses (total number of products in category){Food and Drug Administration, 2010 173 /id}	2010 concentrations of use (active){Personal Care Products Council, 2010 197 /id} (%)	Activity(s) of Raw Material[19] (%)
Other	- (641)	4	-
Noncoloring hair care products			
Conditioners	2 (1313)	-	-
Shampoos	45 (1487)	0.9 - 8	-
Tonics, dressings, etc.	1 (1321)	-	-
Other	- (838)	0.00006	-
Lauramidopropyl Betaine (continued)			
Hair coloring products			
Dyes and colors	27 (2382)	-	-
Shampoos	1 (40)	-	-
Other	- (168)	0.6	-
Makeup preparations			
Other	-(536)	2	-
Nail care products			
Nail polish and enamel	3 (351)	-	-
Personal hygiene products			
Douches	- (13)	-	-
Other	24 (925)	2 - 13[3]	-
Shaving products			
Shaving cream	1 (128)	-	-
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	42 (1528)	3 - 5	-
Face and neck creams, lotions, powder and sprays	2 (1652)	0.7	-
Body and hand creams, lotions, powder and sprays	1 (1875)	2	-
Moisturizers	1 (2750)	-	-
Paste masks/mud packs	1 (462)	-	-
Skin fresheners	- (267)		-
Other	1 (1446)	6[4]	-
Total uses/ranges for Lauramidopropyl Betaine	**227**	**0.00006 - 13**	**-**
Myristamidopropyl Betaine			
Noncoloring hair care products			
Shampoos	1 (1487)		
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	-(1528)	0.3	-
Total uses/ranges for Myristamidopropyl Betaine	**1**	**0.3**	**-**
Oatamidopropyl Betaine			
Suntan products			
Indoor tanning preparations	-(247)	0.3	-
Other	1 (61)	-	-
Total uses/ranges for Oatamidopropyl Betaine	**1**	**0.3**	**-**
Olivamidopropyl Betaine			
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	1 (1528)	-	-
Total uses/ranges for Olivamidopropyl Betaine	**1**	-	-
Palm Kernelamidopropyl Betaine			

Product Category	2010 uses (total number of products in category){Food and Drug Administration, 2010 173 /id}	2010 concentrations of use (active){Personal Care Products Council, 2010 197 /id} (%)	Activity(s) of Raw Material[19] (%)
Noncoloring hair care products			
Shampoos	-(1487)	5	-
Bath products			
Soaps and detergents	-(1781)	0.9	-
Total uses/ranges for Palm Kernelamidopropyl Betaine	-	**0.9 - 5**	-
	Shea Butteramidopropyl Betaine		
Bath products			
Soaps and detergents	3 (1781)	-	-
Other	- (227)	0.6	-
Noncoloring hair care products			
Shampoos	2 (1487)	1	-
Personal hygiene products			
Other	6 (925)	2[5]	-
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	-(1528)	4	-
Total uses/ranges for Shea Butteramidopropyl Betaine	**11**	**0.6 - 4**	-
	Soyamidopropyl Betaine		
Noncoloring hair care products			
Shampoos	-(1487)	1	-
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	3 (1528)	2	-
Moisturizers	1 (2750)	-	-
Total uses/ranges for Soyamidopropyl Betaine	**4**	**1 - 2**	-
	Undecylenamidopropyl Betaine		
Noncoloring hair care products			
Conditioners	1 (1313)	-	-
Shampoos	-(1487)	2	-
Total uses/ranges for Undecylenamidopropyl Betaine [2]	**1**	**2**	-

[1] 6% in a rinse-off baby product.
[2] 2% in a facial exfoliating cleanser, 3% in a body scrub, 3% in a facial cleanser, 3% in a shower gel, 10% in a shower gel.
[3] 5% in a bath and shower gel.
[4] 6% in a foot soak.
[5] 2% in a wipe.

Table 6. Animal skin irritation studies on CAPB.

Concentration	Number and Species	Results	References
50%, diluted 1 part + 1 part (v/v)	3 albino rabbits	No erythema, eschar, or edema; not a primary skin irritant.	[40]
30% active[a]	6 albino rabbits	PII = 0.5. Very slight to well-defined erythema, no edema; mild primary irritant.	[34]
7.5% active[a] solution	3 albino rabbits	No irritation.	[35]
10% active[a] solution, pH 6.1	1 albino rabbit	PII = 0.25; non-irritating.	[36]
10% active[a] solution, pH 4.5	6 NZW rabbits	PII = 0.3. Very slight erythema, no edema.	[37]
30% active[a]	6 NZW rabbits	PII = 3.75. Eschar formation.	[38]
15% active[a] solution	3 albino rabbits	PII - 3.5. Well defined erythema, slight edema; not a primary skin irritant.	[39]

[a] Referenced as full strength

Table 7. Eye irritation studies on CAPB.

Concentration	No./strain of rabbit	Results	Reference
4.5% active[b]	6/albino	Slight conjunctival irritation in 3 unrinsed eyes. Very slight conjunctival irritation in 2 of 3 rinsed eyes.	45
30% active[b]	3/albino	Diffuse corneal opacity at day 3. Mild conjunctival erythema, chemosis, and discharge from day 1. Slight iritis on day 4.	46
6% active solution	3/albino	Mild conjunctival erythema and slight discharge, cleared by day 3.	47
7.5% active, pH 8.3	6/NZW	Mild to moderate conjunctival irritation after 24 h, disappearing by day 6.	48
10% active[b], pH 6.1	1/albino	Max. unrinsed score = 30 after day 3, 7 by day 7.	36
30% active[a]	9/NZW	Max. mean score (unrinsed, n = 6) = 41.7 after 72 h, decreased to 27.2 after 7 days (scale 0 - 110). Minimal irritation in rinsed eyes (n = 3).	49
8.6% active[a]	9/NZW	Max unrinsed score = 25.7 after 24 h, 0 by day 7. Mean score rinsed (n = 3) = 2.0 after 24 h, 0 by 48 h.	50
5%	6/NZW	Draize score = 4.90. Not an ocular irritant.	51
10%	6/NZW	Draize score = 27.3. Moderately irritating.	52
3.0% active	6/albino	Corneal irritation day 3 - 7. Iritis and conjunctival irritation lessens in severity by day 7.	53
3.0% active	6/albino	No corneal irritation. Iritis and conjunctival irritation clear by day 7.	53
3.0% active	6/albino	Average ocular index = 41.6/110. Ocular irritant.	54;55
Soap formulation containing 2.3% active[b] CAPB	9/NZW	Max mean score (unrinsed, n = 6) = 18.7, primarily irritation of iris and conjunctiva. Max mean score (rinsed, n = 3) = 20.0.	56
Soap formulation containing 2.3% active[b] CAPB	9/NZW	Max mean score (unrinsed, n = 6) = 1.7. Max mean score (rinsed, n = 3) = 3.3. Primarily conjunctival irritation.	57
Soap formulation containing 6.5% active[b] CAPB	4/NZW	Max total score = 30.0 (max 110). Irritation of cornea, iris, and conjunctiva. Moderately irritating.	58
Formulation containing 6.0% active[b] CAPB	6/albino	Conjunctival irritation after day 1.	1

[a] Reference cited as % solids.
[b] Referenced as full strength

Table 8. Clinical sensitization studies on CAPB and related amidopropyl betaines.

Exposure	Subjects/Patients	Result	Reference
	Cocamidopropyl Betaine		
0.1872% active CAPB in a shampoo	88	No sensitization	[68]
0.93% active aq. sol. of CAPB	93	No sensitization	[69]
0.3% active CAPB in formulation	100	No sensitization	[70]
1.5% active aq. CAPB changed to 3.0% active CAPB	141	No sensitization	[71]
6% active CAPB in a cleansing cloth	210	No sensitization	{Clinical Research Laboratories, 2002 224 /id;Clinical Research Laboratories, 2002 225 /id}
0.018% active CAPB in a facial cleanser	27	No sensitization	{KGL, 2007 223 /id}
1% aq. CAPB or 0.3% active aq. CAPB	781	56 positive (7.2%)	[72]
1% aq. CAPB or 0.3% active aq. CAPB	10,798	29 positive (0.27%)	[73]
unknown % CAPB	12	Irritation only	[74]
1% aq. CAPB or 0.3% active aq. CAPB	93	4 positive reactions	{Vilaplana J, 1990 119 /id}
1% aq. CAPB or 0.3% active aq. CAPB	210	12 positive (5.75%)	{Fowler JF, 1993 123 /id}
	Almondamidopropyl Betaine and Olivamidopropyl Betaine		
1% active almondamidopropyl betaine and 1% active olivamidopropyl betaine in a body cleanser	103	No sensitization	{AMA Laboratories, 1989 24 /id}
	Capryl/Capramidopropyl Betaine		
1.72% active capryl/capramidopropyl betaine in mousse with SLS co-treatment	26	No sensitization	{KGL, 2005 5 /id}
	Lauramidopropyl Betaine		
14% active lauramidopropyl betaine in a shower gel with SLS co-treatment	25	No sensitization	{KGL, 1996 4 /id}
0.042% active lauramidopropyl betaine in a shampoo	51	No sensitization	{Consumer Product Testing, 2002 217 /id}
0.03955% active aq. sol. of lauramidopropyl betaine in a body cleanser	109	No sensitization	{Clinical Research Laboratories, 2008 216 /id}
	Shea Butteramidopropyl Betaine		
0.54% active shea butteramidopropyl betaine in a body wash	25	No sensitization	{KGL, 2008 6 /id}
0.04% active aq. sol. of shea butteramidopropyl betaine in a body scrub	101	No sensitization	{Clinical Research Laboratories, 2008 216 /id}

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Ref Type: Generic

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Ref Type: Generic

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TEST MATERIAL E9314

BIOLOGICAL TEST FOR SAFETY

ECMBTS No. 597

INSTRUCTIONS: For formulations list full chemical names and percentages in formula for each ingredient. Provide laboratory notebook reference where finished product formula card number, production code, consumer test number, etc., of sample(s) submitted for testing can be found. For ingredients give full chemical and trade names, structure or formula, percent purity, and names and concentrations of known contaminants. Append all toxicological or safety data available from the supplier. DO NOT IDENTIFY BY TRADE NAMES ONLY. Provide laboratory notebook reference where full description of the ingredients can be found. IT IS THE SUBMITTER'S RESPONSIBILITY TO KEEP RECORDS WHICH FULLY DESCRIBE SAMPLES SUBMITTED.

MATERIAL PRODUCT

Name: Palmityl/Stearyl amidopropyl di-methyl amine based on hydrogenated tallow. (ex Goldschmidt)

Formula or nature of product:

25.00 % Palmityl/Stearyl amidopropyl di-methyl amine
8.95 % Phosphoric Acid (85 %)
66.05 % water (dist.)

100.00 %

APPROVAL

[redacted]
P & RS SECT. HD.
ORIG. SECT. HD.
ORIG ASSOC. DIR.
P & RS [redacted] DIRECTOR
RETURN TO P & RS

Physical form : liquid
Density, g/ml : ~ 1
pH : 3.5 - 4.0

For ingredient
% in product : 0.5 %
Est. max in any product : ~ 1 %

TYPE OF CONSUMER EXPOSURE FOR WHICH SAFETY CLEARANCE IS REQUESTED

No. of subjects
Starting date
Duration
Location

PANEL/ BLIND TEST: Feb. '80
SHIP TEST:
TEST MARKET: Oct. '80
NATIONAL:

Safety Clearance/Reassurance Request No.

CHARGE ALLOCATION: Lenor CT

Originating Brand Man: J. Ooms
Discussed with: D.J.G. Müller
Date: 22.11.79

BIOLOGICAL TESTS:

For P & RS use only

1. Acute oral toxicity (rat) ✓
2. Acute percutaneous toxicity (rabbit) ✓
3. Eye irritation (rabbit) ✓
4. Skin irritation (rabbit) ✓
5. Phototoxicity (hairless mice)
6. Pharmacological screen (Irwin screen, mice) ✓
7. Sensitization (guinea pig) ✓
8. DRIPT (after completion of 7.)

SAMPLE IDENTIFICATION

E-9314 = Palmityl/Stearyl amidopropyl di-methyl amine (ex Goldschmidt)

Est. cost: £ 5000
Est. Completion Date: May 1980
P&RS Toxicologist: [redacted]

PDD Notebook No.: E 433 p 34
P&RS Notebook Nos.: E 424 p 7e
Date: 23.11.79

DISTRIBUTION: ORIGINATOR, P&RS (b., F., G., I., UK), ETC (BRUSSELS, NEWCASTLE), MVL.

page 2

6) Skin sensitization (Guinea pig) (LSR 80/PGN 447/202)

Test Material	Concentration		Incidence of sensitization
	Induction	Challenge	
Palmityl/Stearylamido propyldimethylamine (EJ-base, E-9314)	as supplied (25 % active)	as supplied	14/20

A rechallenge programme demonstrated 1 % (i.e. 0.25 % active) as a no-effect level; 2 % (0.5 % active) still elicited reactions in sensitized animals.

The studies on phototoxicity and sensitization in humans were cancelled.

Conclusions :

[redacted] It has a high sensitization potential in Guinea pigs with reactions caused by concentrations as low as 0.5 % active.

[redacted]

[redacted] 19 Feb 81

Distribution :

[redacted]

February 16, 1981 - DM/sv

TEST MATERIAL E9314

LIFE SCIENCE RESEARCH

CONFIDENTIAL

11.06.80

DELAYED CONTACT HYPERSENSITIVITY
IN GUINEA-PIGS
(BUEHLER TEST) :
f- [illegible] BTS 597 E9314

LSR Report No : 80/PGN447/202

Palmityl/Stearyl amidopropyl dimethyl amine
(ex Goldschmidt)

The undersigned hereby declares that the report following constitutes a true and faithful account of the procedures adopted, and the results obtai[illegible]n the performance of this study.

S.A. Buch, B.Sc.
(Head, Short-Term Toxicology)

.....Buch.....

To:
[redacted]

From:
S.A. Buch,
J.R. Gardner,
Life Science Research,
Stock,
Essex.
CM4 9PE

30 May 1980

(1)

1. SUMMARY

1.1 E9314 was examined for provocation of delayed contact hypersensitivity in guinea-pigs. The test material was administered as the undiluted liquid received at all phases of induction and challenge.

The assessment was carried out in accordance with Standard Procedure No. 4 of the Miami Valley Laboratories of the Procter and Gamble Company, dated 15 May 1979.

1.2 Under the conditions of this assessment, E9314 was shown to have the potential to cause delayed contact hypersensitivity.

1.3 The majority of test group animals showed sensitisation responses to challenge which varied from slight patchy erythema to severe erythema. The incidence and severity of the test group responses greatly exceeded those of the control group 24 and 48 hours after challenge.

1.4 All animals remained in overt good health, and made normal bodyweight progress throughout the period of assessment.

2. INTRODUCTION

The objective of the delayed contact hypersensitivity test is the detection of sensitisation potential. This method has been applied to toiletries and household products in screening out of skin sensitisers prior to exposure [illegible].

The experimental work was carried out during the period 21 April - 23 May 1980.

3. MATERIAL

A consignment of approximately 757 g of ECN BTS 598 E9314, a cream-coloured slurry, was received on 29 February 1980.

The identity, strength, stability and purity of the test material as supplied remained the responsibility of the Sponsor.

4. METHOD

The method follows the Standard Procedure No. 4 of the Miami Valley Laboratories of the Procter and Gamble Company, dated 15 May 1979.

4.1 Animals and husbandry

Albino guinea-pigs of the Dunkin-Hartley strain were supplied by Tuck, Rayleigh, Essex. The animals were five to six weeks old on arrival, and within the bodyweight range 269 - 310 g.

Open-topped metal cages (90 x 60 x 22 cm) with solid floors accommodated up to ten animals of the same sex. Autoclaved wood shavings were provided for bedding material and changed for fresh on alternate days.

Animals had free access to a commercially-available standard pelleted cavy diet, Guinea-Pig F.D.1 from BP Nutrition (U.K.) Ltd., Witham, Essex, supplemented daily with autoclaved hay. Water was supplied from two bottles per cage.

Each room within the limited-access building has been assigned to one species alone. All animal rooms were kept at slight positive pressure relative to the outside and each room had its own supply of fresh filtered air. The maximum and minimum temperature of the previous 24-hour period and the relative humidity were recorded at the beginning of each working day. A thermostat in the animal room had target values for temperature and humidity of 18°C ± 2°C and 50% ± 70% R.H. Electric time-switches regulated a cycle of twelve hours of artificial light per day. A stand-by generator was available to maintain electricity supply in the event of a mains power failure.

All personnel entering the building changed into clean protective clothing and wore additional gown, gloves and face-mask to service the animal-holding areas.

Animal preparation

The animals were held for an acclimatisation period of eight days prior to primary induction. During this period, animals were examined daily, and were found to be in good health.

Each animal was weighed on arrival and identified by tattooed ear-mark. Ten males and ten females were allocated to the test group and five males and five females were allocated to the control group.

4.3 Selection of administered concentration of test material

The concentration of E9314 administered was selected as a result of two investigations:

a) The solubility of the test material in distilled water was examined.

b) The primary irritancy of the test material was assessed by exposing four naive animals to dermal applications of E9314 at concentrations of 5%, 10%, 40% and 80% w/v in distilled water.

E9314 was administered as the undiluted liquid at all phases of induction and challenge.

4.4 Induction procedure

The skin overlying the left shoulder of the test group of animals was shaven on the day prior to treatment. Closed patches were applied to the

dermal test sites once each week for three weeks. On each occasion, 0.4 ml of a freshly-prepared suspension of the test material was applied to a double layer of open-weave gauze (20 x 20 mm), overlying the dermal test site. A single layer of occlusive plaster (50 x 50 mm, Blenderm, 3M Company), covered the gauze, and an elastic adhesive bandage (50 x 30 mm, Elastoplast, Smith and Nephew Ltd.), was wrapped around the trunk to secure the dressing during a six-hour exposure period.

The control animals were untreated during the induction period.

4.5 Challenge procedure

Primary challenge of the test and control groups was carried out 15 days after the third phase of the induction period. A six-hour occlusive dressing, as used at induction, was applied to a previously untreated site on the right flank. The challenge sites were depilated 24 hours after challenge, by application of a cream of calcium thioglycolate. Two hours and one day after depilation the exposed sites were examined for incidence and severity of erythematous reactions to treatment.

4.6 Assessment of responses to challenge

The challenge responses were assessed according to the criteria given below:

Skin response to challenge	Score
No reaction	0
Slight patchy erythema	±
Slight, but confluent or moderate patchy erythema	1
Moderate erythema	2
Severe erythema, with or without oedema	3

Grades of 1 or greater in the test group were considered to be indicative of sensitisation, provided grades of less than 1 were seen in control animals. If grades of 1 or greater were noted in control animals, then reactions of test animals that exceeded the most severe reaction in controls were presumed to be due to sensitisation.

The results of challenge were expressed in terms of both incidence and severity of responses.

$$\text{Incidence:} \quad \frac{\text{Number of animals showing responses of 1 or greater}}{\text{Number of animals in group}}$$

$$\text{Severity:} \quad \frac{\text{Sum of test grades}}{\text{Number of animals in group}}$$

(For the purpose of the calculation of severity indices, grades of ± are equal to 0.5).

5. RESULTS

There were no erythematous responses to application of undiluted E9314 after the first phase of induction. Moderate patchy erythema was seen in some animals after the second and third phases of induction.

All but three of the 20 test animals showed erythematous responses 24 and 48 hours after challenge with undiluted E9314. The responses ranged from a slight patchy erythema to severe erythema. Four control animals showed slight or moderate patchy erythema 24 or 48 hours after challenge.

The incidence and severity of the response shown by the test group greatly exceeded that of the control group 24 and 48 hours after challenge.

6. CONCLUSION

Under the conditions of this assessment E9314 was shown to have the potential to cause delayed contact hypersensitivity in the guinea-pig.

TABLE 1

Primary irritation screen

Animal number	Dermal irritation response to applied concentration of E9314			
	5% v/v	10% v/v	40% v/v	80% v/v
[illegible]	0	±	0	±
96♂	0	0	0	0
97♂	0	0	0	0
98♀	0	0	0	0

Dermal sensitisation response to challenge with undiluted E9314 in guinea-pigs

Group	Number of animals challenged	Erythematous response to challenge			
		24 hours		48 hours	
		Incidence[A]	Severity[B]	Incidence	Severity
Control	10	0.00	0.10	0.20	0.13
Test	20	0.55	0.95	0.70	1.43

A Incidence = Number of animals showing responses of 1 or more ÷ number of animals in group.

B Severity = Total score of response grades ÷ number of animals in group.

TABLE 3

Individual skin responses to induction and challenge with undiluted E9314 with bodyweight

Treatment	Animal number & sex	Erythematous response to					Bodyweight				
		1st induction	2nd induction	3rd induction	Challenge (24 hour reading)	Challenge (48 hour reading)	Start	Week 1	Week 2	Week 3	Week 4
Control E9314 undiluted, administered at challenge only	51♂	-	-	-	0	0	356	379	440	490	507
	52♂	-	-	-	0	0	367	431	494	563	638
	53♂	-	-	-	±	1	294	324	388	441	493
	54♂	-	-	-	0	0	304	334	390	441	468
	55♂	-	-	-	0	0	360	419	470	545	589
	56♀	-	-	-	0	1	340	392	422	470	489
	57♀	-	-	-	±	0	336	377	403	447	487
	58♀	-	-	-	0	0	316	364	405	453	464
	59♀	-	-	-	0	0	312	356	376	402	420
	60♀	-	-	-	0	±	364	395	433	467	487
Test E9314 undiluted, administered at induction and challenge	31♂	0	0	0	1	1	377	425	472	517	553
	32♂	0	1	0	1 - 2	2	375	417	470	524	570
	33♂	0	1	0	2	3	336	364	356	440	491
	34♂	0	0	0	0 - 1	0 ± 1	332	357	389	417	480
	35♂	0	0	0	1	1	337	385	411	470	521
	104♂	0	0	1	1	1	357	399	424	480	536
	37♂	0	1	0	0 - 1	0 ± 1	332	371	401	444	496
	38♂	0	0	0	± 1	± 0	286	333	392	460	510
	39♂	0	0	1	1	2	371	416	461	535	580
	40♂	0	0	0	1	2	322	364	395	469	520
	41♀	0	1	0	1	2	341	373	428	476	514
	42♀	0	1	1	±	3	294	322	364	398	420
	43♀	0	0	0	0	±	349	369	405	467	446
	44♀	0	0	0	1	1	326	349	382	419	430
	45♀	0	1	0	2	2	298	331	368	432	466
	46♀	0	0	0	2	2	289	316	350	392	408
	47♀	0	0	0	± 2	2	360	394	431	467	489
	48♀	0	1	0	2	1	302	331	368	414	442
	49♀	0	0	0	1	2	351	380	408	451	490
	50♀	0	0	0	2	3	328	380	420	475	520

REPORT HT 83-1603-21

[redacted]

TEST MATERIAL G0250.01

BIOLOGICAL SAFETY TEST SUMMARY REVIEW

Test Material: Stearamidopropyl dimethyl amine

Corp. H&ES Sample Code No.: G0250.01

Contract Laboratory: Hill Top

Type of Study: Guinea Pig Sensitization Report #: 83-1603-21

Requester: [redacted] Division: BYCR Authorization #: BYCR 0195

Date Report Written: 1/31/84 Date Rec'd by Operations Section: 2/7/84

This report has been reviewed and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

None

H&ES Operations Section Monitor: [redacted] Date: 5/1/84

At primary challenge, one guinea pig elicited delayed contact hypersensitivity to a 0.25% solution of G0250.01 in acetone. Rechallenges were conducted with 0.25, 0.125 and 0.0625% solutions. One guinea pig elicited a positive skin response to 0.25% solution. All remaining test sites were negative.

HSD Scientific Monitor: [redacted] Date Section

This report has been reviewed for scientific quality and is summarized, with comments (if any) as follows:

A group of twenty guinea pigs induced with 1% SAPDMA w/v in 80/20 water : acetone and challenged with 0.25% w/v SAPDMA in 100% acetone produced a weak positive response of 1 in 1/20 test pigs. Six of the twenty guinea pigs were rechallenged with 0.25, 0.125 and 0.0625% w/v SAPDMA in 100% acetone. A positive response was observed in 1/6 guinea pig at 0.25% w/v SAPDMA.

Divisional Toxicologist: [redacted] Date: 5/4/84

CIRCULATE: BACK TO CORP. SCIENTIFIC MONITOR; THEN TO CORP. LIAISON

This report is approved for microfilming and entry into P&G Toxicology Files

HSD Scientific Monitor: [redacted] Date:

Corporate HSD Liaison: [redacted] Date:

Return to HSD Operations Section, [redacted]

Entered into Safety Data System by [redacted] Date: 3-6-85

Microfilming Completed by [redacted] Date:

Hill Top Research, Inc.

P.O. Box 42501, Cincinnati, Ohio 45242 (513) 831-3114

DELAYED CONTACT HYPERSENSITIVITY
STUDY IN GUINEA PIGS
OF
G0250.01 (BYCR 0195)
FOR
[redacted]

Hill Top Research Project No. 83-1603-21

Report Issue Date: 2/20/84

Report Issued By:

HILL TOP RESEARCH, INC.

David L. Conine, Ph.D.
Director of Technical Services,
Acute Toxicology

RECEIVED BY
FEB 23 1984
OPERATIONS SECTION

COPY SENT TO: [redacted]

DATE: 2/23/84

HILL TOP RESEARCH, INC.

IMPORTANT NOTICE

Hill Top Research, Inc. submits this report with the understanding that no portion of it will be used for advertising or promotion without obtaining our prior written consent to the specific proposed use. When such use is desired we will be glad to assist in the preparation of mutually acceptable excerpts or summaries.

Ref.: 83-1603-21 February 9, 1984

REPORT APPROVAL AND CONTRIBUTORS

Report Prepared by:

HILL TOP RESEARCH, INC.

Robert L. Doyle, B.S.
Technical Writer, Acute Toxicology

Report Approved by:

HILL TOP RESEARCH, INC.

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Report Reviewed by Quality Assurance:

HILL TOP RESEARCH, INC.

Alan S. Weiner, B.A.
Auditor, Quality Assurance

Contributors

The following members of Hill Top Research, Inc. contributed to the conduct and reporting of Project No. 83-1603-21:

Name	Title	Function
J. Kreuzmann, B.A.	Section Head, Acute Toxicology	Project Monitor, Interim Study Director, Conduct of Study
D. Conine, Ph.D.	Director of Toxicology	Study Director
J. Fischer, A.S.	Research Technician	Conduct of Study
B. Garling, A.S.	Research Technician	Conduct of Study
T. Hughes	Technician	Conduct of Study
R. Doyle, B.S.	Study Director	Report Preparation

REPORT

Project No.: 83-1603-21

Test/Protocol No.: Delayed Contact Hypersensitivity/G4

Issue Date: May 20, 1983

Sponsor: [redacted]

Sample Identification (Code No.): G0250.01 (BYCR 0195)

Date Sample Received: December 2, 1983

Source of Animals: Murphy Breeding Laboratories, Inc.

Date Study Initiated: December 12, 1983 Date Study Completed: January 27, 1984

Sample Characterization and Stability

The sponsor has assumed responsibility for test substance characterization and stability testing. Unused sample was returned to the sponsor on February 15, 1984.

Sample Preparation and Application

Phase of Study	Material	Concentration	Vehicle
1. Induction	G0250.01	1.0% w/v	80% Ethanol/ 20% Distilled Water
2. Primary Irritation			
Pilot 1	G0250.01	20, 10, 5.0, 2.5% w/v	80% Ethanol/ 20% Distilled Water
Pilot 2	G0250.01	5.0, 2.5, 1.0, 0.5% w/v	Acetone
Pilot 3	G0250.01	1.0, 0.5, 0.25, 0.1% w/v	80% Ethanol/ 20% Distilled Water
Pilot 4	G0250.01	0.5, 0.25% w/v	Acetone
3. Challenge	G0250.01	0.25% w/v	Acetone
4. Rechallenge			
A.	G0250.01	0.25% w/v	Acetone
B.	G0250.01	0.125% w/v	Acetone
C.	G0250.01	0.0625% w/v	Acetone

Summary/Conclusions

The potential of G0250.01 as an 1.0% w/v formulation in 80% ethanol/20% distilled water to produce delayed contact hypersensitivity in guinea pigs was evaluated using the method of Ritz and Buehler[1].

[1] Ritz, H. L., and Buehler, E. V., Current Concepts in Cutaneous Toxicity, ed. Drill, V. A. and Lazar, T. (Academic Press, 1980) pp. 25-40.

Following the primary challenge, the test substance, G0250.01 as a 0.25% w/v formulation in acetone, produced one responder in the test group (1 of 20) and no responders in the control group. Thirteen days after the primary challenge application, G0250.01 was tested as 0.25, 0.125 and 0.0625% w/v formulations in acetone producing one responder in six test animals at the 0.25% concentration and no responders to the other concentrations or in the five control animals.

METHODS (See Appendix 1 for Protocol and Amendments)

1. Protocol followed without deviations: yes X no ____

RESULTS

Results of the primary irritation, challenge and rechallenge applications are given in Tables 1, 2 and 3, respectively.

A concentration of 0.25% w/v in acetone of G0250.01 was determined to be the highest non-irritating concentration for primary challenge.

The incidence and severity indices were calculated as follows:

Group	Sample	Concentration (% w/v in Acetone)	Incidence (No. Responders/ No. Treated)	Mean Severity of Irritation Scores 24-hour	48-hour
			Primary Challenge		
Test	G0250.01	0.25	1/20	0.2	0.2
Control	G0250.01	0.25	0/10	0.1	0.1
			Rechallenge[a]		
1. Test	G0250.01	0.25	1/6	0.4	0.5
Control	G0250.01	0.25	0/5	0.2	0.0
2. Test	G0250.01	0.125	0/6	0.1	0.1
Control	G0250.01	0.125	0/5	0.0	0.1
3. Test	G0250.01	0.0625	0/6	0.1	0.0
Control	G0250.01	0.0625	0/5	0.2	0.1

[a]One common control group was used for all rechallenge applications.

Ref.: 83-1603-21 February 9, 1984

Table 1

PRIMARY IRRITATION

IRRITATION SCORES IN GUINEA PIGS 24 AND 48 HOURS FOLLOWING SIX-HOUR PATCH APPLICATIONS OF G0250.01 AT VARIOUS CONCENTRATIONS IN 80% ETHANOL/20% DISTILLED WATER OR ACETONE

Animal Number	Sex	Irritation Score (Site Location[a]) 20% w/v Solution[b] 24-hr	48-hr	10% w/v Solution[b] 24-hr	48-hr	5.0% w/v Solution[b] 24-hr	48-hr	2.5% w/v Solution[b] 24-hr	48-hr
Pilot 1 in 80% Ethanol/20% Distilled Water									
P-31	ND128 M	2A[c] (1)	2A (1)	2A (2)	2A (2)	2A (3)	2A (3)	1 (4)	1 (4)
P-32	ND128 M	1B (2)	1B (2)	± (3)	±B (3)	± (4)	± (4)	2 (1)	2 (1)
P-33	ND128 M	2A (3)	3A (3)	0 (4)	± (4)	3A (1)	3A (1)	1 (2)	1B (2)
P-34	ND128 M	2 (4)	1 (4)	2A (1)	3A (1)	2 (2)	1B (2)	2A (3)	3A (3)
		5.0% w/v[d] 24-hr	48-hr	2.5% w/v[d] 24-hr	48-hr	1.0% w/v[d] 24-hr	48-hr	0.5% w/v[d] 24-hr	48-hr
Pilot 2 in Acetone									
P-35	ND128 F	1 (1)	1 (1)	1 (2)	1 (2)	1 (3)	± (3)	± (4)	0 (4)
P-36	ND128 F	1 (2)	1 (2)	2 (3)	2 (3)	1 (4)	1 (4)	0 (1)	0 (1)
EP-39	ND128 M	2 (3)	1 (3)	0 (4)	± (4)	1 (1)	± (1)	0 (2)	± (2)
EP-40	ND128 M	2 (4)	2A (4)	2 (1)	2A (1)	± (2)	± (2)	± (3)	± (3)
		1.0% w/v[b] 24-hr	48-hr	0.5% w/v[b] 24-hr	48-hr	0.25% w/v[b] 24-hr	48-hr	0.1% w/v[b] 24-hr	48-hr
Pilot 3 in 80% Ethanol/20% Distilled Water									
EP-41	ND128 M	1 (1)	± (1)	± (2)	0 (2)	0 (3)	0 (3)	0 (4)	0 (4)
EP-42	ND128 M	± (2)	0 (2)	± (3)	± (3)	± (4)	0 (4)	1 (1)	± (1)
EP-43	ND128 F	± (3)	± (3)	± (4)	± (4)	0 (1)	0 (1)	± (2)	0 (2)
EP-44	ND128 F	1 (4)	1 (4)	± (1)	± (1)	0 (2)	0 (2)	0 (3)	0 (3)
		0.5% w/v[d] 24-hr	48-hr	0.25% w/v[d] 24-hr	48-hr				
Pilot 4 in Acetone									
P-37	ND128 F	± (1)	± (1)	0 (2)	0 (2)				
P-38	ND128 F	1 (2)	1 (2)	± (1)	± (1)				
EP-45	ND128 F	± (1)	± (1)	0 (2)	0 (2)				
EP-46	ND128 F	1 (2)	1 (2)	± (1)	± (1)				

[a] Patch site locations are given in parentheses and correspond to those shown in Format #2 or #4, Appendix B, of The Procter & Gamble Company Protocol No. C4, Issue date: May 20, 1983.

[b] In 80% ethanol/20% distilled water.

[c] A=Scab formation.
B=Partial scab formation.

[d] In acetone.

Ref.: 83-1603-21 February 9, 1984

Table 2

IRRITATION DATA FOLLOWING
PRIMARY CHALLENGE OF GO250.01 IN GUINEA PIGS

Animal No.	Sex	Irritation Score 24-hour	Irritation Score 48-hour	Rating[a]
		Test Material (0.25% w/v in Acetone) in Induced Animals		
T- 1 ND128	M	±	±	N
T- 2 ND128	M	0	0	N
T- 3 ND128	M	0	0	N
T- 4 ND128	M	0	0	N
T- 5 ND128	M	±	±	N
T- 6 ND128	M	1	1	R
T- 7 ND128	M	±	±	N
T- 8 ND128	M	0	0	N
T- 9 ND128	M	0	0	N
T-10 ND128	M	0	0	N
T-11 ND128	F	±	0	N
T-12 ND128	F	0	0	N
T-13 ND128	F	0	±	N
T-14 ND128	F	±	0	N
T-15 ND128	F	1	1	N
T-16 ND128	F	±	±	N
T-17 ND128	F	0	0	N
T-18 ND128	F	0	0	N
T-19 ND128	F	0	0	N
T-20 ND128	F	0	0	N
	Mean	0.2	0.2	1 Responder/ 19 Non-Responders
		Test Material (0.25% w/v in Acetone) in Naive Animals		
C-21 ND128	M	0	0	N
C-22 ND128	M	0	0	N
C-23 ND128	M	0	0	N
C-24 ND128	M	0	0	N
C-25 ND128	M	±	±	N
C-26 ND128	F	0	0	N
C-27 ND128	F	0	0	N
C-28 ND128	F	0	0	N
C-29 ND128	F	0	0	N
C-30 ND128	F	0	0	N
	Mean	0.1	0.1	0 Responders/ 10 Non-Responders

[a] R=Responder reaction, N=Non-responder reaction.

Ref.: 83-1603-21 February 9, 1984

Table 3

IRRITATION DATA FOLLOWING
RECHALLENGE OF G0250.01 IN GUINEA PIGS

Animal No.	Sex	Site Order	Irritation Score/Reading Time and Rating[a] 0.25% (A)[b] Score 24	48	Rating	0.125% (B)[b] Score 24	48	Rating	0.0625% (C)[b] Score 24	48	Rating
In Induced Animals											
T- 1 ND128	M	ABC	±	±	N	±	0	N	0	0	N
T- 5 ND128	M	BCA	±	±	N	0	0	N	±	0	N
T- 6 ND128	M	CAB	0	±	N	0	0	N	0	0	N
T- 7 ND128	M	ABC	±	0	N	0	±	N	0	0	N
T-16 ND128	F	BCA	±	±	N	0	0	N	0	0	N
T-17 ND128	F	CAB	±	1	R	0	0	N	0	0	N
Mean			0.4	0.5	1/5[c]	0.1	0.1	0/6	0.1	0.0	0/6
In Naive Animals											
E-47 ND128	M	ABC	0	0	N	0	0	N	0	0	N
E-48 ND128	M	BCA	±	0	N	0	0	N	±	0	N
E-49 ND128	M	CAB	0	0	N	0	0	N	0	±	N
E-52 ND128	F	ABC	±	0	N	0	±	N	±	0	N
E-53 ND128	F	BCA	0	0	N	0	0	N	0	0	N
Mean			0.2	0.0	0/5[c]	0.0	0.1	0/5	0.2	0.1	0/5

[a] 24 = 24-hour reading, 48 = 48-hour reading, R = Responder reaction, N = Non-Responder reaction.
[b] Concentration (w/v) of G0250.01 in acetone, ( ) = application site.
[c] R/N = Responder/Non-responder.

Ref.: 83-1603-21 February 9, 1984

APPENDIX 1

PROTOCOL AND AMENDMENTS

FOR

PROJECT NO. 83-1603-21

PROJECT INSTRUCTION SHEET

83-1603-21

ENTERED 12/5/83 PROJ. NO.

REPORTED 2/2/84 PAGE 1

TYPE OF PROJECT: Delayed Contact Hypersensitivity Study

BY J. Kreuzmann

SUPERVISOR D. Conine

DATE ASSIGNED 12/6/83

CLIENT [redacted]

CLIENT'S REPRESENTATIVE [redacted]

BUDGET QUOTE $ 2,750

CLIENT P.O. NO. [redacted]

DEPT. 21

CODE

BILLING INSTRUCTIONS: Bill through Project No. 83-1603-21

SAMPLES AND DESCRIPTIONS	LOT NO.	DATE RECEIVED
G0250.01 (BYCR 0195)	n/a	12/2/83

PROJECT INSTRUCTIONS

Reference: PROPOSAL/REQUEST dated _____ by _____

Authorized by [redacted] Letter of 12/1/83 Verbally on _____ n/a

Note page numbers of any Supplemental Instructions:

Project Monitor: J. Kreuzmann, B.A.

Protocol Amendment No. 1: Project No. 83-1603-21

1. Proposed Start Date: December 12, 1983
2. Report Date: ~~February 6, 1984~~ February 20, 1984 (correction due to subcontract delivery 1/10/84)
3. RTR Study Director: David L. Conine, Ph.D.
4. Sponsor: [redacted]
5. Protocol Modifications: none

PROJECT REGULATED
☐ YES ☒ NO

REGULATORY AGENCY IF REGULATED
☐ FDA ☐ EPA ☐ ___

Specifics [redacted] 1-30-84 1-30-84

Project Instructions

Run one Delayed Contact Hypersensitivity Study ([redacted] issue date May 20, 1983) using test material G0250.01 (BYCR 0195). A pre-induction primary irritation screen will be run on this test material using four animals, four patches and concentrations of 20%, 10%, 5% and 2.5% w/v in 80% ETOH/30% distilled water per animal. An induction concentration will be chosen either from this initial or a second confirmatory pre-induction primary irritation screen. A separate pre-challenge primary irritation screen will be run on this test material using four animals, four patches and concentrations of 5%, 2.5%, 1.0% and 0.5% w/v in acetone per animal. A primary challenge concentration will be chosen either from this initial or a second confirmatory pre-challenge primary irritation screen. For all four patch primary irritation screens, patch site Nos. 1, 2, 3 and 4 referenced in figure No. 4 of Appendix B of Protocol C4 issue date 5/20/83 will be patched. Patching for induction, primary challenge and possible re-crosschallenge will make use of patch site Nos. 1, 2, 3, 4 and 5 referenced in figure No. 4 of Appendix A of Protocol C4 issue date 5/20/83.

Special Instructions for formulation are as follows:

"G0250.01 is soluable in 100% ethanol up to a concentration of 20% and in acetone up to (Cont.)

PROJECT SHEET PREPARED BY JK | TYPED BY saw

No. Subjects ____
No. Sessions ____
Total Fee Paid ____
Date Requested ____

Size of Finished Project (number of each type of test used):

PROJECT INSTRUCTION SHEET

83-1603-21
ENTERED 12/5/83 PROJ. NO.
REPORTED 2/6/84 PAGE 2
BY J. Kreuzmann
SUPERVISOR D. Conine
DATE ASSIGNED 12/6/83

TYPE OF PROJECT
Delayed Contact Hypersensitivity Study

CLIENT [redacted]
BUDGET QUOTE $2,750
CLIENT'S REPRESENTATIVE [redacted]
DEPT. 21
CODE
CLIENT'S PROJ. OR P. O. NO.
BILLING INSTRUCTIONS Bill through Project No. 83-1603-21.

SAMPLES AND DESCRIPTIONS	LOT NO.	DATE RECEIVED
GO250.01 (BYCR 0195)	n/a	12/2/83

PROJECT INSTRUCTIONS Reference: PROPOSAL/REQUEST dated _____ by _____

Authorized by [redacted] Letter of 12/1/83 Verbally on _____

Note page numbers of any Supplemental Instructions:

Protocol Amendment No. 1: Project No. 83-1603-21 (Cont.)

a concentration of approximately 5%. Dissolution of GO250.01 is easily achieved by heating the test material to a temperature of less than 150°F and then mixing with the diluent. Induce at the highest non-irritating concentration in ethanol/water provided it is less than 20%. Challenge at the highest non-irritating concentration in acetone provided it is less than 5%."

All unused samples are to be returned to Divisional Toxicologist: [redacted].

The invoice for this study should be sent to: [redacted]. Four copies of the final report are needed by February 10, 1984 and are to be sent to [redacted] at the sponsor's address above.

Accounting

20 test and 10 control animals -	$1,810
4 animals for pre-induction primary irritation screening -	125
4 animals for confirmatory preinduction primary irritation screening -	125
4 animals for pre-challenge primary irritation screening -	125
4 animals for confirmatory pre-challenge primary irritation screening -	125
10 animals to be used as naive controls for possible re-crosschallenge -	440
	$2,750

Approved by:

D Conine 12/6/83
David L. Conine, Ph.D.
Director of Technical Services,
Acute Toxicology

D Conine 12/6/83
David L. Conine, Ph.D.
HTR Study Director

PROJECT SHEET PREPARED BY JK
TYPED BY SAC

No. Subjects
No. Sessions
Total Fee Paid
Date Requested

Size of Finished Project (number of each type of test used):

SUPPLEMENTAL INSTRUCTIONS

DATE	12/14/83
PROJ. NO.	83-1603-21
PAGE	2
Supervisor	D. Conine

TYPE OF PROJECT
Delayed Contact Hypersensitivity Study

CLIENT
[redacted]

Changes authorized by [redacted] Letter of n/a Verbally on 12/13/83
Effect on budget or quote: Add $ n/a Subtract $ n/a

SAMPLES AND DESCRIPTIONS	LOT NO.	DATE RECEIVED
G0250.01 (BYCR 0195)	n/a	12/2/83

NEW INSTRUCTIONS:

What was client told would be effect on cost? —

Protocol Amendment No. 2A: Project No. 83-1603-21

Run a second pre-induction primary irritation screen on test material G0250.01 (BYCR 0195) using four animals, four patches and concentrations of 1.0%, 0.5%, 0.25% and 0.1% w/v in 80% ETOH/20% distilled water per animal. Patch site Nos. 1, 2, 3 and 4 referenced in figure No. 4 of Appendix B of Protocol C4 issue date May 20, 1983 will be patched for this second pre-induction primary irritation screen. [redacted]

Approved by:

12-14-83
David L. Conine, Ph.D.
HTR Study Director

[redacted] 15/83
[redacted]

Protocol Amendment No. 2B: Project No. 83-1603-21

The patching of the initial pre-induction primary irritation screen on test material G0250.01 (BYCR 0195) using four animals, four patches and concentrations of 20%, 10%, 5% and 2.5% w/v in 80% ETOH/20% distilled water per animal incorporated four male animals instead of two male and two female animals. The results of this screen led to the above discussed second pre-induction primary irritation screen. This should compromise no aspect of this Delayed Contact Hypersensitivity study. Per J. Kreuzmann 12/14/83.

Approved by:

12-14-83
David L. Conine, Ph.D.
HTR Study Director

[redacted] 12/20/83
[redacted]

PREPARED BY | TYPED BY

SUPPLEMENTAL INSTRUCTIONS

DATE 12/16/83 | PROJ. NO. 83-1603-21 | PAGE 4

Supervisor D. Conine

TYPE OF PROJECT

Delayed Contact Hypersensitivity

CLIENT

[redacted]

Changes authorized by [redacted] Letter of n/a Verbally on 12/15/83

Effect on budget or quote: Add $ n/a, Subtract $ n/a

SAMPLES AND DESCRIPTIONS	LOT NO	DATE RECEIVED
G0250.01 (BYCR 5)	n/a	12/2/83

NEW INSTRUCTIONS:

What was client told would be effect on cost? ~~

Protocol Amendment No. 3A Project No. 83-1603-21

Run a second pre-challenge primary irritation screen on test material G0250.01 (BYCR 0195) using four animals, two patches and concentrations of 0.5% and 0.25% w/v in acetone per animal. Patch site Nos. 1 and 2 referenced in figure No. 2 of Appendix B of protocol C4 issue date May 20, 1983 will be patched for this second pre-challenge primary irritation screen. [redacted]

Approved by:

David L. Conine, Ph.D. 12-16-83
HTR Study Director

[redacted]

Protocol Amendment No. 3B: Project No. 83-1603-21

The patching of the above discussed pre-challenge primary irritation screen on test material G0250.01 (BYCR 0195) using four animals, two patches and concentrations of 0.5% and 0.25% w/v in acetone per animal will incorporate four female animals instead of two male and two female animals. This should compromise no aspect of this Delayed Contact Hypersensitivity study. [redacted]

Approved by:

David L. Conine, Ph.D. 12-16-83
HTR Study Director

[redacted]

83-1603-21

SUPPLEMENTAL INSTRUCTIONS

DATE 1/19/84 PROJ NO PAGE 2

TYPE OF PROJECT
Delayed Contact Hypersensitivity Study

Supervisor D. Conine

CLIENT
[redacted]

Changes authorized by [redacted] Letter of n/a Verbally on 1/17/84

Effect on budget or quote: Add $ 183, Subtract $ n/a

CODE AND DESCRIPTIONS	LOT NO	DATE RECEIVED
G0250.01 (BYC 0195)	n/a	12/2/83

NEW INSTRUCTIONS:

What was client told would be effect on cost? —

Protocol Amendment No. 4: Project No. 83-1603-21

All test animals exhibiting primary challenge scores of 1 or greater at both the 24 and 48 hour readings will be triple rechallenge patched using test material G0250.01 (BYCR 0195) as 0.25%, 0.125% and 0.0625% w/v solutions in acetone. Patch site Nos. 3, 4 and 5 referenced in figure No. 4 of Appendix A of Protocol C4 issue date May 20, 1983 will be patched for this rechallenge. Five naive control animals will be patched identically to and concurrently with the test animals. [redacted]

Approved by:

D Conine 1/22/84
David L. Conine, Ph.D.
Study Director

[redacted]

PROCTER & GAMBLE COMPANY

PROTOCOL NO. C4

Delayed Contact Hypersensitivity

p.7

Issue Date: May 20, 1983
Supersedes Issue Dated: July 15, 1982

Test Substance Identification Number (TSIN) # [redacted]

Divisional Request Document Number (DRD) # [redacted]

Sponsor: [redacted]

Testing Facility: (To be filled in by Operations Section) Hill Top Research, Inc. Cincinnati, Ohio 45242

Study # 83-1603-21 (To be filled in by Testing Facility)

Purpose: To define the allergenic potential of a test substance in guinea pigs.*

Justification for Selection of Test System: The guinea pig is the classical animal for determining delayed contact hypersensitization.

Route of Administration of Test Substance and Reason for Choice: Closed patch on clipped area of intact skin. Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Diet and/or Water Analyses Required: None (no known contaminants expected which would interfere with this study)

Records to be Maintained: All records that would be required to reconstruct the study and demonstrate adherence to protocol.

*Ritz, H. L. and Buehler, E. V., Current Concepts in Cutaneous Toxicity. ed. Drill, V. A. and Lazar, P. (Academic Press, 1980) pp.25-40.

PROTOCOL NO. C4 (Cont'd)

Delayed Contact Hypersensitivity

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Issue Date: May 20, 1983

Test Substance(s)

TSIN #	DRD Number	Description: Color	Description: Physical Form	Expiration Date
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Storage Conditions: (Check one)

[ ] Room temperature [ ] Refrigerator [ ] Freezer
[ ] Other

Hazards: (Check one)

[ ] None known. Take ordinary precautions in handling.
[ ] As follows:

Animals: Use Hartley outbred guinea pigs of a size sufficient to easily fit in the restrainers while the experiment is in progress. Use a minimum of twenty (20) test animals, ten (10) control animals and four or more animals for primary irritation for each test substance. Whenever possible, use equal numbers of males and females.

Animal Care: Follow the approved Standard Operating Procedures of the Test Facility. (Acclimation period must be a minimum of four (4) days.)

Environmental Conditions: Follow the approved Standard Operating Procedures of the Test Facility.

Animal Identification: Mark each cage and restrainer or restraining rubber dental dam with an identifying number. Careful attention must be given to see that the proper animal goes into the proper restrainer during each treatment and back to its original cage after treatment.

CIPE & GAMBLE COMPANY

PROTOCOL NO. C4 (Cont'd)

83-1603-
p.9

Delayed Contact Hypersensitivity

Issue Date: May 20, 1983

Special Instructions:

[ ] None
[✓] As follows:

G0250.01 is ... water in 100% ethanol up to a concentration of 20%
... and in acetone up to a concentration of ~5%. Dissolution of G0250.01
is easily achieved by heating the test material to a temperature of less
than 150°F and then mixing with the diluent.
Induce at the highest non-irritating concentration in EtOH and/water provided it is less than 20%
Dose Preparation: challenge at the highest non-irritating conc. in acetone provided it is less than 5%

[✓] Discussed with Scientific Monitor
[✓] Request that dose preparation information be supplied by the Scientific Monitor
[✓] All solutions should be freshly prepared.

1. Induction

Test Substance(s)	Concentration(s)	Vehicle(s)
a concentration that produces slight primary irritation		in EtOH/H2O vehicle

2. Primary Irritation

Test Substance(s)	Concentration(s)	Vehicle(s)
G0250.01	20, 10, 5 ± 2.5% w/v	EtOH/H2O (80/20)
G0250.01	5, 2.5, 1.0 ± 0.5% w/v	acetone

3. Challenge

4%
Highest nonirritating concentration in acetone

[✓] Reviewed by Scientific Monitor Ethan 12/2/83
Initials and Date

Comments (Scientific Monitor)

[✓] None
[ ] As follows:

Delayed Contact Hypersensitivity

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Issue Date: May 20, 1983

Dose Preparation (Cont'd):

Note

A concentration analysis of the test substance - vehicle mixture(s) will [ ]; will not [/] be required.

If a concentration analysis is required:

Prepare a sufficient quantity of the test substance - vehicle mixture(s) so that a portion can be returned to the Sponsor's Divisional Toxicologist. Store solution/mixture at [ ] room temperature; [ ] refrigerator; [ ] freezer; [ ] other _____

Shipping Instructions

Send approximately _____ml. Send [ ] frozen; [ ] under ambient conditions; [ ] other_____

Dose Level: The dose level chosen for the induction of sensitization is generally one that is greater than the anticipated level of human exposure (if too irritating, this dose level may be decreased during the induction phase). Determine the dose level for elicitation of responses at primary challenge by pre-screening various doses on naive animals as described under "Primary Irritation" on page 5; the eliciting dose will generally be the highest non-irritating concentration.

Procedure: Use twenty (20) test animals and ten (10) control animals in each study unless otherwise specified. Provide about equal numbers of males and females in both test and control groups.

Testing conditions will vary from study to study. Therefore, it should be clearly indicated in the final report which test sites were used for induction, primary challenge, and rechallenge. Use the appended Format for Sensitization Studies (Appendix A) for this purpose (e.g., if the test animals were exposed to two (2) substances at primary challenge and three (3) substances at rechallenge, the reference figure would be #8). When the animals are challenged with several substances at one time, alternate the test substances on the patch sites to prevent bias due to site-to-site variation. Expose control animals for primary challenge and rechallenge at the sites corresponding to those of the test animals.

PROTOCOL NO. C4 (Cont'd)

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Delayed Contact Hypersensitivity

Issue Date: May 20, 1983

Procedure for Primary Sensitization:

Induction of Sensitization - Clip the left shoulder of each animal with a small animal clipper the day before exposure. The area shaved should be approximately one-fourth of the animal's back and side. Apply closed patches to the animals in the test group(s) in the following manner: Apply 0.3 ml of the test substance or freshly prepared solution in a 25 mm Hill Top Chamber® (Hill Top Research Inc., Cincinnati, Ohio). Put the animal in the restrainer and apply the chamber to the clipped surface as quickly as possible after the substance has been applied. Occlude the chamber with a rubber dental dam pulled taut and fastened to the bottom of the restrainer with binder clips or holders.

Adjust the restrainer to minimize movement of the animal during the exposure period. Both edges of the dental dam should be under the front and back adjustable braces of the restrainer. About six (6) hours later, remove the dental dam and chamber, take the animal from the restrainer, and place it in its cage. Remove extremely viscous substances by a gentle rinse with warm water (35-42°C) or other appropriate solvent as specified under special instructions before returning the animals to their cages. Repeat the procedure at the same site once a week for the next two (2) weeks for a total of three 6-hour exposures (the interval between induction exposures may vary from 5 to 9 days). After the last induction exposure, leave the animals untreated for approximately two (2) weeks (12-16 days) before primary challenge.

Primary Irritation #1 - Prior to or during the induction period, determine the primary irritancy of the substance in question if the information is not available. For this purpose, treat four (4) previously unexposed animals by the above-described patching technique to different concentrations (generally 4) of the test substance in the prescribed vehicle. Clearly indicate in the final report which test sites were used. Use the appended Format for Primary Irritation Studies (Appendix B) for this purpose. In this portion of the study, clip the entire back and both sides of the animal the day before application and then expose each animal for one 6-hour period to the various concentrations of the substance. Alternate the different concentrations of the test substance on the various test sites among the animals in order to minimize the site-to-site variation in responsiveness. Grade the responses at 22-26 hours and at 46-50 hours according to the procedure described below for primary challenge.

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Procedure for Primary Sensitization Cont'd):

Primary Irritation #1 (Cont'd)

The highest non-irritating concentration is generally the concentration that induces reponses not exceeding two + and two 0 grades in the group of four (4) animals.

Primary Irritation #2 - An additional screen may be required after reporting the results of the #1 screen to the Scientific Monitor by telephone. Levels will be determined by the Scientific Monitor.

Primary Challenge - Challenge the animals previously exposed during the induction period as well as the previously untreated control animals approximately two (2) weeks after the last induction exposure (the time between the last induction exposure and the primary challenge may vary from 12 to 16 days) using the dose as prescribed by the Scientific Monitor. Use the same patching procedure as for the induction, but apply the patches to a naive skin site. The left posterior quadrant of the side and back of the animal should be clipped for single or double challenge techniques; the entire right side should be clipped for a triple challenge. The site for primary challenge may be varied, if necessary, to achieve the objectives of the experiments (e.g., using multiple samples at primary challenge will require using several sites).

Note any gross skin reactions during induction phase. Notify the Scientific Monitor of any unusual or severe inflammatory skin reactions.

Observations:

Approximately twenty-four (24) hours after primary challenge patches have been removed, depilate all animals with Neet Cream or Lotion Hair Remover (Whitehall Laboratories, Inc., New York). Place the depilatory on the test sites and surrounding areas, and leave it on for no more than thirty (30) minutes. Thoroughly wash off the depilatory with a stream of warm, running water, dry the animals with a towel, and return them to their cages.

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Delayed Contact Hypersensitivity

Issue Date: May 20, 1983

Observations (Cont'd): A minimum of two (2) hours after depilation, grade the test sites on a scale of 0 to 3 (0 = no reaction, ± = slightly patchy erythema, 1 = slight, but confluent or moderate, patchy erythema, 2 = moderate erythema, 3 = severe erythema with or without edema). Repeat the grading 24 hours later (48-hour grades).

Rechallenge: The Sponsor will be notified of primary challenge results. Animals can be rechallenged six (6) days after primary challenge, but not before. Verbal instructions for rechallenge will be given by the Sponsor, followed by written confirmation from the Study Director. At study termination, sacrifice all surviving animals following an order to do so from Sponsor's Scientific Monitor.

Protocol Changes: If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Sponsor. As soon as practical, this change and the reasons for it should be put in writing and signed by both the Study Director and the Sponsor's Divisional Toxicologist or Scientific Monitor. This document is then attached to the protocol as an addendum.

Report: Report should include how the study was conducted, the dates the study was initiated and terminated, and the results of both the primary challenge and the rechallenge in terms of incidence and severity of responses. This report shall conform to all requirements outlined in Section 58.185, Subpart J, Good Laboratory Practices Regulations.

(1) Incidence - The number of animals in each group showing responses at either 24 or 48 hours divided by the total number of animals tested in that group (e.g., 10/20).

...P & GAMBLE COMPANY

PROTOCOL NO. 64 (Cont'd)

83-1603-21
P-14

Delayed Contact Hypersensitivity

Issue Date: May 20, 1983

Report (Cont'd): (2) Severity - The sum of the test grades divided by the total number of animals tested in a given group determined for both 24 and 48 hours (e.g., 0.8-0.7). Grades of ± are equal to 0.5 for calculation of severity indices. All average grades are to be rounded off to the nearest tenth of a unit. (Exceptions: Grades of 0.025-0.044 will be reported as 0.1, not as 0.0.)

Sponsor: [redacted]
Divisional Toxicologist

Date Approved by Sponsor's Divisional Toxicologist 12/6/83

Proposed Starting Date: 12/12/83
Defined as Primary Irritation

Proposed Completion Date: 1/6/83
Defined as Report Mail Date

(To be complet.. by the Test Facility)

Study Director: DD Canine
Date: 12/6/83
Study Cost: $2750.00

PROCTER & GAMBLE COMPANY

Issue Date: 5/20/83

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P.15

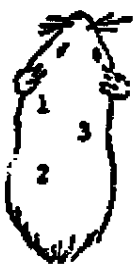
Appendix A

Format for Sensitization Studies

1

tion
ry Challenge

2

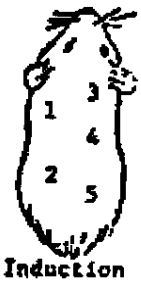


1: Induction
2: Primary Challenge
3: Rechallenge

3

1: Induction
2: Primary Challenge
3,4: Rechallenge

4



1: Induction
2: Primary Challenge
3-5: Rechallenge

5

tion
imary Challenge

6

1: Induction
2,3: Primary Challenge
4: Rechallenge

7

1: Induction
2,3: Primary Challenge
4,5: Rechallenge

8

1: Induction
2,3: Primary Challeng
4-6: Rechallenge

9

duction
rimary Challenge

10

1: Induction
2-4: Primary Challenge
5: Rechallenge

11

1: Induction
2-4: Primary Challenge
5,6: Rechallenge

Issue Date: 5/20/83

PROCTER & GAMBLE COMPANY

Protocol - Appendix B

Format for Primary Irritation Studies

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p.16

#1

One Test Site

#2

Two Test Sites

#3

Three Test Sites

#4

Four Test Sites

Protocol - Page 10 of 10

[redacted]

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[redacted]

December 1, 1983

Dr. David L. Coniine
Hill Top Rese ch, Inc.
P. O. Box 42501
Cincinnati, OH 45242

Dear Dr. Coniine:

This is to authorize you to carry out a Delayed Contact Hypersensitivity Study in Guinea Pigs using the attached Protocol No. C4, issue dated May 20, 1983, with the special instructions and dose preparations provided by [redacted]. This study is to be conducted in conformance with the stipulations of our current Laboratory Services Agreement.

Notice: This study is not expected to be submitted to a regulatory agency. The stipulations of this protocol are to be implemented in conformance with the Good Laboratory Practice Regulations (21 CFR, Part 58) with the following exceptions:

1. The study should not be listed on the Test Facility's master list of regulated studies.

2. If two or more test substances appear on the protocol, it should be conducted as a single study, resulting in a single final report.

3. Quality Assurance Inspections:

   (a) Acute (single dose) studies

   An in-life phase and the final report need not be inspected by the Test Facility's QAU. The Test Facility's Standard Operating Procedure for randomly inspecting all operations should be used to assure study validity and sufficient data should be made a part of each report to allow the Sponsor to check the reported results against the raw data.

   (b) Subchronic/chronic (repeated dose) studies

   All critical phases and the final report will be inspected. The critical phases may be defined by the QAU and Study Director.

83-1603-21
P.34

4. Documentation of the derivation, characterization, and stability testing of the test substance(s) will be the responsibility of the Sponsor. Please indicate this in your final report if you make reference to stability of the test substance.

Test Substance No.: C0250.01
Doc. Req. No.: BYCR 0195
Physical Form: Waxy flake

Verbal results are needed by January 3, 1984. Four copies of the final report are needed by February 10, 1984, and are to be sent to my attention at the above address.

Matters involving the scientific aspects of the work can be handled directly with [redacted] All unused samples are to be returned to the Divisional Toxicologist at the following address (the cost of shipment should be included in the study cost):

[redacted]

Complete both copies of the attached protocol by adding your study number, proposed start and completion dates, and have the Study Director sign and date them. The Study Director should define the start and completion dates on the protocol. Retain one copy and return one copy (which includes the study cost) to me along with a letter stating that you agree to do the work specified in the attached protocol. In addition, if you cannot meet the report dates, please let me know.

The invoice for this study should be sent to:

[redacted]

XC
83-1603-21

Sincerely,

[redacted]

Attachments (2)
cc: Study File
[redacted]

Ref.: 83-1603-21 STUDY REVIEW RECORD February 9, 1984

Reference: 83-1603-21

Date of Study Initiation: December 12, 1983

Date of Study Completion: January 27, 1984

Study Director: David L. Conine, Ph.D.

Project Monitor: James J. Kreuzmann, B.A.

Location of Specimen Storage: n/a

Disposition of Remaining Test Material: At the conclusion of a test program, each sample will be stored for three months. At that time the sample will be returned to the client.

Location of Raw Data: Hill Top Research, Inc.
Main & Mill Streets
Miamiville, OH 45147

Location of Final Report: Hill Top Research, Inc.

Quality Assurance Unit Statement

Date(s) Study Inspected: _____

Date Report Reviewed: 2/16/84

Date(s) Findings Reported to Management: _____

Date(s) Findings Reported to Study Director: _____

Alan S. Weiner
Quality Assurance Auditor

Quality Assurance Director

2/16/84
Date

Date

For Tox Office Use Only
SAHT #
DRD # [illegible]
TSIN # [illegible]

PREPARATION REPORT WORKSHEET/TEST SUBSTANCE CHARACTERIZATION REPORT
(For use with SAHT and in Generating the prep report) (For use with DRD. Submit typed original.)

1. Test Article Name (must agree with SAHT or DRD)
Stearamidopropyl dimethyl amine CAS# 7651-02-7

2. Type of Test: Beauty Shop ____ Building Panel ____ Environmental ____
*HPT ____ *HRD ____ *Clinical ____ Safety X Other ____
*Give Test Request No. ____

3. MAKING DATA: List ingredients in descending order of predominance. (For drug products list active ingredient(s) first.)

Component	Nominal Level (Theoretical % by wt.)	Actual Level (% by wt.) (Complete after batch prep)	Stock Code	Lot No. (Complete after batch prep)
Stearamidopropyl dimethylamine	100%		PX 781	1

Comments on Making Data:

4. Formula Approvals for this test provided the indicated (√) analyses in Section 9 are within limits.
Microbial Susceptibility Classification Approval: [redacted] (Microbiologist's Signature) (Name) OCT 25 1983 (Date)
SAHT Approval: (N/A for TSCA) ____ (Toxicologist's Signature) ____ (Name) ____ (Date)

5. Making Notebook Ref. (with Plant Production Code if applicable) N/A
Date Made N/A Made by Inolex Lab D. L. Coupe, HB 1B24 SW
Adequate shelf testing (no less than the life of the product in the test) in the same container as it is distributed, has been completed for this test: YES/NO/IN PROCESS
1 (no. samples) Finished product samples will be retained by Quality Assurance.

For DRD testing only

6. Physical Form Waxy Flake Color Cream Density .95
Water Solubility 5% (wt/wt) For SENS test only: Ethanol Solubility 5 % (wt/wt); (Dispersion)
(at 4.5 pH in citric or H_3PO_4)
Sample Expiration Date 10/1/84 mineral oil Solubility 10 % (wt/wt). (as Melted)
Melting Point: 150°F

7. Work place hazards skin & eye irritant Storage Conditions ambient (50°-90°F)

8. D.O.T. Classification (see Regulatory personnel) Non Hazardous

Issue Date: 3/83

PREP REPORT WORKSHEET/TEST SUBSTANCE CHARACTERIZATION REPORT

For Tox Office Use Only -
SAHT#
DRD # 82209009
TSINS 60218

9. Characterization, Microbial and Properties Information

	Date Submitted	Submitter Code (if exists); or Lab Notebook #	Component Or Property	(√)*	*Measured Value	Limits	Testing Lab or Data Source
1	10/14/82	82209009	MCT	√	Pass	Must Pass	GMP
2	10/14/82	82209009	Odor		Pass	Must Pass	GMP
3	10/14/82	82209009	IR		Pass	Must Pass	GMP
4	10/14/82	82209009	Acid Value		3.6,3.7,4.0	≤4	GMP
5	10/18/83	7308-0143	Cat SO_4		20.85	15 to 25	HR 1824
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

* Analyses indicated by Toxicologist or Microbiologist.

10. Approvals

The test substance as made and characterized is a representative example of the intended formulation. Making records for plant-made product should be obtained and evaluated by Products Research.

a. Process Development [redacted] (Signature) [redacted] (Name) 10/25/83 (Date)

b. Products Research [redacted] (Signature) [redacted] (Name) 10/27/83 (Date)

(When Prep Report is to be generated by GMP/QA: DISTRIBUTION of Approved Prep Report - Original to Products Research; Copies to GMP/QA, Tox Office, Process and Survey Packing.)

When Prep Report Worksheet is final product release document -

c. GMP-Quality Assurance [redacted] (Signature) [redacted] (Name) 11/2/83 (Date)

GMP-QA will inform the Toxicologist if any checked (√) analyses in Section 9 are out of limits.

DISTRIBUTION: Original to Products Research; Copies to Tox Office, GMP/QA, Process (if involved) and Survey Packing (if involved).

For studies involving a DRD, the test substance is adequately characterized and is acceptable for: acute animal test √; subchronic animal test ____; chronic animal test ____; human safety test ____; in vitro test ____; environmental safety test ____.

d. Safety [redacted] (Toxicologist's Signature) [redacted] (Name) 11/16/83 (Date)

TSCR DISTRIBUTION: Original to Tox Office; Copies to Toxicologist, GMP/QA, Products Research and Process.

Personal Care Products Council
Committed to Safety, Quality & Innovation
Formerly CTFA

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 13, 2009

SUBJECT: Sensitization studies on products containing Cocamidopropyl Betaine

KGL, Inc. 2007. An evaluation of the contact-sensitization potential of a topical coded product (facial cleanser containing 3.6% active CAPB) in human skin by means of the maximization assay.

Study CRL 125301 of cleansing cloths containing 6% active CAPB was conducted in two phases, phase I n = 104 and phase II -= 106.

Clinical Research Laboratories, Inc. 2002. Repeated insult patch test of a cleansing cloth containing 6% active CAPB. Study Number CRL125301 Phase I.

Clinical Research Laboratories, Inc. 2002. Repeated insult patch test of a cleansing cloth containing 6% active CAPB. Study Number CRL125301 Phase II

KGL INC

FINAL REPORT dated October 26, 2007

KGL Protocol: #6383

Sample: Facial Cleanser (0.5% aqueous)

www.kgl-inc.com or www.ivylabs.com

Ivy Laboratories (KGL, INC.)
505 Parkway
Broomall, PA 19008-4204 (USA)

Telephone: [215] 387-8400
FAX: [215] 387-1046

E-mail address: ivystudies@verizon.net

Title: An Evaluation of the Contact-Sensitization Potential of a Topical Coded Product in Human Skin by means of the Maximization Assay

Sponsor: Facial cleanser contains 12% CAPB 30% active (3.6% active CAPB)

Principal Investigator: Kays Kaidbey, M.D. (Board Certified Dermatologist)

Testing Facility:
Ivy Laboratories (KGL, INC.)
505 Parkway
Broomall, PA 19008-4204 (USA)
(Phone: 215-387-8400)
(FAX: 215-387-1046)

Final Report Date: **October 26, 2007**

Kays Kaidbey, M.D.
Principal Investigator

October 26, 2007
Date

FINAL REPORT

KGL PROTOCOL:

Ivy Laboratories - KGL Protocol **#6383**

SPONSOR:

SPONSOR STUDY:

Authorization Letter Dated: August 31, 2007

STUDY TITLE:

Evaluation of the contact-sensitizing potential of a coded topically-applied test agent.

STUDY OBJECTIVE:

The objective of this study is to assess the skin sensitizing potential of any preparation designed for topical use by means of the Maximization Test (see references #1 and #2).

TEST MATERIAL:

The test sample, supplied by the sponsor, was a product labeled Facial Cleanser and

Prior to application, the test product was mixed with distilled water and a 0.5% aqueous dilution was prepared for testing purposes.

TEST PRODUCT ACCOUNTABILITY:

All test samples and materials were received in good condition by our Quality Assurance Department. The test materials and quantities were checked for (1) amount (2) product number or code (3) material container etc. The materials were individually listed on a special sheet (drug/test product log form) signed by the receiver, the laboratory supervisor and the investigator (physician). All test materials were stored under ambient conditions in an inaccessible location under the supervision of the investigator.

PRINCIPAL INVESTIGATOR:

Kays Kaidbey, M.D. (Board Certified Dermatologist)

Medical Director, KGL, INC.

Telephone: (215) 387-8400 (Ivy Labs)

FAX: (215) 387-1046

E-mail: ivystudies@verizon.net

KGL ADMINISTRATIVE STRUCTURE:

Angelit Barnes (Screening, Patch Applications/Removals, Recognize AE's)

John B. Chicchi (Expert Grader)

Bernadette Lonergan (Panel Recruitment/Receptionist)

TESTING FACILITY:

Ivy Laboratories (KGL, INC.)

505 Parkway

Broomall, PA 19008-4204

Telephone: 610-544-1715

CONDUCTION DATES:

This study was conducted from September 10, 2007 through October 12, 2007

PANEL COMPOSITION:

Healthy, adult volunteers over the age of 18 years were recruited for this study. None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics. The criteria for exclusion were:

1 - History of sun hypersensitivity and photosensitive dermatoses

2 - History of drug hypersensitivity or recurrent dermatological diseases

3 - Pregnancy or mothers who are breastfeeding

4 – History of recurrent urticaria or hives

5 - Scars, moles or other blemishes over the test site which can interfere with the study

6 - Subjects receiving systemic or topical drugs or medications, including potential sensitizers within the previous 4 weeks

7 - Other medical conditions considered by the investigator as sound reasons for disqualification from enrollment into the study.

INFORMED CONSENT:

After the protocol, reasons for the study, possible associated risks and potential benefits or risks of the treatment had been completely explained, signed, informed subject consent was obtained from each volunteer prior to the start of the study. Copies of all consent forms are on file at Ivy Laboratories (KGL, INC.).

METHOD:

Patches were applied to the upper outer arm, volar forearm or the back of each subject. The entire test was composed of two distinct phases: (1) an Induction phase and (2) a Challenge phase.

(1) **Induction Phase**:

Approximately 0.05ml of aqueous SLS (0.25%) was applied to a designated site under a 15mm disc of Webril cotton cloth and the patch was fastened to the skin with occlusive tape for a period of 24 hours. After 24 hours, the SLS patch was removed and 0.05ml of the test material was applied to the same site before the site was again covered with occlusive tape (induction patch). The induction patch was left in place for 48 hours (or for 72 hours when placed over a weekend) following which it was removed and the site again examined for irritation. If no irritation was present, a 0.25% aqueous SLS patch was again reapplied to the same site for 24 hours, followed by reapplication of a fresh induction patch with the test material to the same site. This sequence viz. 24 hour SLS pre-treatment followed by 48 hours of test material application was continued for a total of 5 induction exposures.

If irritation developed at any time-point during the induction phase as previously outlined, the 24-hour SLS pre-treatment patch was eliminated and only the test material was reapplied to the same site after a 24-hour rest period during which no patch was applied.

The aim during this phase of the study was to maintain at least a minimal degree of irritation in order to enhance penetration through the corneum barrier.

(2) **Challenge Phase**:

After a ten day rest period which follows the last induction patch application, the subjects were challenged with a single application of the test material to a new skin site on the opposite arm, forearm or side of back in order to determine if sensitization had developed.

Pre-treatment with SLS was performed prior to challenge. Approximately 0.05ml of a 5.0% aqueous solution was applied to a fresh skin site under a 15mm disc of Webril cotton and covered with occlusive tape. The SLS patch was left in place for one hour. It was then removed and the test material was applied to the same site, as outlined above. The challenge patch was then covered by occlusive tape and left in place for 48 hours. After that period, the patch was removed and the site graded 15-30 minutes later and again 24 hours later for any reaction.

SCORING SCALE:

0 = not sensitized

1 = mild sensitization (viz. erythema and a little edema)

2 = moderate sensitization (erythema with infiltration, raised, spreading beyond the borders of the patch, with or without vesiculation)

3 = strong sensitization (large vesiculo-bullous reaction).

Based on these findings the number of subjects with positive responses were tabulated for the test material. The test system shown below was used to classify the allergenic potential of the test substance.

SENSITIZATION RATES:	**GRADES:**	**CLASSIFICATION:**
0 - 2/25	1	Weak
3 - 7/25	2	Mild
8 - 13/25	3	Moderate
14 - 20/25	4	Strong
21 - 25/25	5	Extreme

RESULTS:

A total of twenty-seven (27) healthy, adult volunteers of both sexes who satisfied the inclusion criteria were enrolled into this study. There were 23 females and 4 males. Their ages ranged from 19 to 65 years. All 27 subjects completed this investigation as outlined in the standard protocol. The demographic data are shown in Table 1. No adverse or unexpected reactions were seen in any of the panelists during the induction phase.

The results of the challenge are shown in the enclosed table (Table 2). No instances of contact allergy were recorded at either 48 or 72 hours after the application of the challenge patches.

CONCLUSION:

Under the conditions of this test, the test sample labeled Facial Cleanser (0.5% aqueous) and does not possess a detectable contact-sensitizing potential and hence is not likely to cause contact sensitivity reactions under normal use conditions.

References:

(1) Kligman, A.M.: The Maximization Test. J.I.D., Vol. 47, No. 5, pp. 393-409, 1966.

(2) Kligman, A.M. and Epstein W.: Updating the Maximization Test for Identifying Contact Allergens. Contact Dermatitis. Vol. 1, 231-239, 1975.

TABLE 1

DEMOGRAPHIC DATA

Subject Number:	Subject Initials:	Age:	Sex:	Race:
01	L-I	20	F	C
02	EWF	49	F	C
03	CLL	63	F	B
04	CMA	50	F	C
05	SLH	50	F	C
06	ERG	65	F	C
07	JAV	43	F	C
08	DHA	41	F	C
09	TDM	49	F	C
10	DGW	39	F	C
11	EPM	40	F	C
12	VLL	37	F	C
13	JAG	46	F	C
14	Y-H	39	F	C
15	CAP	50	F	C
16	PMS	56	F	C
17	KGP	45	F	C
18	MGC	37	F	C
19	JMS	40	M	C
20	JJC	53	F	C
21	AAS	20	M	C
22	HWK	19	F	C
23	JSM	42	M	C
24	LVP	38	F	C
25	NED	50	F	C
26	MCW	56	F	C
27	G-Z	30	M	C

C = Caucasian
B = Black

TABLE 2

MAXIMIZATION TESTING RESULTS

Sample: Facial Cleanser

Subject Number:	48-Hour Grading	72-Hour Grading
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0
06	0	0
07	0	0
08	0	0
09	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0

Challenge Readings:

48-Hour Reading – October 11, 2007
72-Hour Reading – October 12, 2007

Clinical
Research
Laboratories, Inc.

COPY

Final Report

Repeated Insult Patch Test

CLIENT:

ATTENTION:

TEST MATERIAL: Cleansing Cloths
Containing 6% active CAPB

CRL STUDY NUMBER: CRL125301
Phase I

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Marilee Candino, R.N.
Director/Clinical Services

George J. Neumaier, M.D.
Diplomate American Board
of Dermatology

Michael J. Muscatiello, Ph.D.
Executive Vice President/C.O.O.

REPORT DATE: January 29, 2002

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical
Research
Laboratories, Inc.

Good Clinical Practice
Final Report Review Statement

Clinical Study Number: CRL125301

Phase I Start Date: November 30, 2001

Phase I Completion Date: January 11, 2002

This clinical study report has been reviewed to assure that it correctly describes the method of testing and that the reported results accurately reflect the data obtained during the study.

Julie R Meyers
Reviewer

1/29/2002
Date

FINAL REPORT

REPEATED INSULT PATCH TEST

PURPOSE

The purpose of this study was to determine the dermal irritation and sensitization potential of a test material.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane
Piscataway, New Jersey 08854

TEST MATERIAL

The following test material was provided by and was received by Clinical Research Laboratories, Inc. on November 29, 2001:

Cleansing Cloths

The test material was coded with the following CRL identification number:

CRL125301

The test material was cut to fit patch ½" x ½" and moistened with distilled water.

STUDY DATES

Phase I of this study was initiated on November 30, 2001 and was completed on January 11, 2002 (112 subjects were impaneled, Phase I). Phase II of this study started on December 17, 2002 (additional 112 subjects) and will be completed on February 1, 2002 (report to follow).

PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects." All subjects completed a Subject Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to the study (Subject Demographics - Appendix I).

No individuals were impaneled if they exhibited or had a history of acute or chronic dermatologic, medical, or physical conditions that could interfere with dermal scoring. No subject was using sympathomimetics, antihistamines, non-steroidal anti-inflammatory agents, or corticosteroids during the study period. No known pregnant or lactating women were impaneled in the study.

TEST METHOD

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied under a semi-occlusive patch* to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of twenty-four (24) hours.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of nine (9) applications during the Induction Period. This schedule may have been modified to allow for missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on two (2) consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation and sensitization twenty-four (24) hours after removal of the patches by the subjects on Tuesday and Thursday and forty-eight (48) hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

* Semi-occlusive Strip (TruMed Technologies Inc., Burnsville, Minnesota)

TEST METHOD (Continued)

The sites were graded according to the following scoring system:

Dermal Scores

0 No visible skin reaction
± Barely perceptible erythema (minimal)
1+ Mild erythema (diffuse)
2+ Well defined erythema
3+ Erythema and edema
4+ Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject was not patched again during the Induction Phase but was challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Following approximately a two (2) week rest period, the challenge patches were applied to previously untreated test sites on the back. After twenty-four (24) hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at forty-eight (48) and seventy-two (72) hours. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a ninety-six (96) hour reading.

RESULTS

Phase I of this study was initiated with one hundred twelve (112) subjects. Eight (8) subjects discontinued study participation for reasons unrelated to the test material. A total of one hundred four (104) subjects completed Phase I of the study.

Individual dermal scores recorded during the Induction and Challenge Phases of Phase I appear in Table I.

CONCLUSION

Based on the test population of one hundred four (104) subjects and under the conditions of Phase I of this study, the sample identified as Cleansing Cloths - did not demonstrate a potential for eliciting dermal irritation or sensitization.

RETENTION

All of the original documents relating to this study will be retained by Clinical Research Laboratories, Inc. for a time period of at least five (5) years or as otherwise required by law.

Test materials shall be archived by Clinical Research Laboratories, Inc. for a period no less than six (6) months, unless otherwise instructed by Sponsor.

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1	LS	08536	58	F
2	FS	14201	39	F
3	AJ	13082	28	F
4	AC	01439	48	F
5	MM	15079	50	F
6	PT	12224	40	F
7	JE	04267	56	M
8	MS	14977	43	F
9	PZ	14943	35	F
10	PD	13068	55	F
11	CE	04167	56	F
12	JC	13875	49	M
13	MT	01614	~~69~~	M
14	KD	15693	54	M
15	AF	07436	63	F
16	LL	15721	66	M
17	LD	13872	38	F
18	JG	11304	55	F
19	WJ	15000	31	F
20	LM	13745	57	F
21	DM	15498	56	F
22	RK	14142	63	M
23	LS	14585	31	F
24	DT	08793	46	F
25	WF	05485	35	F
26	VD	12265	41	F
27	LD	13332	56	F
28	BB	15729	40	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
29	DF	07698	37	F
30	BK	14848	57	F
31	GV	15704	33	M
32	BH	14126	57	F
33	MA	14825	19	F
34	GD	04511	56	F
35	MJ	11163	49	F
36	JR	01117	37	M
37	JM	14829	38	M
38	MM	15719	18	F
39	RG	06155	51	M
40	TA	11100	40	F
41	CL	14050	36	F
42	AE	15466	50	F
43	FJ	06137	56	F
44	EF	13934	66	F
45	RC	08239	18	M
46	PP	13523	33	F
47	HF	07278	25	F
48	BM	14884	22	F
49	GA	15457	43	F
50	MC	12079	36	M
51	MM	13326	43	F
52	TS	01448	31	F
53	MC	13430	42	M
54	PM	09260	46	F
55	LJ	11123	27	F
56	DM	03992	67	F

Appendix I
(Continued)

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
57	KW	12777	36	F
58	SG	11468	41	F
59	ER	15727	25	M
60	SK	15726	21	F
61	JS	03048	37	F
62	HB	14145	40	F
63	JG	05290	56	F
64	MK	11957	48	F
65	JG	11930	21	M
66	MA	15730	22	M
67	QM	15732	30	M
68	MM	15711	45	F
69	HM	15712	44	M
70	HS	07067	46	F
71	MH	01132	63	F
72	CR	13894	30	F
73	MD	01269	59	F
74	DC	12376	46	F
75	CR	01758	65	F
76	PA	14908	39	F
77	CE	12823	40	F
78	AK	15733	23	F
79	AW	06268	28	F
80	DD	04567	42	F
81	JM	07179	41	F
82	SM	03410	27	F
83	JF	15505	19	M
84	WS	11795	38	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
85	BD	06915	48	F
86	JW	13956	41	M
87	DH	02668	39	F
88	KL	03528	28	F
89	CP	13941	41	F
90	EG	10524	67	F
91	BB	04876	52	F
92	BR	15502	45	F
93	ET	04953	70	F
94	KY	15438	20	F
95	JA	15097	45	M
96	SB	14190	39	F
97	RT	14401	33	M
98	SP	14400	27	F
99	MH	11093	43	F
100	SD	15456	53	F
101	UP	15472	39	F
102	DD	15671	42	F
103	BC	01703	69	F
104	DV	15709	38	M
105	DV	15722	18	M
106	EQ	15708	18	F
107	CL	15723	18	F
108	ER	14562	27	M
109	AB	15358	42	F
110	MG	15707	35	M
111	MG	15706	35	F
112	DP	14491	25	F

Clinical Research Laboratories, Inc.

TABLE I

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch/moistened **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
1	LS	08536	0	0	0	0	0	0	0	0	0	0	0	0
2	FS	14201	0	0	0	0	0	0	0	0	0	0	0	0
3	AJ	13082	0	0	0	0	0	0	0	0	0	0	0	0
4	AC	01439	0	0	0	0	0	0	0	0	0	0	0	0
5	MM	15079	0	0	0	0	0	0	0	0	0	0	0	0
6	PT	12224	0	0	0	0	0	0	0	0	0	0	0	0
7	JE	04267	0	0	0	0	0	0	0	0	0	0	0	0
8	MS	14977	0	0	0	0	0	0	0	0	0	0	0	0
9	PZ	14943	0	0	0	0	0	0	0	0	0	0	0	0
10	PD	13068	0	0	0	0	0	0	0	0	0	0	0	0
11	CE	04167	0	0	0	0	0	0	0	0	0	0	0	0
12	JC	13875	0	0	0	0	0	0	0	0	0	0	0	0
13	MT	01614	0	0	0	0	0	0	0	0	0	0	0	0
14	KD	15693	0	0	0	0	0	0	0	0	0	0	0	0
15	AF	07436	0	0	0	0	0	0	0	0	0	0	0	0
16	LL	15721	0	0	0	0	Discontinued Study							
17	LD	13872	0	0	0	0	0	0	0	0	0	0	0	0
18	JG	11304	0	0	0	0	0	0	0	0	0	0	0	0
19	WJ	15000	0	0	0	0	0	0	0	0	0	0	0	0
20	LM	13745	0	0	0	0	0	0	0	0	0	0	0	0
21	DM	15498	0	0	0	0	0	0	0	0	0	0	0	0
22	RK	14142	0	0	0	0	0	0	0	0	0	0	0	0
23	LS	14585	0	0	0	Discontinued Study								
24	DT	08793	0	0	0	0	0	0	0	0	0	0	0	0
25	WF	05485	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch/moistened **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
26	VD	12265	0	0	0	0	0	0	0	0	0	0	0	0
27	LD	13332	0	0	0	0	0	0	0	0	0	0	0	0
28	BB	15729	0	0	0	0	0	0	0	0	0	0	0	0
29	DF	07698	0	0	0	Discontinued Study								
30	BK	14848	0	0	0	0	0	0	0	0	0	0	0	0
31	GV	15704	0	0	0	0	0	0	0	0	0	0	0	0
32	BH	14126	0	0	0	0	0	0	0	0	0	0	0	0
33	MA	14825	0	0	0	0	0	0	0	0	0	0	0	0
34	GD	04511	0	0	Discontinued Study									
35	MJ	11163	0	0	0	0	0	0	0	0	0	0	0	0
36	JR	01117	0	0	0	0	0	0	0	0	0	0	0	0
37	JM	14829	0	0	0	0	0	0	0	0	0	0	0	0
38	MM	15719	0	0	0	0	0	0	0	0	0	0	0	0
39	RG	06155	0	0	0	0	0	0	0	0	0	0	0	0
40	TA	11100	0	0	0	0	0	0	0	0	0	0	0	0
41	CL	14050	0	0	0	0	0	0	0	0	0	0	0	0
42	AE	15466	0	0	0	0	0	0	0	0	0	0	0	0
43	FJ	06137	0	0	0	0	0	0	0	0	0	0	0	0
44	EF	13934	0	0	0	0	0	0	0	0	0	0	0	0
45	RC	08239	0	0	0	0	0	0	0	0	0	0	0	0
46	PP	13523	0	0	0	0	0	0	0	0	0	0	0	0
47	HF	07278	0	0	0	0	0	0	0	0	0	0	0	0
48	BM	14884	0	0	0	0	0	0	0	0	0	0	0	0
49	GA	15457	0	0	0	0	0	0	0	0	0	0	0	0
50	MC	12079	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch/moistened **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
51	MM	13326	0	0	0	0	0	0	0	0	0	0	0	0
52	TS	01448	0	0	0	0	0	0	0	0	0	0	0	0
53	MC	13430	0	0	0	0	0	0	0	0	0	0	0	0
54	PM	09260	0	0	0	0	0	0	0	0	0	0	0	0
55	LJ	11123	0	0	0	0	0	0	0	0	0	0	0	0
56	DM	03992	0	0	0	0	0	0	0	0	0	0	0	0
57	KW	12777	0	0	0	0	0	0	0	0	0	0	0	0
58	SG	11468	0	0	0	0	0	0	0	0	0	0	0	0
59	ER	15727	0	0	0	0	0	0	0	0	0	0	0	0
60	SK	15726	0	0	0	0	0	0	0	0	0	0	0	0
61	JS	03048	0	0	0	0	0	0	0	0	0	0	0	0
62	HB	14145	0	0	0	0	0	0	0	0	0	0	0	0
63	JG	05290	0	0	0	0	0	0	0	0	0	0	0	0
64	MK	11957	0	0	0	0	0	0	0	0	0	0	0	0
65	JG	11930	0	0	0	0	0	0	0	0	0	0	0	0
66	MA	15730	0	0	0	0	0	0	0	0	0	0	0	0
67	QM	15732	0	0	0	0	0	0	0	0	0	0	0	0
68	MM	15711	0	0	0	0	0	0	0	0	0	0	0	0
69	HM	15712	0	0	0	0	0	0	0	0	0	0	0	0
70	HS	07067	0	0	0	0	0	0	0	0	0	0	0	0
71	MH	01132	0	0	0	0	0	0	0	0	0	0	0	0
72	CR	13894	0	0	0	0	0	0	0	0	0	0	0	0
73	MD	01269	0	0	0	0	0	0	0	0	0	0	0	0
74	DC	12376	0	0	0	0	0	0	0	0	0	0	0	0
75	CR	01758	0	0	0	0	0	0	0	0	0	0	0	0

Clinical Research Laboratories, Inc.

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch/moistened **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores*		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
76	PA	14908	0	0	0	0	0	0	0	0	0	0	0	0
77	CE	12823	0	0	0	0	0	0	0	0	0	0	0	0
78	AK	15733	0	0	0	0	0	0	0	0	0	0	0	0
79	AW	06268	0	0	0	0	0	0	0	0	0	0	0	0
80	DD	04567	0	0	0	0	0	0	0	0	0	0	0	0
81	JM	07179	0	0	0	0	0	0	0	0	0	0	0	0
82	SM	03410	0	0	0	0	0	0	0	0	0	0	0	0
83	JF	15505	0	0	0	0	0	0	0	0	0	0	0	0
84	WS	11795	0	0	0	0	0	0	0	0	X	0	0	0
85	BD	06915	0	0	0	0	0	0	0	0	0	0	0	0
86	JW	13956	0	0	0	Discontinued Study								
87	DH	02668	0	0	0	0	0	0	0	0	0	0	0	0
88	KL	03528	0	0	0	0	0	0	0	0	0	0	0	0
89	CP	13941	0	0	0	0	0	0	0	0	0	0	0	0
90	EG	10524	0	0	0	0	0	0	0	0	0	0	0	0
91	BB	04876	0	0	0	0	0	0	0	0	0	0	0	0
92	BR	15502	0	0	0	0	0	0	0	0	0	0	0	0
93	ET	04953	0	0	0	0	0	0	0	0	0	0	0	0
94	KY	15438	0	0	0	0	0	0	0	0	0	0	0	0
95	JA	15097	0	0	0	0	0	0	0	0	0	0	0	0
96	SB	14190	0	0	0	0	0	0	0	0	0	0	0	0
97	RT	14401	0	0	0	0	Discontinued Study							
98	SP	14400	0	0	0	0	Discontinued Study							
99	MH	11093	0	0	0	0	0	0	0	0	0	0	0	0
100	SD	15456	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

Clinical Research Laboratories, Inc.

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch/moistened **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	**Subject Initials**	**CRL ID #**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hr**	**48 Hr**	**72 Hr**
101	UP	15472	0	0	0	0	0	0	0	0	0	0	0	0
102	DD	15671	0	0	0	0	0	0	0	0	0	0	0	0
103	BC	01703	0	0	0	0	0	0	0	0	0	0	0	0
104	DV	15709	0	0	0	0	0	0	0	0	0	0	0	0
105	DV	15722	0	0	0	0	0	0	0	0	0	0	0	0
106	EQ	15708	0	0	0	0	0	0	0	0	0	0	0	0
107	CL	15723	0	0	0	0	Discontinued Study							
108	ER	14562	0	0	0	0	0	0	0	0	0	0	0	0
109	AB	15358	0	0	0	0	0	0	0	0	0	0	0	0
110	MG	15707	0	0	0	0	0	0	0	0	0	0	0	0
111	MG	15706	0	0	0	0	0	0	0	0	0	0	0	0
112	DP	14491	0	0	0	0	0	0	0	0	0	X	0	0

X = Subject Absent

Clinical
Research
Laboratories, Inc.

COPY

Final Report

Repeated Insult Patch Test

CLIENT:

ATTENTION:

TEST MATERIAL: Cleansing Cloths
Containing 6% active CkPB

CRL STUDY NUMBER: CRL125301
Phase II

AUTHORIZED SIGNATURES:

Bruce E Kanengiser MD
Bruce E. Kanengiser, M.D.
President/Medical Director

Marilee Candino RN
Marilee Candino, R.N.
Director/Clinical Services

George J. Neumaier, M.D.
Diplomate American Board of Dermatology

Michael J. Muscatiello, Ph.D.
Executive Vice President/C.O.O.

REPORT DATE: February 14, 2002

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical
Research
Laboratories, Inc.

Good Clinical Practice
Final Report Review Statement

Clinical Study Number: CRL125301

Phase II Start Date: December 17, 2001

Phase II Completion Date: February 1, 2002

This clinical study report has been reviewed to assure that it correctly describes the method of testing and that the reported results accurately reflect the data obtained during the study.

Julie R Meyers
Reviewer

2/14/2002
Date

Clinical Research Laboratories, Inc.

FINAL REPORT

REPEATED INSULT PATCH TEST

PURPOSE

The purpose of this study was to determine the dermal irritation and sensitization potential of a test material.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane
Piscataway, New Jersey 08854

TEST MATERIAL

The following test material was provided by and was received by Clinical Research Laboratories, Inc. on November 30, 2001:

1 Cleansing Cloths

The test material was coded with the following CRL identification number:

CRL125301

The test material was cut to fit patch ½" x ½" and moistened with distilled water.

STUDY DATES

Phase II of this study was initiated on December 17, 2001 and was completed on February 1, 2002 (112 subjects were impaneled). Phase I of this study was initiated on November 30, 2001 and was completed on January 11, 2002 (104 subjects completed the study, report dated January 29, 2002).

PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects." All subjects completed a Subject Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to the study (Subject Demographics - Appendix I).

No individuals were impaneled if they exhibited or had a history of acute or chronic dermatologic, medical, or physical conditions that could interfere with dermal scoring. No subject was using sympathomimetics, antihistamines, non-steroidal anti-inflammatory agents, or corticosteroids during the study period. No known pregnant or lactating women were impaneled in the study.

TEST METHOD

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied under a semi-occlusive patch* to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of twenty-four (24) hours.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of nine (9) applications during the Induction Period. This schedule may have been modified to allow for missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on two (2) consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation and sensitization twenty-four (24) hours after removal of the patches by the subjects on Tuesday and Thursday and forty-eight (48) hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

* Semi-occlusive Strip (TruMed Technologies Inc., Burnsville, Minnesota)

TEST METHOD (Continued)

The sites were graded according to the following scoring system:

Dermal Scores

0 No visible skin reaction
± Barely perceptible erythema (minimal)
1+ Mild erythema (diffuse)
2+ Well defined erythema
3+ Erythema and edema
4+ Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject was not patched again during the Induction Phase but was challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Following approximately a two (2) week rest period, the challenge patches were applied to previously untreated test sites on the back. After twenty-four (24) hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at forty-eight (48) and seventy-two (72) hours. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a ninety-six (96) hour reading.

RESULTS

Phase II of this study was initiated with one hundred twelve (112) subjects. Six (6) subjects discontinued study participation for reasons unrelated to the test material. A total of one hundred six (106) subjects completed Phase II of the study.

Individual dermal scores recorded during the Induction and Challenge Phases of Phase II appear in Table I.

CONCLUSION

Based on the test population of two hundred ten (210) subjects and under the conditions of Phase I and Phase II of this study, the sample identified as Cleansing Cloths did not demonstrate a potential for eliciting dermal irritation or sensitization.

RETENTION

All of the original documents relating to this study will be retained by Clinical Research Laboratories, Inc. for a time period of at least five (5) years or as otherwise required by law.

Test materials shall be archived by Clinical Research Laboratories, Inc. for a period no less than six (6) months, unless otherwise instructed by Sponsor.

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1	HP	07754	68	F
2	RS	09280	43	M
3	LB	08564	34	F
4	DR	13607	52	F
5	MK	15030	42	M
6	CV	15532	31	F
7	AF	14560	45	F
8	PA	02727	62	F
9	KP	03825	41	F
10	CF	08024	50	F
11	BG	06991	64	F
12	DR	14817	43	F
13	HR	15518	52	F
14	JH	10120	42	F
15	ES	14911	47	F
16	IL	00818	50	F
17	MV	15003	48	F
18	JM	10420	32	F
19	HF	00706	67	F
20	ES	09428	53	F
21	JF	15773	42	F
22	VB	14072	66	F
23	HZ	10056	56	F
24	DG	01828	44	F
25	LS	15746	37	F
26	AB	14219	39	F
27	ZM	09802	60	F
28	LM	15349	35	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
29	DB	06105	40	F
30	NK	12925	62	F
31	TJ	14608	31	F
32	KC	09476	55	F
33	AG	14081	70	F
34	KM	09142	47	F
35	ET	09473	63	F
36	DP	08438	45	F
37	MM	08786	63	F
38	CB	11600	46	F
39	MH	13439	38	F
40	DS	14098	50	F
41	BC	15088	56	F
42	AK	10443	68	F
43	MK	11942	29	F
44	PG	00470	65	F
45	NR	02271	58	F
46	AM	14979	40	F
47	JM	15216	30	M
48	JA	14538	54	M
49	RF	10436	69	F
50	KL	01688	49	F
51	LM	14920	45	F
52	RR	10579	42	M
53	JC	14632	40	F
54	FD	13964	32	F
55	AA	14568	57	F
56	TP	14507	41	F

Appendix I
(Continued)

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
57	PP	15120	39	M
58	LB	02950	48	F
59	CJ	15710	29	F
60	MF	01706	69	F
61	MP	12044	57	F
62	BD	14726	56	F
63	LH	13156	42	M
64	PC	11302	37	F
65	SS	03271	38	F
66	BM	00087	57	F
67	EE	12173	48	F
68	JC	15322	21	M
69	RK	12769	60	F
70	CM	04021	67	F
71	RV	03808	50	F
72	JR	15440	46	M
73	JR	04186	66	F
74	MM	08660	57	F
75	LL	07031	45	F
76	AH	14082	26	F
77	PS	03839	47	F
78	BP	10476	47	F
79	PR	07039	64	F
80	CR	15567	18	F
81	JK	03896	31	M
82	LL	09586	43	M
83	CK	14176	43	F
84	CS	05284	49	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
85	DP	10425	54	F
86	JE	13149	57	M
87	SS	14843	47	F
88	JM	14638	42	M
89	LT	05354	42	F
90	CP	05220	48	F
91	AG	15019	23	M
92	RK	15747	46	F
93	MM	13272	45	M
94	NF	14449	45	F
95	SJ	03942	66	F
96	SO	06837	37	F
97	JB	15776	27	F
98	GD	15735	32	F
99	RK	06900	56	M
100	IE	15156	57	F
101	AO	10611	42	F
102	SG	14821	43	F
103	IS	13035	46	F
104	CL	15779	48	F
105	EM	15780	43	F
106	MF	01230	37	F
107	GP	15010	42	F
108	SE	08688	45	F
109	DP	15756	45	F
110	JS	15755	19	F
111	BG	12581	34	F
112	DS	15775	54	M

Clinical Research Laboratories, Inc.

TABLE I

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch ½" x ½"/Moisten **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
1	HP	07754	0	0	0	0	0	0	0	0	0	0	0	0
2	RS	09280	0	0	0	0	0	0	0	0	0	0	0	0
3	LB	08564	Discontinued Study											
4	DR	13607	0	0	0	0	0	0	0	0	0	0	0	0
5	MK	15030	0	0	0	0	0	0	0	0	0	0	0	0
6	CV	15532	0	0	0	0	0	0	0	0	0	0	0	0
7	AF	14560	0	0	0	0	0	0	0	0	0	0	0	0
8	PA	02727	0	0	0	0	0	0	0	0	0	0	0	0
9	KP	03825	0	0	0	0	0	0	0	0	0	0	0	0
10	CF	08024	0	0	0	0	0	0	0	0	0	0	0	0
11	BG	06991	0	0	0	0	0	0	0	0	0	Discontinued Study		
12	DR	14817	0	0	0	0	0	0	0	0	0	0	0	0
13	HR	15518	0	0	0	0	0	0	0	0	0	0	0	X
14	JH	10120	0	0	0	0	0	0	0	0	0	0	0	0
15	ES	14911	0	0	0	0	0	0	0	0	0	0	0	0
16	IL	00818	0	0	0	0	0	0	0	0	0	0	0	0
17	MV	15003	0	0	0	0	0	0	0	0	0	0	0	0
18	JM	10420	0	0	0	0	0	0	0	0	0	0	0	0
19	HF	00706	0	0	0	0	0	0	0	0	0	0	0	0
20	ES	09428	0	0	0	0	0	0	0	0	0	0	0	0
21	JF	15773	0	0	0	0	0	0	0	0	0	0	0	0
22	VB	14072	0	0	0	0	0	0	0	0	0	0	0	0
23	HZ	10056	0	0	0	0	0	0	0	0	0	0	0	0
24	DG	01828	0	0	0	0	0	0	0	0	0	0	0	0
25	LS	15746	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

Clinical Research Laboratories, Inc.

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch ½" x ½"/Moisten **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
26	AB	14219	0	0	0	0	0	0	0	0	0	0	0	0
27	ZM	09802	0	0	0	0	0	0	0	0	0	0	0	0
28	LM	15349	0	0	0	0	0	0	0	0	0	0	0	0
29	DB	06105	0	0	0	0	0	0	0	0	0	0	0	0
30	NK	12925	0	0	0	0	0	0	0	0	0	0	0	0
31	TJ	14608	0	0	0	0	0	0	0	0	0	0	0	0
32	KC	09476	0	0	0	0	0	0	0	0	0	0	0	0
33	AG	14081	0	0	0	0	0	0	0	0	0	0	0	0
34	KM	09142	0	0	0	0	0	0	0	0	0	0	0	0
35	ET	09473	0	0	0	0	0	0	0	0	0	0	0	0
36	DP	08438	0	0	0	0	0	0	0	0	0	0	0	0
37	MM	08786	0	0	0	0	0	0	0	0	0	0	0	0
38	CB	11600	0	0	0	0	0	0	0	0	0	0	0	0
39	MH	13439	0	0	0	0	0	0	0	0	0	0	0	0
40	DS	14098	0	0	0	0	0	0	0	0	0	0	0	0
41	BC	15088	0	0	0	0	0	0	0	0	0	0	0	0
42	AK	10443	0	0	0	0	0	0	0	0	0	0	0	0
43	MK	11942	0	0	0	0	0	0	0	0	0	0	0	0
44	PG	00470	0	0	0	0	0	0	0	0	0	0	0	0
45	NR	02271	0	0	0	0	0	0	0	0	0	0	0	0
46	AM	14979	Discontinued Study											
47	JM	15216	0	0	0	0	0	0	0	0	0	0	0	0
48	JA	14538	0	0	0	0	0	0	0	0	0	0	0	0
49	RF	10436	0	0	0	0	0	0	0	0	0	0	0	0
50	KL	01688	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch ½" x ½"/Moisten **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
51	LM	14920	0	0	0	0	0	0	0	0	0	0	0	0
52	RR	10579	0	0	0	0	0	0	0	0	0	0	0	0
53	JC	14632	0	0	0	0	0	0	0	0	0	0	0	0
54	FD	13964	0	0	0	0	0	0	0	0	0	0	0	0
55	AA	14568	0	0	0	0	0	0	0	0	0	0	0	0
56	TP	14507	0	0	0	0	0	0	0	0	0	0	0	0
57	PP	15120	0	0	0	0	0	0	0	0	0	0	X	0
58	LB	02950	0	0	0	0	0	0	0	0	0	0	0	0
59	CJ	15710	0	0	0	0	0	0	0	0	0	0	0	0
60	MF	01706	0	0	0	0	0	0	0	0	0	0	0	0
61	MP	12044	0	0	0	0	0	0	0	0	0	0	0	0
62	BD	14726	0	0	0	0	0	0	0	0	0	0	0	0
63	LH	13156	0	0	0	0	0	0	0	0	0	0	0	0
64	PC	11302	0	0	0	0	0	0	0	0	0	0	0	0
65	SS	03271	0	0	0	0	0	0	0	0	0	0	0	0
66	BM	00087	0	0	0	0	0	0	0	0	0	0	0	0
67	EE	12173	0	0	0	0	0	0	0	0	0	0	0	0
68	JC	15322	0	0	0	0	0	0	0	0	0	0	0	0
69	RK	12769	0	0	0	0	0	0	0	0	0	0	0	0
70	CM	04021	0	0	0	0	0	0	0	0	0	0	0	0
71	RV	03808	0	0	0	0	0	0	0	0	0	0	0	0
72	JR	15440	0	0	Discontinued Study									
73	JR	04186	0	0	0	0	0	0	0	0	0	0	0	0
74	MM	08660	0	0	0	0	0	0	0	0	0	0	0	0
75	LL	07031	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch ½" x ½"/Moisten **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	Subject Initials	CRL ID #	1	2	3	4	5	6	7	8	9	24 Hr	48 Hr	72 Hr
76	AH	14082	0	0	0	0	0	0	0	0	0	0	0	0
77	PS	03839	0	0	0	0	0	0	0	0	0	0	0	0
78	BP	10476	0	0	0	0	0	0	0	0	0	0	0	0
79	PR	07039	0	0	0	0	0	0	0	0	0	0	0	0
80	CR	15567	0	0	0	0	0	0	0	0	0	0	0	0
81	JK	03896	0	0	0	0	0	0	0	0	0	0	0	0
82	LL	09586	0	0	0	0	0	0	0	0	0	0	0	0
83	CK	14176	0	0	0	0	0	0	0	0	0	0	0	0
84	CS	05284	0	0	0	0	0	0	0	0	0	0	0	0
85	DP	10425	0	0	0	0	0	0	0	0	0	0	0	0
86	JE	13149	0	0	0	0	0	0	0	0	0	0	0	0
87	SS	14843	0	0	0	0	0	0	0	0	0	0	0	0
88	JM	14638	0	0	0	0	0	0	0	0	0	0	0	0
89	LT	05354	0	0	0	0	0	0	0	0	0	0	0	0
90	CP	05220	0	0	0	0	0	0	0	0	0	0	0	0
91	AG	15019	0	0	0	0	0	0	0	0	0	0	0	0
92	RK	15747	0	0	0	0	0	0	0	0	0	0	0	0
93	MM	13272	0	0	0	0	0	0	0	0	0	0	0	0
94	NF	14449	0	0	0	0	0	0	0	0	0	0	0	0
95	SJ	03942	0	0	0	0	0	0	0	0	0	0	0	0
96	SO	06837	0	0	0	0	0	0	0	0	0	0	0	0
97	JB	15776	0	0	0	0	0	0	0	0	0	0	0	0
98	GD	15735	0	0	0	0	0	0	0	0	0	0	0	0
99	RK	06900	0	0	0	0	0	0	0	0	0	0	0	0
100	IE	15156	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Tabulation of Individual Scores

Test Material: Cleansing Cloths (CRL125301)

PATCH TEST CONDITION: Cut to fit patch ½" x ½"/Moisten **PATCH TYPE:** Semi-occlusive

			Induction Scores									Challenge Scores		
Subject Number	**Subject Initials**	**CRL ID #**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hr**	**48 Hr**	**72 Hr**
101	AO	10611	0	Discontinued Study										
102	SG	14821	0	0	0	0	0	0	0	0	0	0	0	0
103	IS	13035	0	0	0	0	0	0	0	0	0	0	0	0
104	CL	15779	0	0	0	0	0	0	0	0	0	0	0	0
105	EM	15780	0	0	0	0	0	0	0	0	0	0	0	0
106	MF	01230	0	0	0	Discontinued Study								
107	GP	15010	0	0	0	0	0	0	0	0	0	0	0	0
108	SE	08688	0	0	0	0	0	0	0	0	0	0	0	0
109	DP	15756	0	0	0	0	0	0	0	0	0	0	0	0
110	JS	15755	0	0	0	0	0	0	0	0	0	0	0	0
111	BG	12581	0	0	0	0	0	0	0	0	0	0	0	0
112	DS	15775	0	0	0	0	0	0	0	0	0	0	0	0

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D. 3/10/10
Industry Liaison to the CIR Expert Panel

DATE: March 10, 2010

SUBJECT: Local Lymph Node Assay on Amidoamine (C12 56.8%)

Calvert Labs. 2010. Local lymph node assay on amidoamine. Calvert Study No. 0787MP72.001.

Please note that the certificate of analysis of the material tested (chain length distribution) can be found in Appendix III of the report.

CALVERT Labs

Customized. Responsive. Proven.

Final Report

Title: Local Lymph Node Assay

Calvert Study No.: 0787MP72.001

Testing Facility: Calvert Laboratories, Inc.
Scott Technology Park
130 Discovery Drive
Scott Township, PA 18447

Study Sponsor: Personal Care Products Council
1101 17th Street NW
Suite300
Washington, DC 20036

Date: 5 Mar 2010

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I. Table of Contents

II. List of Abbreviations

CFR	Code of Federal Regulations
DPM	Disintegrations per minute
EEC	European Economic Community
FDA	Food and Drug Administration
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HCA	Hexylcinnamaldehyde
IACUC	Institutional Animal Care and Use Committee
ILAR	Institute for Laboratory Animal Resources
LLNA	Local Lymph Node Assay
NIH	National Institute of Health
PBS	Phosphate Buffered Saline
OECD	Organisation for Economic Cooperation and Development
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
SI	Stimulation Indices
SEM	Standard error of the mean
SOP	Standard Operating Procedure
TCA	Trichloroacetic Acid
USDA	United States Department of Agriculture
v/v	Volume/volume
w/v	Weight/volume

III. Regulatory Compliance and Signatures

Calvert Study No.: 0787MP72.001

Title: Local Lymph Node Assay

A. Regulatory Compliance

1. Calvert Laboratories-Conducted Study Elements

All parts of this study were performed according to SOP's and the protocol and in compliance with the Good Laboratory Practice Regulations 21 CFR Part 58 and in accordance with OECD Principles of Good Laboratory Practice as revised on 26th November, 1997 [C(97)186/Final] with the following exceptions:

Exceptions:

1) It is unknown if the test article characterization for identity and purity was not done under GLP/GMP. There was no impact on this study since the material was analyzed.

2) The test article was not characterized for stability. The impact on this study is not known since verification of the test article stability cannot be confirmed.

3) The test article and positive control dose formulations were not analyzed for concentration, homogeneity or stability. However, the formulations were prepared fresh on each day of dosing and the procedures were performed by trained technicians as per Calvert SOP's. Therefore, this exception in not considered to have had any impact on the study outcome.

2. Guidelines for Study Design

OECD 429, Skin Sensitization: Local Lymph Node Assay, 24 April 2002

OPPTS Health Effects Test Guidelines 870.2600 – Skin Sensitization, March 2003

EEC Testing Methods Annex V Part B, Skin Sensitization: Local Lymph Node Assay, B.42, 2004/73/EC L152, 2004

B. Study Director Signature

I, the undersigned, hereby declare that this report is a true and accurate record of the results obtained. No circumstances occurred during the study that were considered to have significantly affected the overall quality or integrity of the data obtained. All protocol deviations were documented in the study records and are listed in Appendix IV of this report.

Joan M Chapdelaine 5 Mar 2010

Joan M. Chapdelaine, Ph.D. Date
Study Director
Calvert Laboratories, Inc.

C. Signatures of Other Responsible Personnel

Scientific Oversight and Report Review

Steven D. Slone 5 Mar 2010

Scientific Management Date
Calvert Laboratories, Inc.

IV. Quality Assurance Unit Statement

Calvert Study No.: 0787MP72.001

Title: Local Lymph Node Assay

The following inspections were made in accordance with appropriate Calvert Standard Operating Procedures.

A. Protocol Inspection

Date of Inspection	Report to Study Director	Report to Management
7 Jan 2010	7 Jan 2010	7 Jan 2010

B. Procedures

Inspected Phase	Date of Inspection	Report to Study Director	Report to Management
Lymph node processing	1 Feb 2010	1 Feb 2010	1 Feb 2010

C. Data/Draft Report Inspection

Inspected Phase	Date of Inspection	Report to Study Director	Report to Management
Data/Draft Report #1	24 Feb & 1 Mar 2010	1 Mar 2010	1 Mar 2010
Final Report	5 Mar 2010	5 Mar 2010	5 Mar 2010

This report has been reviewed by the Quality Assurance Unit, employing methods detailed in appropriate Calvert Standard Operating Procedures. The results constitute an accurate representation of recorded data. Any data supplied by the Sponsor were not audited by the Quality Assurance Unit at Calvert.

Darlene Gilpin, RQAP-GLP
Director, Quality Assurance
Calvert Laboratories, Inc.

5 Mar 2010
Date

V. Summary

A. Title

Local Lymph Node Assay

B. Objective

To determine if the test article will induce a hypersensitivity response in mice as measured by the proliferation of lymphocytes in the draining lymph nodes.

C. Methods

Groups of 5 CBA/J female mice were treated on the dorsal surface of both ears once per day for 3 days with the test article (Ameenex 6169) at 0.1%, 0.5%, 1%, 2.5% and 5%, the vehicle (ethanol/water, 7:3 (v/v) neutralized to pH 6.0 with citric acid monohydrate), and the positive control (35% hexylcinnamaldehyde (HCA)). On Day 6, the mice were injected, i.v., with 20 µCi of ^{3}H-thymidine in sterile saline. Five hours later, the mice were euthanized and the draining auricular lymph nodes were removed. The lymph node cells were precipitated with 5% trichloroacetic acid (TCA) and the pellets counted in a ß-scintillation counter to determine incorporation of the ^{3}H-thymidine.

D. Results

Initially a preliminary irritation study was performed by treating ten animals (2 per dose level) with the test article at concentrations of 0.1, 0.5, 1, 2.5 and 5% (w/v). Animals were dosed topically on the dorsal surface of both ears once per day for three consecutive days and were observed for six days for erythema and edema.

On Days 3, 4 and 5 both animals treated with the test article at 5% had very slight erythema and edema. On Day 6, the mice in this group had very slight erythema and slight edema. No erythema or edema was observed at any time in the mice treated with the test article at concentrations of 0.1, 0.5, 1 or 2.5% (w/v).

The ears of the mice treated with the test article at 5% also appeared wet on Days 3-6. There were no other findings in any of the mice (Table 3). After

consultation with the Sponsor, the concentrations used for the main study were 0.1, 0.5, 1, 2.5, and 5% (w/v).

In the main study, there was no mortality and all animals appeared clinically normal throughout the study.

There was no erythema or edema at the application site of any of the animals treated with vehicle, the positive control or the test article at 0.1, 0.5, or 1% (w/v). The mice in the group treated with the test article at 2.5% had no erythema or edema on Days 1 and 2 but had very slight erythema on Day 3 and very slight erythema and very slight edema on Days 4-6. Four of the 5 mice treated with the test article at 5% (w/v) had very slight erythema and very slight edema on Day 2. Days 3-6 the mice treated with the test article at 5% (w/v) had well defined erythema and slight edema.

The ears of the mice treated with HCA appeared wet on Days 2-6, and the ears of the mice treated with test article at 5% (w/v) appeared wet on Days 3-6. There were no other findings.

Mean body weights at Day 1 and Day 6 and mean changes in body weights were evaluated. The only statistically significant differences observed was an increase in change in body weight in the group treated with the test article at 1% (w/v) when compared to the vehicle control group. However, this increase was not biologically relevant. Therefore, the test article did not appear to cause any overt toxicity.

At termination, the lymph nodes from the mice treated with the test article at 2.5 and 5% (w/v) were enlarged relative to the vehicle-treated mice but were otherwise normal in appearance. The lymph nodes from all other test article treated mice and mice in the vehicle treated group were normal in size and appearance. Enlarged lymph nodes relative to the vehicle-treated mice were also observed in the HCA treated group.

The positive control, 35% (v/v) HCA, resulted in a stimulation index (SI) of 26.7. A 3-fold or greater increase in proliferative activity relative to the concurrent vehicle control is considered a positive response. In addition, the response with HCA was also statistically significant ($p < 0.001$) when the log DPM for this group was compared to the vehicle group. Thus, the sensitivity of the test system was demonstrated.

Exposure to the test article at concentrations of 0.1, 0.5, 1, 2.5 and 5% (w/v) resulted in stimulation indices of 1.8, 1.0, 3.1, 24.5, and 60.6, respectively. Statistically significant differences were found between the groups treated with the test article at 1, 2.5, and 5% and the vehicle control ($p = 0.01$, 0.001 and 0.001, respectively. The EC3 was calculated to be 0.98%.

E. Conclusion

A test material is considered to have skin sensitizing activity if, at one or more concentrations, it induces a 3-fold or greater increase in proliferative activity relative to the concurrent vehicle treated control. Thus, a stimulation index ≥ 3.0 is regarded as a positive response. Treatment with AMEENEX 6169 at concentrations of 1, 2.5 and 5% (w/v) resulted in stimulation indices greater than 3.0. Therefore, AMEENEX 6169 is considered to have skin sensitizing activity. The EC3 was calculated to be 0.98%

VI. General Information

A. Key Study Dates

Study Initiation Date:	4 Jan 2010
Animal Receipt Date:	14 Jan 2010
Experimental Start Date:	19 Jan 2010
First Day of Dosing:	27 Jan 2010
Last Day of In-life:	1 Feb 2010
Experimental Completion Date:	3 Feb 2010

B. Responsible Personnel Testing Facility

Study Director:	Joan M. Chapdelaine, Ph.D. Calvert Laboratories, Inc. 130 Discovery Drive Scott Township, PA 18447
Primary Technicians:	Maura Boyarsky and Yvonne Coccetti, LAT
Pharmacy Technicians:	Nicholas Acri, B.S. and Christina Jackson, B.S.

C. Contributing Scientist

Study Monitor:	Linda Loretz, Ph.D., DABT

D. Objective

To determine if the test article will induce a hypersensitivity response in mice as measured by the proliferation of lymphocytes in the draining lymph nodes.

E. Experimental Design Overview

1. General Description

To assess the potential contact hypersensitivity response of mice to the test articles, groups of 5 CBA/J female mice were treated on the dorsal surface of both ears once per day for 3 days with the test article (Ameenex 6169) at 0.1%, 0.5%, 1%, 2.5% and 5%, the vehicle (ethanol/water, 7:3 (v/v) neutralized to pH 6.0 with citric acid monohydrate) and the positive control

(35% hexylcinnamaldehyde (HCA)). On Day 6, the mice were injected, i.v., with 20 µCi of ^{3}H-thymidine in sterile saline. Five hours later, the mice were euthanized and the draining auricular lymph nodes were removed. The lymph node cells were precipitated with 5% trichloroacetic acid (TCA) and the pellets counted in a ß-scintillation counter to determine incorporation of the ^{3}H-thymidine.

VII. Materials and Methods

A. Test Articles, Vehicles and Preparation

1. Test Article

Identification:	Ameenex 6169
Supplier:	Personal Care Products Council
Lot/Batch No.:	9265
Expiration Date:	not declared
Physical Description:	Off white solid
Storage:	Room temperature
Composition/Purity/ Stability:	A Certificate of Analysis was provided (Appendix III), but stability data was not provided.

2. Positive Control Article

Identification:	Hexylcinnamaldehyde (HCA)
Supplier:	Aldrich, St Louis, MO
Batch No.:	MKAA2596
Expiration Date:	3 Mar 2012
Physical Description:	Clear yellow liquid
Storage Conditions:	Room temperature

3. *Vehicle*

Identification:	Ethanol/water, 7:3 (v/v) neutralized to pH 6.0 with citric acid monohydrate
Supplier:	Ethanol: Pharmco Water: Baxter
Lot/Batch No.:	Ethanol: 0801282 Water: C780122 Ethanol/water, 7:3 (v/v): 19 Jan 2010
Expiration Date:	Ethanol: Feb 2011 Water: Aug 2110 Ethanol/water, 7:3 (v/v): 19 Feb 2010
Physical Description:	Ethanol: Clear, colorless liquid Water: Clear colorless liquid Ethanol/water, 7:3 (v/v): Clear colorless liquid
Storage:	Room temperature

4. *Dose Preparation*

On each day of dosing, the test article was prepared at the appropriate concentrations (w/v) in volumetric flasks by dissolving the appropriate amount of test article in the vehicle. All preparations were vortexed to mix. Test article dosing formulations were dosed as clear colorless liquids.

The positive control (HCA) was prepared daily as a 35% (v/v) solution in vehicle. It was dosed as a clear pale yellow liquid.

5. *Formulated Test Article Analysis*

Samples of the test article dosing solutions were not collected for analysis. Good Laboratory Practice (GLP) regulations require that all formulated test articles be appropriately verified for concentration, homogeneity, and stability. Compliance necessitates documentation that verification has been done. Since verification was not performed, this fact was reflected in the compliance statement in the report.

6. *Accountability and Disposition*

Unused test article will be retained for use on possible related future studies.

B. Test System (Animals and Animal Care)

1. *Description*

Species:	Mouse
Strain/Substrain:	CBA/J
Total Number:	Preliminary Irritation Screen: 2/dose level Main Study: 35
Gender:	Female
Age Range:	9-10 weeks
Body Weight Range:	20 to 26 grams at the outset (Day 1) of the study.
Animal Source:	Jackson Laboratories, Bar Harbor, ME
Experimental History:	Purpose-bred and experimentally naïve at the outset of the study.
Identification:	Tail marked with an indelible marker

2. *Rationale for Choice of Species and Number of Animals*

The mouse is the standard species used in the local lymph node assay (LLNA), which has been developed as an alternative to Guinea Pig Sensitization Assays. The LLNA is a refinement in terms of reducing or eliminating distress in the animals compared to the guinea pig tests. The number used is the minimum number recommended. (NIH Publication No. 99-4494).

3. *Husbandry*

Housing:	Animals were group housed (5 per cage) upon receipt in compliance with National Research Council "Guide for the Care and Use of Laboratory Animals". The room in which the animals were

kept was documented in the study records. No other species were kept in the same room.

Lighting:	12 hours light/12 hours dark
Room Temperature:	21.6 to 26.7°C
Relative Humidity:	12 to 48%
Food:	Animals had access to Harlan Teklad Rodent Diet (certified) or equivalent *ad libitum*. The lot number(s) and specifications of each lot used are archived at Calvert. No contaminants were known to be present in the certified diet at levels that would be expected to interfere with the results of this study. Analysis of the diet was limited to that performed by the manufacturer, records of which are maintained in the Calvert archives.
Water:	Tap water was available *ad libitum,* via water bottles. The water is routinely analyzed for contaminants as per Calvert SOP's. No contaminants were known to be present in the water at levels that would be expected to interfere with the results of this study. Results of the water analysis are maintained in the Calvert archives.
Acclimation:	Study animals were acclimated to their housing for a minimum of 6 days prior to their first day of dosing.

4. *Prestudy Health Screen and Selection Criteria*

All animals used in this study were assessed as to their general health by a member of the technical staff or other authorized personnel. During the acclimation period, each animal was observed at least once daily for any abnormalities or for the development of infectious disease. Only animals that were determined to be suitable for use were assigned to this study.

5. Assignment to Study Groups

Mice were selected and randomly assigned to study groups based on body weight and apparent good health. Mice were given the following identification numbers and identified by tail mark with an indelible marker:

Group Number	Number
1	1-5
2	6-10
3	11-15
4	16-20
5	21-25
6	26-30
7	31-35

6. Humane Care of Animals

Treatment of animals was in accordance with the study protocol and also in accordance with Calvert SOP's which adhere to the regulations outlined in the USDA Animal Welfare Act (9 CFR Parts 1, 2 and 3) and the conditions specified in the Guide for the Care and Use of Laboratory Animals (ILAR publication, 1996, National Academy Press). The Calvert IACUC approved the study protocol prior to finalization.

C. Test Article Administration

1. Preliminary Study (Irritation Screen)

Each test article concentration was applied to the ears of two mice. Mice were treated with the test article on the dorsal surface of both ears (25 µl/ear), once per day for three consecutive days using a micropipette. The following concentrations were used: 0.1, 0.5, 1, 2.5 and 5 (w/v).

Animals were scored approximately 24 hours after each dose for irritation using the Draize scoring system (Appendix I). Doses for the main study were selected based on the results of the irritation screen. After discussion with the Sponsor, it was decided that these doses would also be used in the main study.

2. *Group Assignments and Dose Levels*

Group	Treatment	Dose*	Number of Animals
1	Vehicle	-	5
2	Test Article	0.1%	5
3	Test Article	0.5%	5
4	Test Article	1.0%	5
5	Test Article	2.5%	5
6	Test Article	5.0%	5
7	HCA	35%	5

3. *Dosing*

Route: Topically on the dorsal surface of both ears

Frequency: Once daily for 3 consecutive days (Days 1-3). The timing of dose administration remained consistent (± 2 hours) during the dosing phase.

Procedure: The test/control article dosing solutions were applied using a mechanical pipette to the dorsal surfaces of both ears once a day for 3 consecutive days (Days 1, 2, and 3) to each animal. A volume of 25 µl/ear was used.

4. *Justification for Route and Dose Levels*

The dermal route was selected as this is the route required for this model of contact hypersensitivity.

The recommended doses for this assay are selected from the concentration series 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5% etc. Three consecutive concentrations should be chosen, the top one being the highest level that could be achieved while avoiding systemic toxicity and excessive local irritation. The doses for the irritation screen were selected by the Sponsor based on results with other similar materials. The doses for the main study were based on the results of the irritation screen and discussions with the Sponsor.

The frequency of dosing is the convention for this type of study.

D. In-Life Observations and Measurements

1. Mortality/Morbidity

Frequency: Daily on Days 1 to 6

2. Clinical Observations

Frequency: Observations were performed prior to dose administration and following dose administration. Clinical observations were also performed once daily on Days 4-6. Particular attention was given to the application sites. Any significant alterations to the application sites, and the general appearance of the pinnae, including build up of test article, was recorded.

3. Dermal Irritation

Frequency: Animals were examined daily for signs of erythema and edema. Irritation was scored and recorded using the Draize scoring system (Appendix 1). On Days 1-3 scoring was performed prior to dosing.

4. Body Weight

Frequency: Animals were weighed on Days 1 and 6.

E. Method of Performance

Mice were treated on the dorsal surfaces of ears, once per day on Days 1, 2, and 3. Approximately 24±2 hours between applications of test article were maintained. On Day 6 the mice were injected, i.v., with 20 µCi of ^{3}H-thymidine in 250 µl of sterile saline. Five hours later the mice were euthanized with CO_2 and the draining auricular lymph nodes removed. At removal, the number of nodes collected per animal was recorded, and the nodes were examined for size/appearance. Any unexpected observations were noted in study records. A single cell suspension was prepared from the lymph nodes of each mouse. Cells were washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloroacetic acid (TCA) overnight at 2-8°C. The pellets were

recovered by centrifugation and resuspended in 1 ml of TCA and transferred to 12 ml of scintillation fluid. An additional 1 ml of TCA was used to rinse the tube, and it was also transferred to the 12 ml of scintillation fluid. Incorporation of ^{3}H-thymidine was measured in a ß-scintillation counter.

F. Terminal Procedures

1. Termination

a) Scheduled Sacrifice

All surviving animals were euthanized by CO_2 asphyxiation.

b) Unscheduled Sacrifice

All animals survived to the end of the study.

VIII. Records and Reports

A. Data Collection and Analysis

Data was manually collected except for the data generated by the scintillation counter (Beckman LS 6000 SC). The mean DPM for each group was calculated using SYSTAT version 9.01, developed by SPSS, Inc. Increases in ^{3}H-thymidine incorporation relative to the vehicle-treated control were derived for each group and recorded as stimulation indices (SI). The criterion for a positive response was that one or more concentrations of a test article elicited a 3-fold or greater increase in isotope incorporation relative to the vehicle control.

The mean body weights and changes in body weight were also calculated and evaluated using SYSTAT.

Individual DPM values were analyzed by log transformation (base 10) of the data. The evaluation of the equality of means for the DPM and body weight data was made by a one-way analysis of variance using the F distribution to assess statistical significance. If statistically significant differences between the means were found, a Dunnett's test was used to determine the degree of significance from the control means.

The data indicated that the test article was positive, therefore the EC3 was calculated using the formula:

$$EC3 = c+[(3-d)/(b-d)](a-c)$$

where the data points lying immediately above and below the SI value of 3 have the co-ordinates (a,b) and (c,d) respectively.

B. Storage of Records

Test article preparation, test article tracking, in-life data, protocol and the original final report generated as a result of this study will be archived at Calvert, 105 Edella Road, Suite 100, Clarks Summit, PA 18411. After 2 years, the Sponsor will be contacted to determine final disposition of all study materials.

IX. Results

A. Irritation Screen

Initially a preliminary irritation study was performed by treating ten animals (2 per dose level) with the test article at concentrations of 0.1, 0.5, 1, 2.5 and 5% (w/v). Animals were dosed topically on the dorsal surface of both ears once per day for three consecutive days and were observed for six days for erythema and edema.

On Days 3, 4 and 5 both animals treated with the test article at 5% had very slight erythema and edema (Table 1). On Day 6, the mice in this group had very slight erythema and slight edema. No erythema or edema was observed at any time in the mice treated with the test article at concentrations of 0.1, 0.5, 1 or 2.5% (w/v).

The ears of the mice treated with the test article at 5% also appeared wet on Days 3-6 (Table 2). There were no other findings in any of the mice (Table 3). After consultation with the Sponsor, the concentrations used for the main study were 0.1, 0.5, 1, 2.5, and 5% (w/v).

B. Main Study

There was no mortality and all animals appeared clinically normal throughout the study (Table 4).

There was no erythema or edema at the application site of any of the animals treated with vehicle, the positive control or the test article at 0.1, 0.5, or 1% (w/v) (Table 5). The mice in the group treated with the test article at 2.5% had no erythema or edema on Days 1 and 2 but had very slight erythema on Day 3 and very slight erythema and very slight edema on Days 4-6. Four of the 5 mice treated with the test article at 5% (w/v) had very slight erythema and very slight edema on Day 2. Days 3-6 the mice treated with the test article at 5% (w/v) had well defined erythema and slight edema.

The ears of the mice treated with HCA appeared wet on Days 2-6, and the ears of the mice treated with test article at 5% (w/v) appeared wet on Days 3-6. There were no other findings (Table 6).

Mean body weights at Day 1 and Day 6 and mean changes in body weights were evaluated (Table 7). The only statistically significant differences observed was an increase in change in body weight in the group treated with the test article at 1% (w/v) when compared to the vehicle control group. However, this increase was not biologically relevant. Therefore, the test article did not appear to cause any overt toxicity.

At termination, the lymph nodes from the mice treated with the test article at 2.5 and 5% (w/v) were enlarged relative to the vehicle-treated mice but were otherwise normal in appearance. The lymph nodes from all other test article treated mice and mice in the vehicle treated group were normal in size and appearance. Enlarged lymph nodes relative to the vehicle-treated mice were also observed in the HCA treated group.

The positive control, 35% (v/v) HCA, resulted in a stimulation index (SI) of 26.7 (Table 8). A 3-fold or greater increase in proliferative activity relative to the concurrent vehicle control is considered a positive response. In addition, the response with HCA was also statistically significant ($p < 0.001$) when the log DPM for this group was compared to the vehicle group. Thus, the sensitivity of the test system was demonstrated.

Exposure to the test article at concentrations of 0.1, 0.5, 1, 2.5 and 5% (w/v) resulted in stimulation indices of 1.8, 1.0, 3.1, 24.5, and 60.6, respectively (Table 8). Statistically significant differences were found between the groups

treated with the test article at 1, 2.5, and 5% and the vehicle control ($p = 0.01$, 0.001 and 0.001, respectively. The EC3 was calculated to be 0.98%.

X. Conclusion

A test material is considered to have skin sensitizing activity if, at one or more concentrations, it induces a 3-fold or greater increase in proliferative activity relative to the concurrent vehicle treated control. Thus, a stimulation index ≥ 3.0 is regarded as a positive response. Treatment with AMEENEX 6169 at concentrations of 1, 2.5 and 5% (w/v) resulted in stimulation indices greater than 3.0. Therefore, AMEENEX 6169 is considered to have skin sensitizing activity. The EC3 was calculated to be 0.98%

XI. Reference

1. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. NIH Publication No. 99-4494, Feb. 1999.

XII. Tables

A. Table 1: Irritation Screen – Dermal Irritation Scores

Group	Treatment	Dose % (w/v)	Animal No.	Day 2		Day 3		Day 4		Day 5		Day 6	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
1	AMEENEX 6169	0.1	1	0	0	0	0	0	0	0	0	0	0
			2	0	0	0	0	0	0	0	0	0	0
2	AMEENEX 6169	0.5	3	0	0	0	0	0	0	0	0	0	0
			4	0	0	0	0	0	0	0	0	0	0
3	AMEENEX 6169	1	5	0	0	0	0	0	0	0	0	0	0
			6	0	0	0	0	0	0	0	0	0	0
4	AMEENEX 6169	2.5	7	0	0	0	0	0	0	0	0	0	0
			8	0	0	0	0	0	0	0	0	0	0
5	AMEENEX 6169	5	9	0	0	1	1	1	1	1	1	1	2
			10	0	0	1	1	1	1	1	1	1	2

ER = Erythema
ED = Edema

B. Table 2: Irritation Screen - Observations of Dose Application Sites

Group 1

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	2/2	2/2	2/2	2/2	2/2	2/2

Group 2

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	2/2	2/2	2/2	2/2	2/2	2/2

Group 3

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	2/2	2/2	2/2	2/2	2/2	2/2

Group 4

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	2/2	2/2	2/2	2/2	2/2	2/2

Group 5

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	2/2	2/2	0/2	0/2	0/2	0/2
Ears appear wet	0/2	0/2	2/2	2/2	2/2	2/2

On Days 1-3, observations were performed both pre and post dose. The observations were the same for both observation periods unless otherwise indicated.

C. Table 3: Irritation Screen – Clinical Observations

Group 1

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 2

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 3

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 4

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 5

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

On Days 1-3, clinical observations were performed both pre and post dose. The observations were the same for both observation periods.

D. Table 4: Main Study – Clinical Observations

Group 1

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 2

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 3

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 4

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 5

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 6

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 7

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

On Days 1-3, clinical observations were performed both pre and post dose. The observations were the same for both observation periods.

E. Table 5: Dermal Irritation Scores (Main Study)

Group	Treatment	Dose (%) (w/v)	Animal No.	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
1	Ethanol/water (7:3)	-	1	0	0	0	0	0	0	0	0	0	0	0	0
			2	0	0	0	0	0	0	0	0	0	0	0	0
			3	0	0	0	0	0	0	0	0	0	0	0	0
			4	0	0	0	0	0	0	0	0	0	0	0	0
			5	0	0	0	0	0	0	0	0	0	0	0	0
2	AMEENEX 6169	0.1	6	0	0	0	0	0	0	0	0	0	0	0	0
			7	0	0	0	0	0	0	0	0	0	0	0	0
			8	0	0	0	0	0	0	0	0	0	0	0	0
			9	0	0	0	0	0	0	0	0	0	0	0	0
			10	0	0	0	0	0	0	0	0	0	0	0	0
3	AMEENEX 6169	0.5	11	0	0	0	0	0	0	0	0	0	0	0	0
			12	0	0	0	0	0	0	0	0	0	0	0	0
			13	0	0	0	0	0	0	0	0	0	0	0	0
			14	0	0	0	0	0	0	0	0	0	0	0	0
			15	0	0	0	0	0	0	0	0	0	0	0	0
4	AMEENEX 6169	1	16	0	0	0	0	0	0	0	0	0	0	0	0
			17	0	0	0	0	0	0	0	0	0	0	0	0
			18	0	0	0	0	0	0	0	0	0	0	0	0
			19	0	0	0	0	0	0	0	0	0	0	0	0
			20	0	0	0	0	0	0	0	0	0	0	0	0
5	AMEENEX 6169	2.5	21	0	0	0	0	1	0	1	1	1	1	1	1
			22	0	0	0	0	1	0	1	1	1	1	1	1
			23	0	0	0	0	1	0	1	1	1	1	1	1
			24	0	0	0	0	1	0	1	1	1	1	1	1
			25	0	0	0	0	1	0	1	1	1	1	1	1
6	AMEENEX 6169	5	26	0	0	1	1	2	2	2	2	2	2	2	2
			27	0	0	0	0	2	2	2	2	2	2	2	2
			28	0	0	1	1	2	2	2	2	2	2	2	2
			29	0	0	1	1	2	2	2	2	2	2	2	2
			30	0	0	1	1	2	2	2	2	2	2	2	2

Table 5 (continued)

Group	Treatment	Dose (%) (v/v)	Animal No.	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
7	HCA	35	31	0	0	0	0	0	0	0	0	0	0	0	0
			32	0	0	0	0	0	0	0	0	0	0	0	0
			33	0	0	0	0	0	0	0	0	0	0	0	0
			34	0	0	0	0	0	0	0	0	0	0	0	0
			35	0	0	0	0	0	0	0	0	0	0	0	0

ER = Erythema
ED = Edema

F. Table 6: Main Study - Observations of Dose Application Sites

Group 1

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 2

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 3

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 4

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 5

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	5/5	5/5	5/5	5/5

Group 6

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	5/5	0/5	0/5	0/5	0/5
Ears appear wet	0/5	0/5	5/5	5/5	5/5	5/5

Group 7

Observation	Number of Animals/Total Number in Group					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	5/5	0/5	0/5	0/5	0/5	0/5
Ears appear wet	0/5	5/5	5/5	5/5	5/5	5/5

On Days 1-3, observations were performed both pre and post dose. The observations were the same for both observation periods unless otherwise indicated.

G. Table 7: Body Weights

Group	Treatment	Dose % (w/v)	Body Weights (g) (mean ± sem)		Change in Body Weight (g) (mean ± sem)
			Day 1	Day 6	
1	Vehicle	-	24.0 ± 0.7	23.4 ± 0.8	-0.6 ± 0.2
2	AMEENEX 6169	0.1	23.4 ± 1.1	22.6 ± 1.3	-0.8 ± 0.4
3	AMEENEX 6169	0.5	24.4 ± 0.5	24.4 ± 0.5	0.0 ± 0.0
4	AMEENEX 6169	1.0	23.4 ± 0.7	24.2 ± 0.8	0.8 ± 0.2*
5	AMEENEX 6169	2.5	23.8 ± 0.4	24.2 ± 0.7	0.4 ± 0.4
6	AMEENEX 6169	5	24.0 ± 0.6	22.4 ± 0.4	-1.6 ± 0.4
7	HCA	35	24.6 ± 0.2	24.2 ± 0.4	-0.4 ± 0.2

[1] Ethanol/water (7:3)

*Statistically significant difference ($p < 0.05$) when compared to the vehicle control group.

H. Table 8: Local Lymph Node Assay

Group	Treatment	Dose (%)	DPM (mean ± sem)	SI (Test/control Ratio)	Results[1]
1	Vehicle	-	273 ± 34	-	-
2	AMEENEX 6169	0.1	488 ± 124	1.8	-
3	AMEENEX 6169	0.5	265 ± 44	1.0	-
4	AMEENEX 6169	1.0	854 ± 201**	3.1	+
5	AMEENEX 6169	2.5	6680 ± 1136***	24.5	+
6	AMEENEX 6169	5.0	16544 ± 1199***	60.6	+
7	HCA	35	7285 ± 1021***	26.7	+

[1]ethanol/water (7:3)

**Statistically significant difference when Log DPM compared to the corresponding vehicle control group ($p < 0.01$)

***Statistically significant difference when Log DPM was compared to the corresponding vehicle control group ($p = 0.001$).

XIII. Appendices

A. Appendix I—Irritation Scoring

Draize Definition for Scoring Dermal Irritation[1]

I. Dermal Observations

Erythema and Eschar Formation (Most severely affected area graded):

- No erythema 0
- Very slight erythema (barely perceptible) 1
- Well-defined erythema 2
- Moderate to severe erythema 3
- Severe erythema (beet redness) to slight eschar formation (injuries in depth) 4

Edema Formation (Most severely affected area graded):

- No edema 0
- Very slight edema (barely perceptible) 1
- Slight edema (edges of area well-defined by definite raising) 2
- Moderate edema (raised approximately 1mm) 3
- Severe edema (raised more than 1mm and extending beyond area of exposure) 4

[1]Draize, J.H. 1959. The Appraisal of Chemicals in Foods, Drugs and Cosmetics, pp. 36-45. Association of Food and Drug Officials of the United States, Austin, Texas.

B. Appendix II - Individual Animal Data

Individual Animal DPM and Body Weight Data

Animal #	Group	DPM	Log DPM	BWT1 (grams)	BWT6 (grams)	Change (grams)
1	1	262.97	2.420	24	24	0
2	1	200.59	2.302	26	25	-1
3	1	210.22	2.323	22	21	-1
4	1	300.29	2.478	25	25	0
5	1	390.19	2.591	23	22	-1
6	2	504.21	2.703	20	18	-2
7	2	226.73	2.356	26	25	-1
8	2	424.39	2.628	24	23	-1
9	2	335.80	2.526	25	25	0
10	2	949.65	2.978	22	22	0
11	3	315.12	2.498	24	24	0
12	3	409.51	2.612	24	24	0
13	3	162.05	2.210	23	23	0
14	3	234.02	2.369	26	26	0
15	3	203.36	2.308	25	25	0
16	4	1011.91	3.005	22	22	0
17	4	639.84	2.806	25	26	1
18	4	1476.39	3.169	22	23	1
19	4	876.82	2.943	25	26	1
20	4	263.35	2.421	23	24	1
21	5	10256.68	4.011	23	23	0
22	5	4042.73	3.607	25	26	1
23	5	7829.53	3.894	23	22	-1
24	5	6765.65	3.830	24	25	1
25	5	4506.40	3.654	24	25	1
26	6	16911.25	4.228	22	21	-1
27	6	13318.65	4.124	24	22	-2
28	6	14283.73	4.155	24	23	-1
29	6	19065.40	4.280	24	23	-1
30	6	19142.53	4.282	26	23	-3
31	7	6448.37	3.809	25	25	0
32	7	5769.44	3.761	25	25	0
33	7	4815.56	3.683	24	24	0
34	7	9422.40	3.974	25	24	-1
35	7	9968.65	3.999	24	23	-1

DPM=Disintegrations per minute
BWT1 = Body weight on Day 1
BWT6 = Body weight on Day 6
Change = Change in body weight (Day 6 – Day1)

C. Appendix III – Certificate of Analysis

Lubrizol

14:50:00 1/12/10

Certificate of Analysis

Send To:	Ship To:
W/ Ship:	

Customer Order Number	Order Number	Date Ship
Customer Product Code	Quantity Shipped UM	Vehicle ID
Product Description Ameenex 6169		Lot Number 9265

Test Description	Test Result	Minimum	Maximum
Date of Manufacture	10/15/09		
Recommended Retest Date	10/15/10		
AMINE EQUIVALENT	305.5	294.0	307.0
DMAPA, %	0.0		0.34
COLOR, VCS	1.0	0	2.0

Chain Length Distribution:

	%
C10	0.5
C12	56.8
C14	21.3
C16	9.9
C18	10.6
C18:1	0.8

It is hereby certified that the material indicated above has been tested in accordance with the testing parameters set forth in the product specification and, unless agreed otherwise, conforms in all respects to the specification.

common
Acid Value 252.75
W/KF = 0.04 / 0.03

Procter&Gamble Chemicals

4501172302

Produced by Twin Rivers Technologies Quincy LLC, Quincy, MA. 02169
Questions? Call Customer Service: 800-477-8899 or 513-626-6882

INSP. LOT 0100793255

C-1218DH FATTY ACID

CERTIFICATE OF ANALYSIS

LUBRIZOL ADVANCED MATERIALS
(Customer or Consignee)

(Dept. if applicable)
Quality Control Manager
(Attention)
3115 Propeller Drive
(Delivery Address)
Paso Robles, CA 93446
(City, State, Zip)
45,000 LB
(Approximate weight Lb. KG MT)
10245058
(P&G Material Code No.)

P&G Invoice No. 68669840
Customer P.O. No. 4501172302
Customer Code G436/
Vessel No. Dana
(Method TC TT Ship Iso)
P&G Lot No. TQ1432-9265
Delivery No. 80005948
Seals

Acid Value (mg KOH/g sample) (AOCS Te 1a-64)	254.5	(250-260)
Iodine Value (cg I/g sample) (AOCS Tg 1a-64)	0.9	(1.5 max)
Moisture, (Weight%) (AOCS Ca 2e-84)	0.02	(0.3 max)
Color (AOCS Td 2a-64)		
% transmittance (440 nm)	93	(90 min)
(550nm)	99	(95 min)
1963 Gardner (AOCS Td 1a-64)	1-	(1 max)
Saponification Value (AOCS Cd 3b-75)	256.0	(------)
Unsaponifiable, % weight	< 0.15	(------)

G.C. Chain Length Distribution (Weight %) (AOCS Ce 1e-91)

C10 and lower	0.5	(1 max)
C12	56.8	(57 max)
C14	21.3	(18-26)
C16	9.9	(5-12)
C18	10.6	(8-12)
C18:1 and higher	0.8	(1 max)
C20 and higher	0.0	(------)
Appearance @ melt	Pass	(Clear, clean liquid)

Nicholas Roberts — **Signature: Nicholas Roberts**
9/22/2009 — **Date**
Quincy — **Shipping Location**

This analysis is not to be constructed as a warranty. Customer is responsible to verify the lot and code numbers of product received with the numbers contained on this report and perform any other analyses necessary to determine suitability of the product described above for the use intended by the customer. No representations as to FDA regulted use are made for this product unless it is designated as meeting either USP, NF, Cosmetic grade or Food Grade Status. The foregoing statements are valid up to, but not beyond, delivery to our primary customer. Any subsequent handling, repackaging, storage, processing, ect. render these claims void and unsubstatiated by Procter & Gamble Chemicals.

D. Appendix IV– Protocol and Deviation

CALVERT
LABORATORIES, INC.

Study Protocol

TITLE: Local Lymph Node Assay

Calvert Study No.: 0787MP72.001

Testing Facility: Calvert Laboratories, Inc.
Scott Technology Park
130 Discovery Drive
Scott Township, PA 18447

Study Sponsor: Personal Care Products Council
1101 17th Street NW
Suite 300
Washington, DC 20036

16 November 2009

I. Table Of Contents

II. Introduction

A. Title

Local Lymph Node Assay

B. Objective

To determine if the test article will induce a hypersensitivity response in mice as measured by the proliferation of lymphocytes in the draining lymph nodes.

C. Regulatory Compliance

This study will be conducted in compliance with the Good Laboratory Practice Regulations 21 CFR Part 58 and in accordance with OECD Principles of Good Laboratory Practice as revised on 26th November, 1997 [C(97)186/Final], and in accordance with the appropriate Standard Operating Procedures (SOP) of Calvert.

D. Declaration of Intent

This study may be submitted to the United States Food and Drug Administration (FDA), an Organization for Economic Cooperation and Development (OECD) member country, and/or the Japanese Ministry of Health, Labor and Welfare (JMHLW).

E. Testing Guidelines

OECD 429, Skin Sensitization: Local Lymph Node Assay, 24 April 2002

OPPTS Health Effects Test Guidelines 870.2600 – Skin Sensitization, March 2003

EEC Testing Methods Annex V Part B, Skin Sensitization: Local Lymph Node Assay, B.42, 2004/73/EC L152, 2004

F. Calvert Study Number

0787MP72.001

G. Testing Facility

Calvert Laboratories, Inc. (Calvert)
Scott Technology Park
130 Discovery Drive
Scott Township PA 18447

H. Sponsor

Personal Care Products Council
1101 17th Street NW
Suite 300
Washington, DC 20036

I. Study Director

Joan M. Chapdelaine, Ph.D.
Calvert Laboratories, Inc. (Calvert)
Scott Technology Park
130 Discovery Drive
Scott Township PA 18447
Phone: (570) 585-2211
Fax: (570) 585-2383
E-mail: joan.chapdelaine@calvertlabs.com

J. Study Monitor

Linda Loretz, Ph.D. DABT
Personal Care Products Council
1101 17th Street NW
Suite 300
Washington, DC 20036
Phone: (202) 466-0493
Fax: (202) 331-1969
E-mail: loretzl@personalcarecouncil.org

K. Key Study Dates

Proposed Experimental Start Date:	Dec 2009
Proposed First Day of Dosing:	Dec 2009

Last Day of In-Life:	Dec 2009
Proposed Experimental Completion Date:	Dec 2009

III. Materials and Methods

A. Test Article

1. Test Article

Identification:	Ameenex 6169
Lot/Batch No.:	Will be documented in the raw data
Physical Description:	Pale yellow pasty solid
Composition/Purity Stability:	Good Laboratory Practice (GLP) regulations require that all test articles be appropriately characterized for identity, purity, composition and stability. Compliance necessitates documentation that characterization and stability testing have been performed. The sponsor will provide the Study Director with a certificate of analysis/results of stability testing and whether analysis/testing was conducted under GLP/GMP. If characterization/stability is not provided or was not performed under GLP/GMP, this fact will be reflected in the compliance statement.
Storage Conditions:	Room temperature not to exceed 49°C; well ventilated.

2. Vehicle

Identification:	Ethanol/water, 7:3 (v/v) neutralized to pH 6.0 with citric acid monohydrate
Lot/Batch No.:	Will be documented in the raw data

Physical Description: Will be documented in the raw data

Storage Conditions: Will be documented in the raw data

3. *Positive Control*

Identification: Hexylcinnamaldehyde (HCA)

Lot/Batch No.: Will be documented in the raw data

Physical Description: Will be documented in the raw data

Storage Conditions: Will be documented in the raw data

4. *Dose Preparation*

The test article formulations will be prepared on each day of dosing by dissolving the appropriate amount of test article in vehicle using a volumetric flask for each concentration. The positive control will be prepared as a 35% solution in the vehicle.

5. *Dose Formulation Analysis*

Samples of the test article dosing solutions will not be collected for analysis. Good Laboratory Practice (GLP) regulations require that all formulated test articles be appropriately verified for concentration, homogeneity, and stability. Compliance necessitates documentation that verification has been done. Since verification will not be performed, this fact will be reflected in the compliance statement in the report.

6. *Accountability and Disposition*

Unused test article will be returned to the Sponsor or designee at the termination of this study or, if necessary, retained for use on related future studies. The Sponsor will be notified in advance of shipping and a transmittal letter will accompany the shipment. The material will be packed in a suitable container to maintain the conditions specified by the Sponsor during transit plus an adequate margin of safety to account for any possible transit delays. If returned to the Sponsor, unused material will be returned to:

Linda Loretz, Ph.D. DABT
Personal Care Products Council
1101 17th Street NW
Suite 300
Washington, DC 20036
Phone: (202) 466-0493
Fax: (202) 331-1969
E-mail: loretzl@personalcarecouncil.org

B. Test System (Animals and Animal Care)

1. Description

Species:	Mouse
Strain/Substrain:	CBA/J or CBA/Ca
Total Number:	Preliminary Irritation Screen: 2/dose level Main Study: 35
Gender:	Female
Age Range:	8-12 weeks at start of dosing; records of dates of birth for animals used in this study will be retained in the Calvert archives.
Body Weight Range:	18-25 grams at the outset (Day 1) of the study.
Animal Source:	Jackson Laboratories, Bar Harbor, ME or Harlan Indianapolis, IN
Experimental History:	Purpose-bred and experimentally naïve at the outset of the study.
Identification:	Tail marked with an indelible marker.

2. Rationale for Choice of Species and Number of Animals

The mouse is the standard species used in the local lymph node assay (LLNA), which has been developed as an alternative to the Guinea Pig sensitization tests. The LLNA is a refinement in terms of reducing or eliminating distress in the animals compared to the Guinea Pig tests. The

number used is the minimum number recommended. (NIH Publication No. 99-4494).

3. *Husbandry*

Housing:	Animals will be group housed (5 per cage) upon receipt in compliance with National Research Council "Guide for the Care and Use of Laboratory Animals". The room in which the animals will be kept will be documented in the study records. No other species will be kept in the same room.
Lighting:	12 hours light/12 hours dark.
Room Temperature:	19 to 25°C
Relative Humidity:	30-70%
Food:	All animals will have access to Harlan Teklad Rodent Diet (certified) or equivalent *ad libitum*, unless otherwise specified. The lot number(s) and specifications of each lot used are archived at Calvert. No contaminants are known to be present in the certified diet at levels that would be expected to interfere with the results of this study. Analysis of the diet was limited to that performed by the manufacturer, records of which will be maintained in the Calvert archives.
Water:	Tap water will be available *ad libitum,* to each animal via water bottles. The water is routinely analyzed for contaminants as per Calvert SOP's. No contaminants are known to be present in the water at levels that would be expected to interfere with the results of this study. Results of the water analysis will be maintained in the Calvert archives.

Acclimation: Study animals will be acclimated to their housing for a minimum of 5 days prior to their first day of dosing.

4. Prestudy Health Screen and Selection Criteria

All animals received for this study will be assessed as to their general health by a member of the technical staff or other authorized personnel. During the acclimation period, each animal will be observed at least once daily for any abnormalities or for the development of infectious disease. All animals will be examined to ensure they have no observable skin lesions prior to the start of the study. Only animals that were determined to be suitable for use will be assigned to this study. Any animals considered unacceptable for use in this study will be replaced with animals of similar age and weight from the same vendor.

5. Assignment to Study Groups

Mice will be selected by body weight and apparent good health. Mice will be randomly assigned to study groups.

6. Humane Care of Animals

Treatment of animals will be in accordance with the study protocol and also in accordance with Calvert SOP's which adhere to the regulations outlined in the USDA Animal Welfare Act (9 CFR Parts 1, 2 and 3) and the conditions specified in the Guide for the Care and Use of Laboratory Animals (ILAR publication, 1996, National Academy Press). The Calvert Institutional Animal Care and Use Committee (IACUC) will approve the study protocol prior to finalization to insure compliance with acceptable standard animal welfare and humane care.

No alternative test systems exist which have been adequately validated to permit replacement of the use of live animals in this study. Every effort has been made to obtain the maximum amount of information while reducing to a minimum the number of animals required for this study. The assessment of pain and distress in study animals and the use or non-use of pain alleviating medications will be in accordance with Standard Operating Procedure VET-19, Criteria for Assessing Pain and

Distress in Laboratory Animals. The study will be terminated in part or whole for humane reasons if unnecessary pain occurs. To the best of our knowledge, this study is not unnecessary or duplicative.

C. Test Article Administration

1. Preliminary Study (Irritation Screen)

Each test article concentration and vehicle will be applied to the ears of two mice. Mice will be treated with the test article on the dorsal surface of both ears (25 µl/ear), once per day for three consecutive days using a micropipette. Concentrations used will be 0.1, 0.5, 1, 2.5, and 5%. Animals will be scored daily approximately 24 hour after each dose for irritation using the Draize scoring system (Appendix 1). If no irritation is observed over 6 days, these doses will be used for the main study. If irritation is observed, further concentrations may be tested until a dose is found where there is little or no irritation.

2. Group Assignments and Dose Levels for Main Study

Group	Treatment	Dose*	Number of Animals
1	Vehicle	-	5
2	Test Article	0.1%	5
3	Test Article	0.5%	5
4	Test Article	1.0%	5
5	Test Article	2.5%	5
6	Test Article	5.0%	5
7	HCA	35%	5

*Doses will be confirmed based on irritation screen

3. Dosing

Route: Topically on the dorsal surface of both ears

Frequency: Once daily for 3 consecutive days (Days 1-3). The timing of dose administration will remain consistent (± 2 hours) during the dosing phase.

Procedure: A volume of 25 µl/ear will be applied using a micro pipette.

4. Justification for Route, Dose Levels and Dosing Schedule

The dermal route was selected as this is the route required for this model of hypersensitivity.

The recommended doses for this assay are selected from the concentration series 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5% etc. Three consecutive concentrations should be chosen, the top one being the highest level that can be achieved while avoiding systemic toxicity and excessive local irritation. The doses for this study were selected by the Sponsor based on results with other similar material.

The frequency of dosing is the convention for this type of study.

D. In-Life Observations and Measurements

1. Mortality/Morbidity

Frequency: Daily on Days 1 to 6.

Each animal observed for evidence of death or impending death (as per Calvert SOP VET-14). A gross necropsy will be performed on any animal that dies during the study.

2. Clinical Observations

Frequency: Observations will be performed prior to dose administration and following dose administration. Clinical observations will also be performed once daily on Days 4-6. Particular attention will be given to the application sites. Any significant alterations to the application sites, and the general appearance of the pinnae, including build up of test article, will be recorded.

3. Dermal Irritation

Frequency: Animals will be examined daily for signs of erythema and edema. Irritation will be scored and recorded using the Draize scoring system

(Appendix 1). On Days 1-3 scoring will be performed prior to dosing.

4. Body Weight

Frequency: Animals will be weighed on Days 1 and 6.

E. Method of Performance

Mice will be treated on the dorsal surface of both ears, once per day on Days 1, 2, and 3. Approximately 24±2 hours between applications of test article will be maintained. On Day 6 the mice will be injected i.v. with 20 µCi of ^{3}H-thymidine in 250 µl of sterile saline. Five hours later the mice will be euthanized with CO_2 asphyxiation and the draining auricular lymph nodes removed. At removal, the number of nodes collected per animal will be recorded, and the nodes will be examined for size/appearance and the data recorded. Any unexpected observations will be noted in study records. A single cell suspension will be prepared from the lymph nodes of each mouse. Cells will be washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloroacetic acid (TCA) overnight at 2-8°C. The pellets will be recovered by centrifugation and resuspended in 1 ml of TCA and transferred to a vial containing scintillation fluid. An additional 1ml of TCA will be used to rinse the tube, and it will also be transferred to the scintillation fluid. Incorporation of ^{3}H-thymidine will be measured in a ß-scintillation counter.

F. Terminal Procedures

1. Termination

a) Scheduled Sacrifice

All surviving animals will be euthanized by CO_2 asphyxiation.

b) Unscheduled Sacrifice

Any animals sacrificed for humane reasons will be euthanized by CO_2 asphyxiation.

IV. Records and Reports

A. Data Collection and Analysis

Data will be manually collected except for the data generated by the scintillation counter (Beckman LS 6000 SC). The mean DPM for each group will be evaluated using SYSTAT version 9.01, developed by SPSS, Inc. Increases in ^{3}H-thymidine incorporation relative to the vehicle-treated control will be derived for each group and recorded as stimulation indices (SI). The criterion for a positive response is that one or more concentrations of a test article elicits a 3-fold or greater increase in isotope incorporation relative to the vehicle control.

The mean body weights and changes in body weight will also be calculated and evaluated using SYSTAT.

Individual DPM values will be analyzed by log transformation (base 10) of the data. The evaluation of the equality of means for the DPM and body weight data will be made by a one-way analysis of variance using the F distribution to assess statistical significance. If statistically significant differences between the means are found, a Dunnett's test will be used to determine the degree of significance from the control means.

If the data indicates that the test article is positive, the EC3 will be calculated using the formula:

$$EC3 = c+[(3-d)/(b-d)](a-c)$$

where the data points lying immediately above and below the SI value of 3 have the co-ordinates (a,b) and (c,d) respectively.

For test articles for which the lowest concentration tested results in a stimulation index of greater than 3, an EC3 value will be extrapolated from the two lowest doses utilized (C. A. Ryan et al., 2007). The extrapolated EC3 value will be calculated by log-linear interpolation between these two points on a plane where the x-axis represents the dose level and the y-axis represents the SI. The point with the higher SI is denoted (a,b) and the point with the lower SI is denoted (c,d). The formula for the extrapolated EC3 value is as follows:

$$EC3 = 2^{(\log2(c) + (3-d)/(b-d)*(\log2(a) - \log2(c)))}$$

B. Storage of Records

Test article preparation, test article tracking, in-life data, protocol, protocol amendments (if applicable), and the original final report generated as a result of this study will be archived at Calvert, 105 Edella Road, Suite 100, Clarks Summit, PA 18411. After 2 years, the Sponsor will be contacted to determine final disposition of all study materials.

One bound, one unbound copy of the final report and a scanned PDF of the final report will be provided to the Sponsor.

Six months following the submission of the audited draft report, if there are no client comments generated by the Sponsor and/or Study Monitor, the Sponsor/Study Monitor will be notified and the report will be finalized and archived according to the terms stated in the protocol.

V. Miscellaneous

A. Confidentiality Statement

The information contained herein is for the personal use of the intended recipient(s).

B. References

1. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. NIH Publication No. 99-4494, Feb. 1999.

2. C. A. Ryan, J. G. Chaney, P. S. Kern, R. J. Dearman, I. Kimber, D. A. Basketter, and G. F. Gerberick. Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency. Cutaneous and Ocular Toxicology, 26:135-145, 2007.

VI. Appendix I

Draize Definition for Scoring Dermal Irritation[1]

I. Dermal Observations

Erythema and Eschar Formation (Most severely affected area graded):

- No erythema....0
- Very slight erythema (barely perceptible)....1
- Well-defined erythema....2
- Moderate to severe erythema....3
- Severe erythema (beet redness) to slight eschar formation (injuries in depth)....4

Edema Formation (Most severely affected area graded):

- No edema....0
- Very slight edema (barely perceptible)....1
- Slight edema (edges of area well-defined by definite raising)....2
- Moderate edema (raised approximately 1mm)....3
- Severe edema (raised more than 1mm and extending beyond area of exposure)....4

[1]Draize, J.H. 1959. The Appraisal of Chemicals in Foods, Drugs and Cosmetics, pp. 36-45. Association of Food and Drug Officials of the United States, Austin, Texas.

VII. Protocol Approval Signatures

Joan M Chapdelaine
Study Director's Signature
Joan M. Chapdelaine, Ph.D.
Calvert Laboratories, Inc.

4 Jan 2010
Date

Steven D. Slone
Scientific Management
Calvert Laboratories, Inc.

4 JAN 2010
Date

Terri L. Lyon
IACUC
Calvert Laboratories, Inc.

4 Jan 2010
Date

Linda Loretz
Linda Loretz, Ph.D., DABT
Sponsor's Approval Signature

12/23/09
Date

Protocol Deviation

1. The test article used on this study was an off-white solid not a pale yellow pasty solid as described in the study protocol. As this was the test article provided by the Sponsor and since the Certificate of Analysis confirms that this was the correct test article, this deviation is not considered to have any impact on the outcome of the study.
2. Body weights of 20-26 grams for the mice were recorded at the outset (Day 1) of the study. Three mice in the preliminary screen and 4 mice in the main study weighed 26 grams. All other animals were within the protocol-specified body weight range of 18-25 grams at the outset (Day 1) of the study. Because animals were within the protocol-specified age range and the deviation was minor, this deviation did not adversely affect the outcome of the study.
3. The room temperature was out of the protocol specified range (19-25°C) on several occasions during the study with a high of 26.7°C. The relative humidity was out of the protocol specified range (30-70%) on several occasions during the study with a low of 12%. The mice were housed in micro-isolator cages providing the animals with a micro-environment and the animals appeared healthy. Therefore, these protocol deviations had no impact on the quality, integrity or validity of the study.

Personal Care Products Council
Committed to Safety,
Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 24, 2010

SUBJECT: Risk Assessment Regarding Amidoamines in Amidopropyl Betaine Ingredients

Personal Care Product Council Task Force. 2010. Amidoamine skin sensitization risk assessment.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Amidoamine Skin Sensitization Quantitative Risk Assessment

Representing Views of the Personal Care Products Council Task Force
24 March 2010

Summary: The Personal Care Products Council Task Force on Amidoamine Skin Sensitization recommends that the Cosmetic Ingredient Review Expert Panel evaluation of Cocamidopropyl Betaine (CAPB) consider a quantitative risk assessment (QRA) approach for assessing the skin sensitization potential of the impurity amidoamine to establish a safe level of amidoamine in CAPB, based upon induction of skin sensitisation. This approach has been well accepted for fragrance ingredients and is equally applicable to cosmetic ingredients and impurities (Api et al., 2008).

The Task Force has reviewed all of the existing sensitization data on amidoamine, including murine local lymph node assay (LLNA), Guinea-pig maximisation test (GPMT) and human repeat insult patch test (HRIPT) on amidoamine of varying fatty acid chain length. Amidoamine is considered to be a skin sensitizer of moderate potency. The test data is summarized in this document.

No expected sensitization induction levels (NESIL) values have been calculated using both human skin sensitization test data and murine LLNA data. Use of human data to assign a NESIL is customary (ECETOC Monograph No. 32, Use of Human Data in Hazard Classification for Irritation and Sensitization, 2002 and summarized in Kimber et al., 2001), and results in a NESIL of 180 $\mu g/cm^2$. The newly generated murine LLNA data was also used to assign a NESIL, based on the relevance of the test material (predominant amidoamine species C12/C14). Using these data resulted in a NESIL of 245 $\mu g/cm^2$.

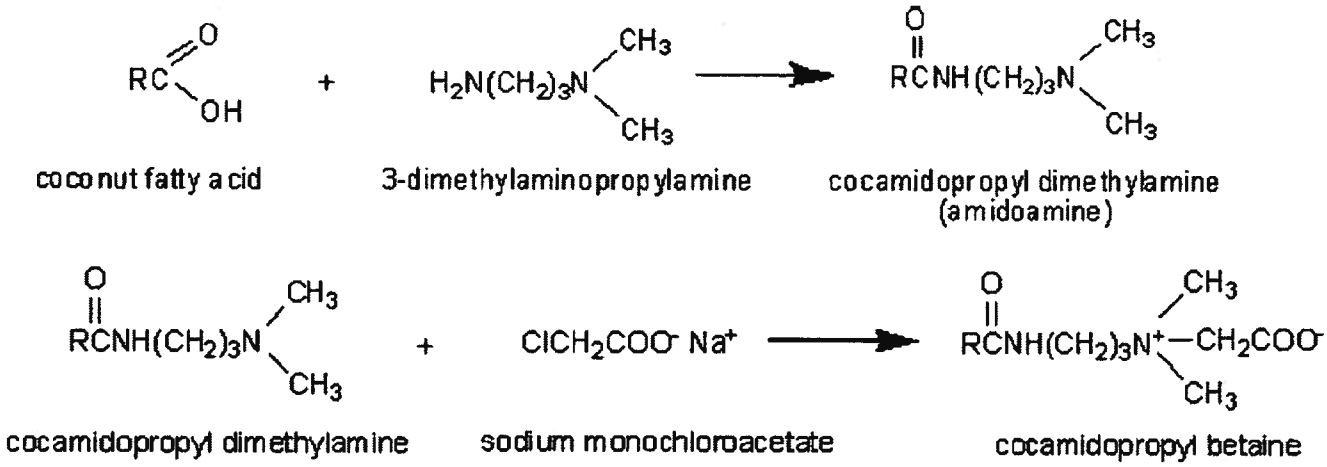
Also included in this submission is an example risk assessment to assess the potential for amidoamine impurity in CAPB to induce skin sensitization from normal product usage. The assessment is based upon use of CAPB containing amidoamine in cosmetic products using the following conservative exposure assumptions:

- the suggested limit of 1.5% amidoamine in CAPB as compared to the level typically present (≤ 0.5%)
- use of 90th percentile product exposure data
- use of the highest dermal exposure in a cosmetic product based on the 2008 Tentative CIR report for CAPB (i.e., 20% CAPB in a skin cleansing liquid applied at 0.15 mg/cm^2 per day)
- assuming 100% dermal penetration of amidoamine
- the human patch testing was done with completely occlusive patches, while the typical cosmetic exposures are rinse-off applications

The outcome of this risk assessment is favorable when compared to the NESIL values calculated using either the human HRIPT data or the murine LLNA data. As such it is unlikely that use of up to 20% CAPB containing up to 1.5% amidoamine impurity in a facial wash product will induce skin sensitisation. This is the product type which represents the highest consumer exposure to CAPB from personal care products, thereby supporting the safe incorporation of CAPB containing up to a maximum of 1.5% amidoamine in personal care products.

The Task Force greatly appreciates the opportunity to provide input for the present evaluation and welcomes the opportunity to provide further input if requested.

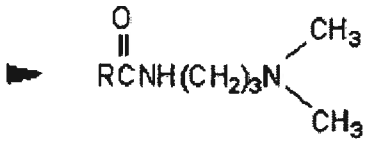
Background: Cocamidopropyl betaine (CAS No. 61789-40-0) is a surfactant widely used in cosmetics and is currently under review by the Cosmetic Ingredient Review Expert Panel. The potential for skin sensitization is included in this review and has been previously called into question in the literature (Fowler et al., 1997; Hunter and Fowler, 1998; Fartash et al., 1999). Two impurities that have been associated with sensitization are 3-dimethylaminopropylamine (DMAPA, CAS 109-55-7) and cocamidopropyl dimethylamine, more commonly referred to as amidoamine. The results do not rule out the possibility that cocamidopropyl betaine itself may be an allergen in rare cases (Fowler et al., 1997; Li, 2008). The diagram below demonstrates the method of manufacture of cocamidopropyl betaine, with R representing the coconut fatty acid chain that varies between C-8 and C-18 (per the Tentative Amended Safety Assessment of the Cosmetic Ingredient Review Expert Panel for Cocamidopropyl Betaine, September 23, 2008). The present document provides a quantitative risk assessment for the skin sensitization potential from amidoamine.



Method of Manufacture

Amidoamine characterization:

Amidoamine is described by the 2008 Tentative Review of Cocamidopropyl betaine as the compound demonstrated below with the R group representing the coconut fatty acid chain that varies between C-8 and C-18 (this includes the carbonyl carbon). Industry survey results demonstrated amidoamine to be present in cocamidopropyl betaine (CAPB) at between 0.5% and 5%, with 0.5% typical and a suggested maximum level of 1.5%.



cocamidopropyl dimethylamine
(amidoamine)

The Tentative Review lists the distribution of fatty acids in cosmetic grade cocamidopropyl betaine (CAPB), which presumably represents the same fatty acid profile of amidoamine.

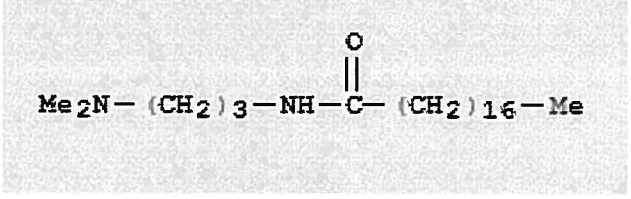
Fatty Acids

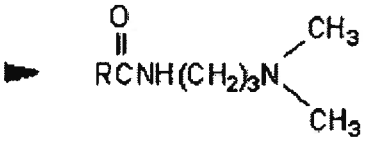
C_8	5.6 - 6.0%
C_{10}	5.4 - 5.7%
C_{12}	53.1 - 53.2%
C_{14}	16.1 - 17.4%
C_{16}	8.1 - 8.3%
C_{18}	10.0 - 10.2%

Human sensitization data on amidoamine:

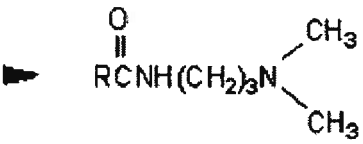
Two human repeat insult patch test (HRIPT) studies have been conducted to assess the skin sensitization potential of amidoamine. One study was conducted with the amidoamine stearylamidopropyl dimethylamine in mineral oil (Study 1) and one study was conducted with a mixture of 2 amidoamines, stearylamidopropyl dimethylamine and palmitylamidopropyl dimethylamine in a liquid fabric softener matrix diluted in distilled water. The summaries of these studies are provided below.

Study 1 – Amidoamine 1

Test Substance	Stearylamidopropyl dimethylamine (CAS No. 7651-02-7)  Equivalent to :

	 cocamidopropyl dimethylamine (amidoamine) with R = 17.
Test substance remarks	Amidoamine is one of the impurities of CAPB. The test substance (stearylamidopropyl dimethylamine) is an amidoamine containing an 18-carbon fatty acid chain (this includes the carbonyl carbon in the amide).
Method	Human Repeat Insult Patch Test (HRIPT)
Test type	Topical induction and topical challenge using completely occlusive patches with 0.3 mL of 0.25% w/v of stearylamidopropyl dimethylamine in mineral oil applied to 4 cm^2 occlusive patch for a topical dose of 187.5 $\mu g/cm^2$.
Year	1984
Test Conditions	
Species	Human, 120 subjects enrolled with 112 subjects completing.
Sex	78 females and 34 males completing all phases of the study.
Route of administration	Topical on the dorsal surface of upper arm under occlusive patch for 24 hours at induction (3 patches per week for 3 consecutive weeks); two week rest period; topical patch for 24 hours at challenge (original site and alternate naïve site).
Vehicle	Mineral oil.
Grading system	0 = no visible reaction 1 = mild erythema, faint pink to definite pink 2 = moderate erythema, definite pink to red 3 = strong erythema, beet red 4 = severe erythema with edema, papules, and vesicles 5 = bullous reaction E = edema or papules During induction, the site is graded at 48 hours after patch application (72 hours after application of Friday patches). During challenge, the site is evaluated at 48 and 96 hours after patch application.
Results	
	Several incidences of slight to moderate irritation was observed during induction, but were transient and therefore indicative of primary irritation. During challenge, five subjects had mild irritation responses (scores of 1 or 1E) at the 24-hour reading. These were indicative of primary irritation and not sensitization since the responses dissipated by the 96 hour challenge reading.
Conclusions	This is the key human study to define the NESIL. No reactions were evident which were considered to be a contact hypersensitivity response. By convention, the NESIL is rounded down to 180 $\mu g/cm^2$.

Study 2 – Amidoamine 2

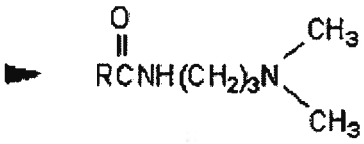
Test Substance	Stearyl/palmitylamidopropyl dimethylamine in a liquid fabric softener formula.  cocamidopropyl dimethylamine (amidoamine) with R = 15 and 17 carbons.
Test substance remarks	Amidoamine is one of the impurities of CAPB. The test substance (stearyl/palmitylamidopropyl dimethylamine) are amidoamines containing a 16- and 18-carbon fatty acid chains (this includes the carbonyl carbon in the amide).
Method	Human Repeat Insult Patch Test (HRIPT)
Test type	Topical induction and topical challenge using completely occlusive patches with 0.5 mL of a 4% test material containing 0.6% stearyl/palmitylamidopropyl dimethylamine in distilled water applied to 0.75 in^2 occlusive patch for a topical dose of stearyl/palmitylamidopropyl dimethylamine of 25 $\mu g/cm^2$.
Year	1981
Test Conditions	
Species	Human, 85 subjects enrolled with 77 subjects completing.
Sex	81 females and 4 males enrolled.
Route of administration	Topical on the arm under occlusive patch for 24 hours at induction (3 patches per week for 3 consecutive weeks); two week rest period; topical patch for 24 hours at challenge (original site and alternate naïve site).
Vehicle	Distilled water
Grading system	0 = no visible reaction 1 = mild erythema, faint pink to definite pink 2 = moderate erythema, definite pink to red 3 = strong erythema, beet red 4 = severe erythema with edema, papules, and vesicles 5 = bullous reaction E = edema or papules During induction, the site is graded at 48 hours after patch application (72 hours after application of Friday patches). During challenge, the site is evaluated at 48 and 96 hours after patch application.
Results	
	Several subjects experienced mild to moderate erythema during induction patching. Eight subjects reacted at challenge with mild to moderate erythema and/or edema and 7 were re-challenged (with 4% and 0.4% concentrations of the formula, equal to 25 $\mu g/cm^2$ and 2.5 $\mu g/cm^2$ of stearyl/palmitylamidopropyl dimethylamine). There was evidence of mild erythema in 4 of the 7 subjects rechallenged at the higher concentration and none at the lower concentration. There was no evidence of sensitization during re-challenge. These subjects then

	participated in a 4-week use test with the test material formulation (liquid fabric softener) and no adverse events were observed or reported.
Conclusions	No reactions were evident which were considered to be a contact hypersensitivity response.

Rodent sensitization data on amidoamine:

A mouse Local Lymph Node Assay (LLNA) and two Magnusson and Kligman Guinea-pig maximisation studies have been conducted to assess the skin sensitization potential of amidoamine representative of the impurity found in CAPB. In addition, two guinea pig Buehler studies were conducted on amidoamines. The summaries of these studies are provided below.

Study 3 – Amidoamine 1

Test Substance	Cocamidopropyl dimethylamine  cocamidopropyl dimethylamine (amidoamine) Fatty Acids % ≤ C10 0.5; C12 56.8; C14 21.3; C16 9.9; C18 10.6; ≥ C20 0.0
Test substance remarks	Amidoamine representative of the impurity in CAPB (Ameenex 6169 Lot 9265 from Lubrizol).
Method	Local Lymph Node Assay (OECD 429)
Test type	Topical application to dorsal surface of ears once per day for 3 days. Hypersensitivity assessed by measurement of lymphocyte proliferation in the draining lymph nodes.
GLP	Yes
Year	2010
Test Conditions	
Species	Mouse
Strain	CBA/J
Sex	Female
Number of animals per dose	2/dose for preliminary irritation study (0.1, 0.5, 1, 2.5, and 5%) 5/dose for sensitization assessment (0, 0.1, 0.5, 1, 2.5, and 5%) 5/dose for sensitization assessment positive control, 35% hexylcinnamaldehyde (HCA).
Vehicle	Ethanol/water, 7:3 (v/v) neutralized to pH 6.0 with citric acid monohydrate.
Route of administration	Topical to dorsal surface of both ears once per day for 3 days. On Day 6, the mice were injected iv with 20 µCi of ^{3}H-thymidine in sterile saline. Five hours later, the mice were euthanized and the draining auricular lymph nodes removed, processed and assessed for lymphocyte proliferation.
Preliminary studies (irritation screen)	0.1%, 0.5%, 1.0%, 2.5%, and 5.0% (w/v) was applied topically to the dorsal surface of both ears once per day for three consecutive days and mice were observed for erythema and edema for six days. Result: On Days 3, 4, and 5 both animals treated with the test article at 5% had very slight erythema and edema. On Day 6, the 5% group had

	very slight erythema and slight edema. No erythema or edema was noted at any time in the 0.1%, 0.5%, 1.0%, and 2.5% dose groups.
Grading system used for irritation screen	Erythema and eschar formation (most severely affected area): No erythema 0 Very slight erythema (barely perceptible) 1 Well-defined erythema 2 Moderate to severe erythema 3 Severe erythema (beet redness) to slight eschar formation (injuries in depth) 4 Edema formation (most severely affected area): No edema 0 Very slight edema (barely perceptible) 1 Slight edema (edges of area well-defined by definite raising) 2 Moderate edema (raised ~ 1mm) 3 Severe edema (raised > 1mm and extending beyond area of exposure) 4
Results	
Main study (sensitization)	No mortality and all animals appeared clinically normal throughout the study. The test article at 2.5% demonstrated very slight erythema on Day 3 and very slight erythema and very slight edema on Days 4-6. Four of the five mice treated with 5% test article had very slight erythema and very slight edema on Day 2. Mice treated with 5% test article had well defined erythema and slight edema on Days 3-6. Group — Stimulation Index Vehicle — --- 0.1% test material — 1.8 0.5% test material — 1.0 1.0% test material — 3.1 2.5% test material — 24.5 5.0% test material — 60.6 Positive control (HCA) — 26.7 The EC3 for amidoamine was calculated to be 0.98%.
Grading system used for the main study	Erythema and eschar formation (most severely affected area): No erythema 0 Very slight erythema (barely perceptible) 1 Well-defined erythema 2 Moderate to severe erythema 3 Severe erythema (beet redness) to slight eschar formation (injuries in depth) 4 Edema formation (most severely affected area): No edema 0 Very slight edema (barely perceptible) 1 Slight edema (edges of area well-defined by definite raising) 2 Moderate edema (raised ~ 1mm) 3

	Severe edema (raised > 1mm and extending beyond area of exposure) 4
Conclusions	This is the key animal study to define the NESIL. A test material is considered to have skin sensitizing activity if, at one or more concentrations, it induces a 3-fold or greater increase in proliferative activity relative to concurrent vehicle treated control. Amidoamine is considered to have skin sensitizing activity and the EC3 was calculated to be 0.98%. This corresponds to a dose of 245 µg/cm^2 (based on a volume of 25 µL of a 0.98% solution applied to a mouse ear of 1 cm^2).

Study 4 – Amidoamine 2

Test Substance	Amidoamine
Test substance remarks	Amidoamine is one of the impurities of CAPB. The test substance (Amidoamine) was isolated from a sample of CAPB. The associated analytic report for the test item associated with this study states the test item is 100% free amine, and contains no residual betaine.
Method	Skin sensitization (Maximisation test) OECD 406
Test type	Intra-dermal and topical induction and topical challenge
GLP	No
Year	1987
Test Conditions	
Species	Guinea pigs
Strain	Albino Dunkin/Hartley
Sex	Preliminary tests - females Main study - Animals 1-6 (inclusive) female and animals 7-10 (inclusive) male. Treated controls (4) male.
Number of animals per dose	4 – preliminary study 10 – main study treatment group 4 – main study control
Route of administration	Intra-dermal and topical at induction; topical at challenge
Preliminary studies: A. Topical application (induction and challenge) B. Intradermal application (induction)	 0.5%, 1% and 2.5%, 7.5%, 15% and 30% of test substance was applied topically under occlusion to the shaved flank of the animals and left in place for 24-hours. Reactions were observed at 24- and 48-hours after removal of patch. Result: Irritation observed at 2.5% was considered insufficient and 7.5% too high for the purposes of this study. 5% was selected as minimally irritating and chosen for topical induction. 0.5% was chosen as the highest non-irritant concentration for challenge. Test substance was administered intradermally at 0.1%, 0.25%, 0.5% and 1% on the clipped flanks of the animals. Reactions were examined 24-hours later. Result: Each concentration gave an irritant response in the animals. 0.1% considered as minimally irritating and chosen for intradermal induction.
Main study: Induction	**Day 0:** Treatment group

	• Two 0.1ml injections of 50% FCA in vehicle • Two 0.1ml of test substance at 0.1% • Two 0.1ml injections of test substance in vehicle mixed 50/50 with FCA such that the final concentration of test substance injected is 0.1%. **Day 7:** Treatment group On the same test site as the Intradermal induction, 0.2-0.3ml test item (at 5%) was applied to a filter paper 2x4cm and applied to shaved skin. The patch was held in place for 48-hours by adhesive plaster.
Induction vehicle	DOBS/Saline (injection), Acetone/PEG 400 (topical)
Challenge 1 Challenge 2 Challenge 3	**Day 21**: An 8mm diameter filter paper patch in an 11mm aluminium patch test cup was saturated with the test substance (0.5%) and applied to the clipped and shaved flank. The patch was held in place for 24hours by an adhesive plaster wound around the trunk. The treatment sites were examined for evidence of skin sensitization 24- and 48-hours after the removal of the patch. **Day 28**: One week after the first (or subsequent challenge), a second challenge was made on the opposite flank, as for the first challenge. **Day 35**: One week after challenge 2, a third challenge was made.
Challenge vehicle	Acetone/PEG 400
Grading system	Dermal reactions graded for erythema and oedema by blind reading according to grading scale: No reaction 0 Mild erythema or oedema 1 Moderate and diffuse erythema or oedema 2 Severe erythema and oedema 3
Method remarks	The method is generally in line with OECD guideline 406 (skin sensitization). Minor deviations: Use of 4 animals in control group. Topical induction patch was a 2 x 4 cm occluded filter paper, whilst challenge was an 8 mm diameter filter paper, occluded using a Finn chamber.
Results	
Challenge 1 Challenge 2 Challenge 3	7 animals elicited a reaction with a score >/= 0.5 24-hours after removal of the occluded patch. After 48 hours, 6 animals elicited a reaction, score >/= 0.5. Animal 10 elicited a reaction scored at 0.5 at 24 hours, response resolved (0) at 48 hours post patch removal. All other animals remained the same in terms of their score. The highest score for reactions observed was 2 (3 /10 animals). Overall conclusion 6/10 positive responses at challenge 1. 7 animals elicited a reaction which was scored >/= 0.5 24 hours after patch removal. Scores were consistent at 48 hour reading. Stronger reactions were observed at challenge 2 compared with challenge 1 (5/10 animals were scored 2 at challenge 2) All 10 animals elicited a reaction, with a score of >/= 1 24 hours after patch removal. Scores were generally consistent at the 48 hour reading (exception of animal 4, which was scored at 2 at 24-hours and then 1-2 at 48-hours). Stronger reactions were seen at challenge 3 compared with challenge 2 (8/10 animals were scored at 2 at challenge 3)

Conclusions	6/10 animals elicited a positive response at challenge 1 (increasing at subsequent challenge). Induction concentration (topical 5%). The study indicates Amidoamine is a moderate sensitiser.
Reliability	Klimisch grade: 2 – fairly reliable (see method remarks above)

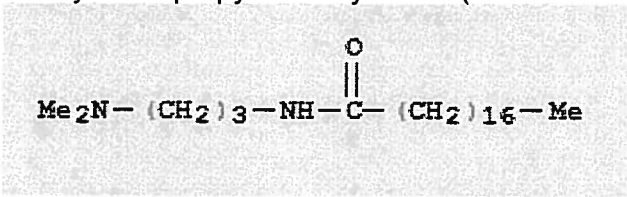
Study 5 – Amidoamine 3

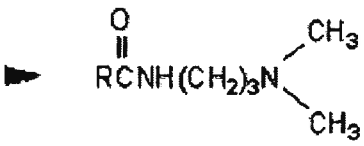
Test Substance	Amidoamine
Test substance remarks	Amidoamine purified from CAPB.
Method	Skin sensitization (Maximisation test) OECD 406
Test type	Intra-dermal induction and topical application at challenge
GLP	No
Year	1987
Test Conditions	
Species	Guinea pigs
Strain	Albino Dunkin/Hartley
Sex	Intradermal injection prelim– males used Occluded patch prelim – females used Main study – Animals 1-6 (inclusive) females, animals 7-10 (inclusive) male. Treated Control (4) female
Number of animals per dose	4 – preliminary study 10 – main study treatment group 4 – main study control
Route of administration	Intra-dermal and topical at induction; topical at challenge
Preliminary studies A. Topical application (induction & challenge)	30%, 15%, 7.5%, 0.5%, 1% and 2.5% was applied topically under occlusion to the shaved flank of the animals and left in place for 24-hours. Reactions were observed at 24- and 48-hours after removal of patch. Result: 1% was identified as the dose providing minimal irritation and was selected as the dose for induction. 0.5% was identified as the lowest does not eliciting irritation and was selected for challenge.
B. Intra-dermal application (induction)	Test substance was administered intradermally at 0.01%, 0.025%, 0.05%, 0.1%, 0.25%, 0.5% and 1% on the clipped flanks of the animals. Reactions were examined 24-hours later. Result: 0.025% considered minimally irritating and chosen for intra-dermal induction.
Induction (intradermal injection) Induction (topical application)	**Day 0:** Treatment group • Two 0.1ml injections of 50% FCA in vehicle • Two 0.1ml of test substance (0.025%) at the chosen concentration. • Two 0.1ml injections of test substance in vehicle mixed 50/50 with FCA such that the final concentration of test substance injected was 0.025% **Day 7:** Treatment group On the same test site as the Intradermal induction, 0.2-0.3ml test item (at 1%) was applied to a filter paper 2x4cm and applied to shaved skin. The patch was held in place for 48-hours by adhesive plaster.
Induction vehicle	DOBS/Saline (injection), Acetone/PEG400 (topical)

Challenge 1 Challenge 2	**Day 21**: Guinea pigs were challenged on the clipped and shaved flank by an occluded patch. An 8mm diameter filter paper patch in an 11mm aluminium patch test cup was saturated with the test substance (0.5%) and applied to the clipped and shaved flank. The patch was held in place for 24 hours by an adhesive plaster wound around the trunk. The treatment sites were examined for evidence of skin sensitization 24- and 48-hours after the removal of the patch. **Day 28:** One week after the first challenge, a second was made by applying test item (0.5%) as described above to the opposite flank, as for the first challenge.
Challenge vehicle	Acetone/PEG400
Grading system used	Dermal reactions graded for erythema and oedema by blind reading according to grading scale: No reaction 0 Mild erythema or oedema 1 Moderate and diffuse erythema or oedema 2 Severe erythema and oedema 3
Method remarks	The method is generally in line with OECD guideline 406 (skin sensitization). Minor deviations: Use of 4 animals in control group. Topical induction patch was a 2 x 4 cm occluded filter paper, whilst challenge was an 8 mm diameter filter paper, occluded using a Finn chamber.
Results	
Challenge 1 Challenge 2	3 animals elicited a reaction scored at >/= 1 at both 24-hours and 48-hours after patch removal. 1/10 animals elicited a reaction which was scored at 2. 3 animals elicited a reaction scored at >/= 1 at both 24- and 48-hours. 2 of these animals had a consistent response when observed at 24- and 48-hours. However, 1 animal elicited a reaction at 24-hours but not 48-hours and another elicited no reaction at 24-hour but a reaction was visible at 48-hours.
Conclusions	3/10 animals showed reactions at challenge 1 (and challenge 2). Induction concentration (topical) 1%. This study indicates Amidoamine is a moderate sensitiser.
Reliability	Klimisch grade: 2 – fairly reliable (see method remarks above).

Two guinea pig Buehler tests were conducted with amidoamines. One study with stearylamidopropyl dimethylamine (CAS 7651-02-7) and one study with palmityl/stearylamidopropyl dimethylamine.

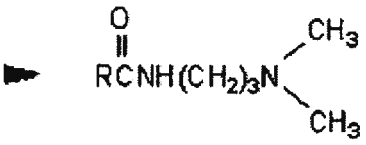
Study 6 – Amidoamine 4

Test Substance	Stearylamidopropyl dimethylamine (CAS No. 7651-02-7) 

	Equivalent to :  cocamidopropyl dimethylamine (amidoamine) with R = 17 carbons.
Test substance remarks	Amidoamine is one of the impurities of CAPB. The test substance (stearylamidopropyl dimethylamine) is an amidoamine containing a 18-carbon fatty acid chain (also referred to as C18 amidoamine).
Method	Guinea pig Buehler test
Test type	Dermal topical induction and challenge with occlusive patches.
Year	1984
Test Conditions	
Species	Guinea pigs
Strain	Hartley outbred guinea pigs
Sex	Primary irritation pilots utilized 8 males plus 8 females. Sensitization study = 10 males plus 10 females for induction and challenge along with 10 naïve animals (5 males plus 5 females) at challenge. At re-challenge, 6 induced animals (4 male, 2 female) and 5 naïve animals (3 male, 2 female) were utilized.
Number of animals per dose	Preliminary studies for primary irritation = 16 Main study treatment group = 20 Main study control group = 10 Re-challenge treatment group = 6 Re-challenge control group = 5
Route of administration	Dermal topical at induction and at challenge using occlusive patches.
Preliminary studies: Primary irritation pilots	Pilot 1 = 20, 10, 5, and 2.5% w/v stearylamidopropyl dimethylamine in 80/20 ethanol/water vehicle. Pilot 2 = 5, 2.5, 1, and 0.5% w/v stearylamidopropyl dimethylamine in acetone vehicle. Pilot 3 = 1, 0.5, 0.25, and 0.1% w/v stearylamidopropyl dimethylamine in 80/20 ethanol/water vehicle. Pilot 4 = 0.5 and 0.25% w/v stearylamidopropyl dimethylamine in acetone vehicle.
Main study: Induction	Animals are shaved the day before dosing. A volume of 0.3 mL of test material (1% stearylamidopropyl dimethylamine in ethanol/water) is applied to a 25 mm Hill Top chamber and patched on the animals for 6 hours each. A total of 3 induction patches are applied, once weekly for 3 consecutive weeks. After induction, there is a 2-week rest period with no test material exposure.
Induction vehicle	80% ethanol and 20% water mixture.

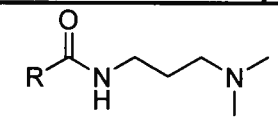
Primary challenge Re-challenge	A volume of 0.3 mL of test material (0.25% stearylamidopropyl dimethylamine in acetone) is applied to a 25 mm Hill Top chamber and patched on the animals for 6 hours each. 24 hours after patch application, animals are depilated and the skin graded a minimum of 2 hours after depilation. A second grading occurs 24 hours after the first grading (i.e., 48-hour grade). Animals were re-challenged at least 6 days after primary challenge. Re-challenge dose concentrations were 0.25, 0.125, and 0.0625% w/v stearylamidopropyl dimethylamine in acetone vehicle.
Challenge vehicle	Acetone.
Grading system	Dermal reactions graded for erythema and oedema by blind reading according to grading scale: No reaction 0 Slight patchy erythema ± Slight but confluent or moderate patchy erythema 1 Moderate erythema 2 Severe erythema with or without edema 3 Grades of 1 or greater in the test group are considered to be indicative of a sensitization responder.
Method remarks	Ritz, H. L., and Buehler, E. V., Current Concepts in Cutaneous Toxicity, ed. Drill, V. A. and Lazar, T. (Academic Press, 1980) pp. 25-40.
Results	
Primary challenge Re-challenge	Induced animals: 1 animal responded at challenge with slight erythema at 24- and 48-hour grades. 10 animals had slight patchy erythema at 24-hour and/or 48-hour grades. Mean irritation score was 0.2 at 24 and 48-hour grades. In total, there was 1 responder and 19 non-responders. Control animals: no animals responded at challenge. 1 animal had slight patchy erythema at 24 and 48-hour grades. In total, all 10 animals were non-responders. At 0.25% test material there was one responder and 5 non-responders in the treatment group. There were no other responders in treatment or control animals at 0.25%, 0.125%, or 0.0625% stearylamidopropyl dimethylamine in acetone.
Conclusions	A 1% stearylamidopropyl dimethylamine solution (in an 80/20 ethanol/water vehicle) was used for induction and a 0.25% solution (in acetone) used at challenge. One of the 20 guinea pigs responded at challenge and re-challenge. The incidence of animals reacting to the test material was less than 15% (i.e., 1 out of 20 responded), which in accordance with the ECETOC Technical Report No. 87 and as summarized in Kimber et al., 2003, would not result in the material being classified as a skin sensitizer.

Study 7 – Amidoamine 5

Test Substance	Stearyl/palmitylamidopropyl dimethylamine  cocamidopropyl dimethylamine (amidoamine) with R = 15 and 17 carbons.
Test substance remarks	Amidoamine is one of the impurities of CAPB. The test substance (stearyl/palmitylamidopropyl dimethylamine) are amidoamines containing a 16- and 18-carbon fatty acid chains (also referred to as C16 amidoamine and C18 amidoamine).
Method	Guinea pig Buehler test
Test type	Dermal topical induction and challenge with occlusive patches.
Year	1980
Test Conditions	
Species	Guinea pigs
Strain	Albino Dunkin/Hartley guinea pigs
Sex	Primary irritation pilots utilized 8 males plus 8 females. Sensitization study = 10 males plus 10 females for induction and challenge along with 10 naïve animals (5 males plus 5 females) at challenge.
Number of animals per dose	Preliminary studies for primary irritation = 4 Main study treatment group = 20 Main study control group = 10
Route of administration	Dermal topical at induction and at challenge using occlusive patches.
Preliminary studies: Primary irritation	80, 40, 10, and 5% w/v in distilled water vehicle.
Main study: Induction	Animals are shaved the day before dosing. A volume of 0.4 mL of test material (25% stearylamidopropyl dimethylamine) is applied to a 4 cm^2 patch and applied to the animals for 6 hours each. A total of 3 induction patches are applied, once weekly for 3 consecutive weeks. After induction, there is a 2-week rest period with no test material exposure.
Induction vehicle	Water and phosphoric acid. Test material is 25% palmityl/stearylamidopropyl dimethyl amine in 8.95% phosphoric acid and 66.05% water.
Primary challenge	Animals are shaved the day before dosing. A volume of 0.4 mL of test material (25% stearylamidopropyl dimethylamine) is applied to a 4 cm^2 patch and applied to the animals for 6 hours. Grading occurs 24- and 48-hours after patch application.
Re-challenge	Re-challenge occurred at concentrations of 0.25% and 0.5%.

Challenge vehicle	Water and phosphoric acid. Test material for challenge is 25% palmityl/stearylamidopropyl dimethyl amine in 8.95% phosphoric acid and 66.05% water.
Grading system	Dermal reactions graded for erythema and oedema by blind reading according to grading scale: No reaction 0 Slight patchy erythema ± Slight but confluent or moderate patchy erythema 1 Moderate erythema 2 Severe erythema with or without edema 3 Grades of 1 or greater in the test group are considered to be indicative of a sensitization responder.
Method remarks	Ritz, H. L., and Buehler, E. V., Current Concepts in Cutaneous Toxicity, ed. Drill, V. A. and Lazar, T. (Academic Press, 1980) pp. 25-40.
Results	
Primary challenge	Induced animals: All but 3 of the 20 test animals demonstrated erythema at 24 and 48-hours after challenge. The responses ranged from slight patchy erythema to severe erythema. Fourteen of the animals were considered responders and 6 non-responders. Control animals: Four control animals demonstrated slight or moderate patchy erythema 24 or 48 hours after challenge. Two of the control animals were considered responders and 8 non-responders.
Re-challenge	The re-challenge program demonstrated that 0.25% did not elicit a sensitization response, but that 0.5% did elicit reactions in sensitized animals.
Conclusions	A guinea pig Buehler test was conducted with an amidoamine containing a mixture of 18-carbon and 16-carbon fatty acid chains. The test for palmityl/stearylamidopropyl dimethylamine was conducted with a 25% solution both at induction and at challenge. Fourteen of the 20 guinea pigs elicited a response at challenge. Re-challenge with a 0.25% solution elicited no response, whilst re-challenge with a 0.5% solution did elicit a positive response. In accordance with the ECETOC Technical Report No. 87 and as summarized in Kimber et al., 2003, the test material is considered a weak skin sensitizer.

Relevance of fatty acid chain length on sensitization potential :



1, Amidoamine

Amidoamine, **1**, is a precursor used to make cocamidopropyl betaine, CAPB, and is known to remain as a residual impurity in CAPB. Generally found as a mixture of straight chain aliphatic amides of lengths varying from 8 to 18 carbons, the major component of the mixture is C12 amidoamine (see Table 1, **1c**). The potential for **1** to be a skin sensitizer has drawn the attention of the Personal Care Products Council (PCPC). Of particular interest is the effect of chain length on the potential for sensitization.

Skin sensitization data are presented in this document for C18 amidoamine (**1f**), a mixture of C18 amidoamine and C16 amidoamine (**1f** and **1e**) in a formulation, and amidoamine purified from CAPB presumably with the distribution of fatty acid chain lengths discussed in the section titled "Amidoamine Characterization". The Review Committee appears to be focusing on **1c**, as it is the major component of the mixture. From a chemical reactivity standpoint the aliphatic chain is non-reactive, regardless of the chain length. All alkyl-amidoamine species have identical reactive functionality, namely from the tertiary amine and the amide. They would all undergo identical metabolic pathways, and have the same reactivity patterns. The potential for divergent sensitization could come from differences in physical properties of the chemicals and how dermal penetration might be affected, which could be dependant upon alkyl chain length.

In the absence of experimental data on dermal penetration, some calculations were performed to estimate the permeation coefficient, Kp, of the components of **1** (Table 1 below) with different aliphatic chain lengths. While there is a trend towards increased permeability with increased chain length, even the shorter chain lengths would be considered to be "good" to "very good" skin permeants, and current guidelines support an assumption of 100% penetration without additional experimental data. The amphiphilic nature of these molecules (having the hydrophobic alkyl chains coupled with the hydrophilic amines) increases the skin penetration. There will be a point where increasing the chain length will overwhelm any hydrophilicity of the amine and the chemical would no longer be absorbed into the skin but this is likely outside the range presented below. The Kp models used are most accurate when LogP is between -1 and 4 and molecular weights less than 500. Kp greater than 0.01 are considered "very good" skin permeants. Thus, the C18 fatty acid amidoamine would be expected to have the greatest dermal penetration based on these calculations and thus is thought to represent the worst case scenario for sensitizaton potential. Following the same logic, the existing data on the C18 amidoamine would be expected to have a similar potential for skin sensitization (or greater) than the C12 amidoamine.

Table 1.

1	**Fatty Acid**	**MW**	**chemsilico LogP**	**dermwin LogP**	**Kp (cm/hr)**
a	C8	228.37	1.51	2.44	4.15 e-3
b	C10	256.44	1.98	3.42	1.4 e-2
c	C12	284.49	2.41	4.4	4.69 e-2
d	C14	312.54	2.83	5.39	0.157
e	C16	340.6	3.18	6.37	0.529
f	C18	368.65	3.34	7.35	1.78

Summary of sensitization data and determination of WoE NESIL for amidoamine :
The hazard characterisation data are summarised below.

Hazard characterisation test	Amidoamine fatty acid (predominant chain length)	Potency	NESIL (µg/cm^2)	Comment
HRIPT (study 1)	C18	No sensitization	187	Key human study for NESIL determination. Vehicle = mineral oil
HRIPT (study 2)	C18 and C16	No sensitization	25	Vehicle = liquid fabric softener product
LLNA (study 3)	C12 and C14 (see detailed description above for complete chain length analysis)	Moderate/Strong	245	Key animal study for NESIL determination.
GPMT (study 4)	C12 and C14	Moderate	100 – 1,000	Predominant chain length assumed to be C12/14 as test item derived from CAPB. NESIL established in accordance with Gerberick et al 2001.
GPMT (study 5)	C12 and C14	Moderate	100 – 1,000	Predominant chain length assumed to be c12/14 as test item derived from CAPB. NESIL established in accordance with Gerbrick et al 2001.
Buehler (study 6)	C18	Non sensitiser	NA	
Buehler (study 7)	C18 and C16	Weak	1,000 – 10,000	NESIL established in accordance with Gerberick et al 2001.

Example of how the WoE NESIL for amidoamine is used in a Quantitative Risk Assessment for Skin Sensitization :
Use of sensitization data in Quantitative Risk Assessment (QRA) for skin sensitization has been described earlier (Api et al., 2008). Using this approach, a QRA for amidoamine present as a

residual in CAPB at the suggested limit of 1.5% in a face wash cosmetic product containing 20% CAPB is summarized in the table below using NESIL values from both HRIPT and LLNA data. Although CAPB can be found in a range of product types, the QRA presented has been conducted on a facial wash product as this product format represents the product resulting in the highest consumer exposure to CAPB. Any limits for amidoamine impurity in CAPB should be established based upon its highest exposure level.

WoE NESIL = Weight of Evidence No Expected Sensitization Induction Level for amidoamine	180 $\mu g/cm^2$ and 245 $\mu g/cm^2$, based on HRIPT data and murine LLNA data, respectively)
SAF = Sensitization Assessment Factor	100 (10X inter-individual, 3X matrix, 3X use because face can have increased permeability)
AEL = Acceptable Exposure Level = (WoE NESIL/SAF)	1.80 $\mu g/cm^2$ (from HRIPT)2.45 $\mu g/cm^2$ (from LLNA)
Product Exposure	0.15 mg/cm^2/day of face wash product (CTFA 90th percentile data as noted for face washes, gels, and scrubs in the 2006 IFRA Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients document).
Concentration of amidoamine in the product	CAPB is present in face wash formula at 20%, which is the maximum level listed in the 2008 Tentative CIR for CAPB under "skin cleansing creams, lotions, liquids, and pads". Assume amidoamine is present at the suggested maximum level of 1.5%. Thus, amidoamine is present at ≤ 0.30% in final products.
CEL = Consumer Exposure Level	0.45 $\mu g/cm^2$ [based on 0.30% of 0.15 mg/cm^2]
Risk Assessment	**Acceptable risk** because AEL > CEL; MOS = Margin of Safety for sensitization = AEL/CEL = 4 and 5, based on HRIPT data and murine LLNA data, respectively.

The outcome of this risk assessment, using worst case exposure scenarios, is favorable. An AEL/CEL ratio of 1 or more indicates that this exposure is unlikely to induce skin sensitization.

Recommendation to Expert Review Panel on CAPB with respect to amidoamine skin senstization potential :

The Personal Care Products Council Task Force to evaluate residual amidoamine as a sensitizer in CAPB has reviewed the available sensitization data and recommends that QRA be included as an acceptable tool for evaluation of the potential for sensitization from amidoamine. The NESIL values calculated for amidoamine using either HRIPT data or murine LLNA data are broadly equivalent and demonstrate acceptable risk.

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Tentative Amended Safety Assessment of the Cosmetic Ingredient Review Expert Panel. Cocamidopropyl Betaine. September 23, 2008.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: May 7, 2010

SUBJECT: Updated concentration of use information - Cocamidopropyl Betaine and related ingredients

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use - Cocamidopropyl Betaine

Product Category	Concentration of Use (active)	Activity(s) of Raw Material
Baby shampoo	2-4%	30%
Other baby products[1]	3-6%	30%
Bath oils, tablets and salts	0.06-7%	30%
Bubble baths	0.2-6%	8%, 10%, 30%
Bath capsules	0.9%	30%
Other bath preparations	3-6%	6%, 28%, 30%, 35%
Eye shadow	3%	35%
Eye makeup remover	0.005%	1%
Hair conditioners	2-4%	28%, 30%, 35%
Hair sprays (aerosol fixatives)	0.2%	36%
Hair straighteners	0.4-9%	30%, 35%, 36%
Permanent waves	2-9%	30%, 35%
Rinses (noncoloring)	9%	30%
Shampoos (noncoloring)	3-9%	13%, 28%, 30%, 35%, 36%, 38%
Tonics, dressings and other hair grooming aids	0.2-5%	28%, 30% 35%
Hair dyes and colors (all types requiring caution statement and patch testing)	0.6-6%	30%
Hair tints	6%	30%
Hair rinses (coloring)	1-6%	4%, 30%
Hair color sprays (aerosol)	6%	30%
Hair lighteners with color	6%	30%
Hair bleaches	6%	30%
Other hair coloring preparations	0.6-3%	30%

Other manicuring preparations	0.8%	39%
Dentifrices (aerosol, liquid, pastes and powders)	0.6-6%	Not reported
Bath soaps and detergents	0.5-10%	28%, 30%, 32%, 34%
Deodorants (underarm)	2%	31%
Douches	3.8%	30%
Other personal cleanliness products[2]	2-10%	30%, 32%, 35%, 36%
Shaving cream (aerosol, brushless and lather)	0.03-9%	30%, 35%
Shaving soaps (cakes, sticks, etc.)	9%	30%
Other shaving preparations	11%	32%
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	1-7%	28%, 30%, 31%, 35%, 38%, 40%
Body and hand creams, lotions and powders	0.4-3%	35%
Foot powders and sprays	4%	30%
Paste masks (mud packs)	0.2%	35%

[1]6% in a rinse-off baby product

[2]2% in an facial exfoliating cleanser, 3% in a body scrub, 3% in a facial cleanser, 3% in a shower gel, 10% in a shower gel

Information collected in 2008
Table prepared December 1, 2008
Table updated May 7, 2010

Concentration of Use - Potential Additions to the Cocamidopropyl Betaine Report

Almondamidopropyl Betaine, Apricotamidopropyl Betaine, Avocadamidopropyl Betaine. Babassuamidopropyl Betaine. Behenamidopropyl Betaine, Canolamidopropyl Betaine, Capryl/Capramidopropyl Betaine, Coco/Oleamidopropyl Betaine, Coco/Sunfloweramidopropyl Betaine, Cupuassuamidopropyl Betaine, Isostearamidopropyl Betaine, Lauramidopropyl Betaine, Meadowfoamamidopropyl Betaine, Milkamidopropyl Betaine, Minkamidopropyl Betaine, Myristamidopropyl Betaine, Oatamidopropyl Betaine, Oleamidopropyl Betaine, Olivamidopropyl Betaine, Palmamidopropyl Betaine, Palmitamidopropyl Betaine, Palm Kernelamidopropyl Betaine, Ricinoleamidopropyl Betaine, Sesamidopropyl Betaine, Shea Butteramidopropyl Betaine, Soyamidopropyl Betaine, Stearamidopropyl Betaine, Tallowamidopropyl Betaine, Undecylenamidopropyl Betaine and Wheat Germamidopropyl Betaine*

Ingredient	Product Category	Concentration of Use
Almondamidopropyl Betaine	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	3%
Babassuamidopropyl Betaine	Hair conditioners	0.9%
Babassuamidopropyl Betaine	Shampoos (noncoloring)	4%
Babassuamidopropyl Betaine	Bath soaps and detergents	2%
Babassuamidopropyl Betaine	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.9%
Capryl/Capramidopropyl Betaine	Shampoos (non coloring)	0.3%
Capryl/Capramidopropyl Betaine	Suntan gels, creams and liquids	2%
Capryl/Capramidopropyl Betaine	Other suntan preparations	2%
Lauramidopropyl Betaine	Bubble baths	4-5%
Lauramidopropyl Betaine	Other bath preparations	3-8%
Lauramidopropyl Betaine	Other fragrance preparations	4%
Lauramidopropyl Betaine	Shampoos (noncoloring)	0.9-8%
Lauramidopropyl Betaine	Other hair preparations (noncoloring)	0.00006%
Lauramidopropyl Betaine	Other hair coloring preparations	0.6%
Lauramidopropyl Betaine	Other makeup preparations	2%

Lauramidopropyl Betaine	Bath soaps and detergents	2-8%
Lauramidopropyl Betaine	Other personal cleanliness products[1]	2-13%
Lauramidopropyl Betaine	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	3-5%
Lauramidopropyl Betaine	Face and neck creams, lotions and powders	0.7%
Lauramidopropyl Betaine	Body and hand creams, lotions and powders	2%
Lauramidopropyl Betaine	Other skin care preparations[2]	6%
Myristamidopropyl Betaine	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.3%
Oatamidopropyl Betaine	Indoor tanning preparations	0.3%
Palm Kernelamidopropyl Betaine	Shampoos (noncoloring)	5%
Palm Kernelamidopropyl Betaine	Bath soaps and detergents	0.9%
Shea Butteramidopropyl Betaine	Other bath preparations	0.6%
Shea Butteramidopropyl Betaine	Other personal cleanliness products[3]	2%
Shea Butteramidopropyl Betaine	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	4%
Soyamidopropyl Betaine	Shampoo	1%
Soyamidopropyl Betaine	Skin cleansing (cold, creams, cleansing lotions, liquids and pads)	2%
Undecylenaminopropyl Betaine	Shampoos (noncoloring)	2%

*Ingredients included in the title of the table, but not found in the table were included in the concentration of use survey, but no uses were reported.

[1]5% in a bath and shower gel

[2]6% in a foot soak

[3]2% in a wipe

Information collected in 2009
Table prepared February 16, 2009
Updated March 9, 2009
Updated April 23, 2010

Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D. 3/29/10
Industry Liaison to the CIR Expert Panel

DATE: March 29, 2010

SUBJECT: Comments on the Draft Report on Cocamidopropyl Betaine and Related Amidopropyl Betaines
CIR Expert Panel Meeting April 5-6, 2010

p.1, 44 - The Abstract and Conclusion should not have been written for this draft report as this is the first time the CIR Expert Panel has seen the report with all the ingredients in the report. Even without the LLNA on the predominantly C12 amidoamine (recently submitted), the CIR Expert Panel may have been able to reach a safe with qualifications conclusion on compounds that are predominantly C18 e.g., Stearamidopropyl Betaine, Shea Butteramidopropyl Betaine, based on the LLNA on the C18 amidoamine.

p.5 - Please change "Gottschalck and Bailey described the current uses.." to "Gottschalck and Bailey described the functions.."

p.6 - It would be helpful to note the European status of the additional ingredients that have been added to the report.

p.10 - The summary of the Dermal Irritation section does not appear to accurately reflect the information in the section. Only one study examined 50% CAPB and it was diluted before application (1 part plus 1part (v/v) with distilled water). A 4 hour study of 30% active found it to be a mild irritant, while another study with a 24 hour exposure reported moderate irritation.

p.11 - Because CAPB was diluted before application, perhaps the following sentence should be revised. "The authors concluded that 50% CAPB was not a primary irritant in this study."

p.12, 13 - If available, the concentration used in the LLNA study should be added to the report (p.13) and the summary of the Dermal Sensitization section.

p.19 - In the Summary of the Dermal Irritation section, it would be helpful to note that it was a formulation that contained the 1% Wheatgermamidopropyl Betaine.

p.19 - In the Dermal Sensitization section, please provide at least one example of a dose per unit area calculation. It would be helpful to know what default values were used. Attempts to calculate some of these doses did not result in the same values.

p.22 - Please check the KGL, Inc. study (ref. 77). The shower gel was diluted to 0.5% before application. Was this dilution taken into account in the calculation of the dose/unit area?

p.22 - Please check the KGL, Inc. study (ref. 80). The body wash was diluted to 0.5% before application. Was this dilution taken into account in the calculation of the dose/unit area?

p.28 - In the summary of the Animal Studies subsection, it would be helpful to indicate the average chain length of the amidoamine tested.

p.40 - Please add use information about the additional ingredients to the Summary.

p.41 - Please summarize the limited information regarding the potential sensitization of the additional ingredients.

p.44 - The last two sentences of the Summary need to be deleted, as the CIR Expert Panel has not had the opportunity to adequately review the LLNAs (C18-amidoamine, C12-amidoamine) and determine the impact of these studies on the safety of all the ingredients included in this report.

p.45, Figures 1 and 2 - Please provide references for Figures 1 and 2.

p.46, Table 1 - What does NA with Coco/Sunfloweramidopropyl Betaine mean?