Supplement Wave 3

PCPC Comments
- 6-Amino-m-Cresol
- Ginger
- Mallow
- Phytosteryl Glutamates
- ReReviews
- RR Summaries
- *Zanthoxylum Piperitum*

References on *Zanthoxylum Piperitum*

EXPERT PANEL MEETING
December 5-6, 2022

Personal Care Products Council
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Memorandum

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

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

DATE: December 1, 2022

SUBJECT: Draft Amended Report: Safety Assessment of 6-Amino-m-Cresol as Used in Cosmetics (December 2022 meeting draft)

The Personal Care Products Council respectfully submits the following comments on the draft amended report, Safety Assessment of 6-Amino-m-Cresol as Used in Cosmetics.

Dermal Absorption – The identity of the receptor fluid (Dulbecco's minimum eagle medium) should be stated. Please state what is meant by "dermal delivery" (amount in the receptor fluid plus the amount in the skin).

Acute Toxicity, old report summary – Please correct: "dosed for 2 consecutive days with up to mg/kg 6-Amino-m-Cresol" (1500 needs to be added).

Short-Term, old report summary – "25 mg/kg/day" needs to be corrected to "250 mg/kg/day"

Summary – Please correct: "but not genotoxicity" ("not" should be "no")

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Memorandum

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

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

DATE: December 1, 2022

SUBJECT: Draft Final Report: Safety Assessment of *Zingiber officinale* (Ginger) – Derived Ingredients as Used in Cosmetics (December 2022 meeting draft)

The Personal Care Products Council respectfully submits the following comments on the draft final report, Safety Assessment of *Zingiber officinale* (Ginger)–Derived Ingredients as Used in Cosmetics.

Abstract – Please correct: "data are insufficient data" (delete second data)

Cosmetic Use – Please correct: "reportedly used pump spray body and hand formulations" (add "in")

Non-Cosmetic Use – Please correct: "essential oils at concentrations of 100% would be used in cosmetic products" ("in" should be "as" – small amounts of pure essential oils are used in cosmetics, but pure essential oils themselves should not be used directly on the skin (as cosmetics))

DART – Although the authors may have said "Post-implantation loss (abortifacient effects) were evaluated in mice treated 20 d before, and throughout gestation", it does not make sense. The timing of treatment includes pre-implantation, so it would not be possible to determine if it was a "post-implantation loss" using this protocol.

Anti-Carcinogenicity, Animal – The study described in reference 56 would make more sense if group V (choline-deficient diet + ginger extract) was also given ethionine. Currently, it indicates that only group IV was given ethionine.

UV-Protective Effects – Please revise: "The dorsal skin was removed and measured for cytokines and hematoxylin and eosin staining." The current wording suggests that hematoxylin and eosin staining was measured. It is likely that the skin was "stained" with hematoxylin and eosin.

Ocular Irritation – The ECHA dossier indicated that the dimethylformamide used as the positive control was undiluted. It also indicated that the test item, ginger extract, was tested, which indicates that it was tested in this assay undiluted. The statement that "The concentrations of the test agents used were not reported" is not correct.

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Memorandum

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

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

DATE: December 1, 2022

SUBJECT: Draft Report: Safety Assessment of *Malva sylvestris* (Mallow)-Derived Ingredients as Used in Cosmetics (December 2022 meeting draft)

The Personal Care Products Council respectfully submits the following comments on the draft report, Safety Assessment of *Malva sylvestris* (Mallow)-Derived Ingredients as Used in Cosmetics.

Cosmetic Use; Summary – As use in body and hand products would result in greater exposure than use in depilatories, it is that use (rather than the depilatory use) that should be highlighted in the Cosmetic Use section and Summary.

Cytotoxicity; Summary – Please check the units. Was it µl/ml or µl/plate? Units of just µl do not represent a concentration.

Photoprotective Effects; Summary – What volume or dose was applied dermally? What were the results of the dermal exposure?

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Memorandum

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

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

DATE: November 30, 2022

SUBJECT: Draft Tentative Report: Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics (December 2022 meeting draft)

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

Dermal Irritation and Sensitization; Summary – The material tested in the human patch test (reference 4) was the ingredient itself - 100% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (the word "Glutamate" is missing from the name of the ingredient in the description of this study).

Dermal Irritation and Sensitization - In the HRIPT from reference 16 (in 219 subjects), the mixture was tested neat, but it should state that the mixture contained 5.99% Phytosteryl/Octyldodecyl Lauroyl Glutamate (as noted in Table 4).

Summary – Please revise the following sentence: "The short-term oral toxicity of Phytosteryl/Octyldodecyl Lauroyl Glutamate was administered by gavage to SPF-bred Wister rats of both sexes at dose levels of 50, 200, or 1000 mg/kg for 28 d."

Summary – In the description of the EpiOcular™ assay, please add the units for 1-16 (perhaps hours). This information is not provided earlier in the report.

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Memorandum

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

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

DATE: December 1, 2022

SUBJECT: Re-Reviews (December 2022 meeting drafts)

The Personal Care Products Council respectfully submits the following comments on the re-reviews prepared for the December 2022 CIR meeting.

Ingredient Specific Comments

Methyl Alcohol
ADME – As presented in the table, the first study under ADME is not actually an ADME study. Did they measure methanol or its metabolites in the blood or excreta? In the last ADME study, in the results column, the following is not clear: "Methyl Alcohol concentration in blood reached 1.9 h after the last exposure."

Carcinogenicity – In the first study in this section (oral study in Swiss mice), what was the NOAEL or NOEL? The adverse effects observed at the LOAEL are not clear. The meaning of "based on lack of negative effects" is not clear. Is a "negative effect" the same as an adverse effect? If there were no "negative effects", why is it a LOAEL?

Phytantriol
Memo – It is not clear why the original CIR report on Phytantriol is being called a "final amended report".

Memo; Summary table – Please state the FDA product category in which the maximum use concentration was reported. Since the maximum use concentration was in an aerosol hair spray, it would also be helpful to state the maximum use concentration in a product intended to be left on the skin (0.2% in moisturizing products).

No Comments on the Following Re-Reviews

Basic Blue 99
HC Yellow No. 5
Peanut Glycerides
Brown Algae
Choleth-24

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Memorandum

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

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

DATE: December 1, 2022

SUBJECT: Re-Review Summaries (December 2022 meeting draft)

The Personal Care Products Council respectfully submits the following comments on the re-review summaries prepared for the December 2022 CIR meeting.

Hexamidine and Hexamidine Diisethionate
In the text, please note the most significant new information found, e.g., the ECHA dossier or the SCCNFP opinion.

Glyceryl Diesters
There should be a published record of what information was considered in this re-review. In the Reference section, please add the references for the studies that were included in the table reviewed by the Expert Panel during the September 2022 meeting. The numbers of these references should then be included in the text when new information found is mentioned.

Sodium Lauryl Sulfoacetate
Please state clearly that no new data were found. The following sentence suggests that some data were found but it did not raise concerns to open the report. "The Panel agreed, however, that that the published literature did not reveal toxicity or other data that warrant re-evaluation of the safety of this ingredient in cosmetic products." (if this sentence is left in the document, the second "that" needs to be deleted)

Acid Orange 3
In the re-review summary, saying "new data were found" is a bit misleading as there was really only one additional study found, the guinea sensitization study. This study should be specifically mentioned. The other reports review data that are already in the CIR report.

No Comments on the Following Re-review Summaries

Chloroxylenol
Erythorbic Acid and Sodium Erythorbate
Mink Oil

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Memorandum

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

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

DATE: December 1, 2022

SUBJECT: Draft Report: Safety Assessment of *Zanthoxylum piperitum*-Derived Ingredients as Used in Cosmetics (December 2022 meeting draft)

The Personal Care Products Council respectfully submits the following comments on the draft report, Safety Assessment of *Zanthoxylum piperitum*-Derived Ingredients as Used in Cosmetics.

Cosmetic Use; Summary – Please state a product category for which the highest use concentration of the Peel Extract was reported. Because there are multiple product categories with the highest use concentration, identifying the product category with the greatest likely exposure, e.g., body and hand products, would be helpful.

ADME – In the human ADME study, did they determine the amount of hydroxy-α-sanshool in the mixture that was tested?

Cytotoxicity – Please revise the following: "This activity was studied on human cancer cell lines was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay." (delete "was measured")

Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Regina Tucker, M.S., Scientific Analyst/Writer, CIR
Date: December 1, 2022
Subject: Wave 3 - Safety Assessment of *Zanthoxylum piperitum*-Derived Ingredients as Used in Cosmetics

Dr. Belsito has provided CIR with the 6 published studies on *Zanthoxylum piperitum*. Below, please find the reference for each article, along with the published abstract that is included in each paper.

Gwon SY, Ahn JY, Kim TW, Ha TY. *Zanthoxylum piperitum* DC ethanol extract suppresses fat accumulation in adipocytes and high fat-diet-induced obese mice by reguating adipogenesis. *J. Nutr Sci Vitaminol*. 2012; 58: 393-401.

This study was conducted to determine the anti-obesity effects of *Zanthoxylum piperitum* DC fruit ethanol extract (ZPE) in 3T3-L1 adipocytes and obese mice fed a high-fat diet. We evaluated the influence of the addition of ZPE to a high-fat diet on body weight, adipose tissue weight, serum and hepatic lipids in C57BL/6 mice. In addition, adipogenic gene expression was determined by Western blot and real-time reverse transcription-PCR analysis. We assessed the effect of ZPE on 3T3-L1 preadipocyte differentiation. ZPE reduced weight gain, white adipose tissue mass, and serum triglyceride and cholesterol levels ($p<0.05$) in high-fat diet-fed C57BL/6 mice. ZPE decreased lipid accumulation and PPARγ, C/EBPα, SREBP-1, and FAS protein and mRNA levels in the liver. ZPE inhibited in vitro adipocyte differentiation in a dose-dependent manner and significantly attenuated adipogenic transcription factors, such as PPARγ, C/EBPα, and SREBP-1 in 3T3L1 cells. These findings suggest that *Z. piperitum* DC exerts an anti-obesity effect by inhibiting adipogenesis through the downregulation of genes involved in the adipogenesis pathway.

Hwang W, Kim D, Kwon OS, Kim YS, Ahn B, Kang NG. Topical application of *Zanthoxylum piperitum* extract improves lateral canthal rhytides by inhibiting muscle contractions. *Scientific Reports* 2020;10(1):21514.

Facial wrinkles are the predominant phenotypes of skin aging. To date, one of the most effective ways to improve wrinkles is botulinum toxin type A (BoNT/A) injection, which inhibits muscle contractions by reducing acetylcholine release from neurons. However, since BoNT/A is a hazardous neurotoxin, the injection can only be performed by medical doctors and the procedure is only possible through invasive injection, causing inconveniences such as pain. To overcome these inconveniences, we tried to find a way to reduce wrinkles non-invasively via mechanisms similar to BoNT/A. We first designed in vitro assays to test BoNT/A-like muscle contraction inhibition in two different model systems. By using the assays, we identified *Zanthoxylum piperitum* (*Z. piperitum*) fruit extract as a BoNT-like reagent (27.7% decrease of muscle contraction rates by 1000 ppm of Z. piperitum extract treatment). Next, we determined mechanisms of how *Z. piperitum* extract decreases muscle contraction rates and found that the extract treatment inhibits electrical signal transduction in neurons. We also showed that among known components of *Z. piperitum* extract, quercitrin is responsible for muscle contraction inhibition. We further identified that Z. piperitum extract has synergistic effects with acetyl hexapeptide-8 and BoNT/A light chain, which are well-known BoNT-like peptides. Finally, we showed that topical treatment of the *Z. piperitum* extract indeed decreases facial wrinkles and treatment of *Z. piperitum* extract with acetyl hexapeptide-8 has a tendency to improve wrinkles synergistically (14.5% improvement on average). The synergistic effect of the combination is expected to improve wrinkles effectively by implementing the BoNT/A mechanisms in a non-invasive way.

Kim MH, Lee H, Ha IJ, Yang WM. *Zanthoxylum piperitum* alleviates the bone loss in osteoporosis via inhibition of RANKL-induced c-fos/NFATc1/NF-κB pathway. Phytomedicine. 2021; 80:153397.

The fruit of *Zanthoxylum piperitum* (ZP) is an herbal medicine as well as a spice agent in Asia to treat carminative, stomachic, anthelmintic and degenerative diseases. *Z. piperitum* was reported to have anti-oxidant, anti-inflammatory, anti-osteoarthritic and osteosarcoma proliferation-control effects. A study was conducted to determine the anti-osteoporotic effects and mechanisms of action of ZP. Female ICR mice underwent ovariectomies (OVX) and were orally administered ZP at 1, 10 and 100 mg/kg for 6 weeks. The femoral and tibial bones were assessed by dual-energy X-ray absorptiometry and histology to analyze the bone mineral density (BMD) and the number of osteoclasts. Raw 264.7 cells were stimulated by 100 ng/ml receptor activator of nuclear factor-κB ligand (RANKL) for 7 days in the presence of ZP. RANKL-induced signaling molecules were analyzed in osteoclasts. The levels of femoral and tibial BMD were significantly increased by ZP administration. In addition, the number of osteoclasts in the head, trochanter and body of the femur was obviously decreased in the ZP treatment groups. Moreover, ZP treated-cells showed a reduction in the number of TRAP-positive multinuclear cells in RANKL-stimulated Raw 264.7 cells. ZP decreased the RANKL-activated NFATc1 and c-fos, transcription factors of osteoclast formation. The nuclear translocation of NF-κB and phosphorylation of ERK42/44 were inhibited by the ZP treatment in RANKL-induced osteoclasts. Collectively, ZP exerts its inhibitory effect against bone resorption by regulating RANKL-mediated c-fos/NFATc1/NF-κB in osteoclast. ZP may prove to be a therapeutic agent for osteoporosis.

Kim MH, Lee HJ, Park JC, Hong J, Yang WM. *Zanthoxylum piperitum* reversed alveolar bone loss of periodontitis via regulation of bone remodeling-related factors. J Ethnopharmacol. 2017;195:137-142.

Ethnopharmacological relevance: *Zanthoxylum piperitum* (ZP) has been used to prevent toothache in East Asia.
Aim of study: In this study, we investigated the effects of ZP on periodontitis along with alveolar bone loss.
Materials and methods: Twenty-eight male Sprague-Dawley rats were assigned into 4 groups; non-ligated (NOR), ligated and treated vehicle (CTR), ligated and treated 1 mg/mL ZP (ZP1), and ligated and treated 100 mg/mL ZP (ZP100). Sterilized 3-0 nylon ligature was placed into the subgingival sulcus around the both sides of mandibular first molar. After topical application of 1 and 100 mg/mL ZP for 2 weeks, mandibles was removed for histology. In addition, SaOS-2 osteoblast cells were treated 1, 10 and 100 μg/mL ZP for 24 h to analyze the expressions of alveolar bone-related markers.
Results: Several alveolar bone resorption pits, which indicate cementum demineralization were decreased by ZP treatment. Topical ZP treatment inhibited periodontitis-induced alveolar bone loss. In addition, there were significant reduction of osteoclastic activities following topical ZP treatment in periodontium. The expression of RANKL was decreased in SaOS-2 osteoblast cells by treating ZP, while that of OPG was increased. ZP treatment increased the expressions of Runx2 and Osterix in SaOS-2 cells.
Conclusion: In summary, ZP treatment inhibited alveolar bone loss as well as maintained the integrity of periodontal structures via regulation of bone remodeling. ZP may be a therapeutic target for treating periodontitis.

Nozaki R, Kono T, Bochimoto H, et al. Zanthoxylum fruit extract from Japanese pepper promotes autophagic cell death in cancer cells. *Oncotarget*. 2016;7(43): 70437-70446.

Zanthoxylum fruit, obtained from the Japanese pepper plant (*Zanthoxylum piperitum* De Candolle), and its extract (Zanthoxylum fruit extract, ZFE) have multiple physiological activities (e.g., antiviral activity). However, the potential anticancer activity of ZFE has not been fully examined. In this study, we investigated the ability of ZFE to induce autophagic cell death (ACD). ZFE caused remarkable autophagy-like cytoplasmic vacuolization, inhibited cell proliferation, and ultimately induced cell death in the human cancer cell lines DLD-1, HepG2, and Caco-2, but not in A549, MCF-7, or WiDr cells. ZFE increased the level of LC3-II protein, a marker of autophagy. Knockdown of ATG5 using siRNA inhibited ZFE-induced cytoplasmic vacuolization and cell death. Moreover, in cancer cells that could be induced to undergo cell death by ZFE, the extract increased the phosphorylation of c-Jun N-terminal kinase (JNK), and the JNK inhibitor SP600125 attenuated both vacuolization and cell death. Based on morphology and expression of marker proteins, ZFE-induced cell death was neither apoptosis nor necrosis. Normal intestinal cells were not affected by ZFE. Taken together, our findings show that ZFE induces JNK-dependent ACD, which appears to be the main mechanism underlying its anticancer activity, suggesting a promising starting point for anticancer drug development.

Oh K, Adnan M, Cho D. Uncovering Mechanisms of *Zanthoxylum piperitum* Fruits for the Alleviation of Rheumatoid Arthritis Based on Network Pharmacology. Biology (Basel). 2021;10(8):703.

Zanthoxylum piperitum fruits (ZPFs) have been demonstrated favorable clinical efficacy on rheumatoid arthritis (RA), but its compounds and mechanisms against RA have not been elucidated. This study was to investigate the compounds and mechanisms of ZPFs to alleviate RA via network pharmacology. The compounds from ZPFs were detected by gas chromatography-mass spectrometry (GC-MS) and screened to select drug-likeness compounds through SwissADME. Targets associated with bioactive compounds or RA were identified utilizing bioinformatics databases. The signaling pathways related to RA were constructed; interactions among targets; and signaling pathways-targets-compounds (STC) were analyzed by RPackage. Finally, a molecular docking test (MDT) was performed to validate affinity between targets and compounds on key signaling pathway(s). GC-MS detected a total of 85 compounds from ZPFs, and drug-likeness properties accepted all compounds. A total of 216 targets associated with compounds 3377 RA targets and 101 targets between them were finally identified. Then, a bubble chart exhibited that inactivation of MAPK (mitogen-activated protein kinase) and activation of PPAR (peroxisome proliferator-activated receptor) signaling pathway might be key pathways against RA. Overall, this work suggests that seven compounds from ZPFs and eight targets might be multiple targets on RA and provide integrated pharmacological evidence to support the clinical efficacy of ZPFs on RA.