Communications
Supplement Wave 3

PCPC Comments On ReReviews
WVE Comments On Clays

EXPERT PANEL MEETING
September 26-27, 2022

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: September 22, 2022

SUBJECT: Re-Reviews (September 2022 meeting drafts)

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

General Comment for Re-Reviews with New Data

Notable New Data Table – The title of the data tables suggests that there are additional data about these ingredients that was found but it was not considered "notable". What makes a study "notable"? If there are other references that were identified but not included in the table, perhaps a list of these references should be included. If the table includes all the new information identified, please consider not including the word "Notable" for future re-reviews.

Ingredient Specific Comments

Chloroxylenol

In addition to the published re-review, it would be helpful if the summaries of the studies prepared for the original re-review could also be provided. The re-review summary lists a percutaneous absorption study that may be useful. The re-review summary also includes the following reference:

Momma, J., K. Takada, Y. Aida, H. Yoshimoto, K. Naito, Y. Suzuki, Y. Nakaji, Kurokawa, and M. Tobe. 1988. Combined long-term toxicity and carcinogenicity test of p-chloro-m-xylenol (PCMX) applied to female mouse skin. Eisei Shikenjo Hokoku 106:39–47.

Perhaps this is the same long-term study in the new data table cited to Yost et al. (2016).

Use Table – Cosmetic use information (frequency and concentrations) from the re-review summary should also be presented in the use table. The statement in the Notes column ("slight increase in frequency of use decreased") is not clear.

Dermal Carcinogenicity Study – The results should be stated in the table.

Skin Sensitization, LLNA – The following does not belong in the results of an LLNA "test material was shown to be mutagenic to L5178Y cells" (it should be presented for the results of a mutagenicity test).

Risk Assessment – It would have been helpful if the details, e.g., exposure scenarios considered, and the results of the Yost et al (2016) risk assessment would have been presented. The abstract of the study states: "The identified NOAELs were used together with exposure estimates to derive margin of exposure (MOE) estimates for chloroxylenol (i.e., estimates of exposure over NOAELs). These estimates were designed with conservative assumptions and likely overestimate exposure and risk (i.e., highest frequency, 100% dermal penetration). The resulting MOEs ranged from 178 to over 100,000,000 indicating negligibly small potential for harm related to consumer or health-care worker exposure to chloroxylenol in liquid soaps used in dish washing and hand washing."

Glyceryl Diesters

Activity of the diesters is dependent on chain length in addition to the amount of 1,2-diester. From the information provided in the table, it is not clear if the studies on diacylglycerol oil identified the chain lengths of the material tested, or the ratio of the 1,3- to 1,2- diesters. This review paper (https://nutritionj.biomedcentral.com/track/pdf/10.1186/1475-2891-6-43.pdf) suggests that the ratio is 7:3, 1,3- to 1,2-diesters.

> Yanai H, Tomono Y, Ito K, et al. 2007. Diacylglycerol oil for the metabolic syndrome. Nutrition Journal, 6:43 (doi:10.1186/1475-2891-6-43.

In the AICIS risk assessment, did they consider the estimated cosmetic exposure to Glyceryl Dibehenate of 5.1162 mg/kg/day safe? It should be made clear that the material considered was a mixture of mono, di and triesters (it states "Glycerol dibehenate predominates at > 50% by weight of the total mixture based on analysis provided by the notifier.")

Tumor promotion – In the Results – Brief Overview column it says "(%DAG/%TG) at 0, 1.375%/5.5%/4.125%..." what does the third value represent? (perhaps 5.5% should be deleted as the total oil exposure for each group appears to have been 5.5%)

No Comments on the Following Re-Reviews

Hexamidine and Hexamidine Diisethionate
Erythorbic Acid and Sodium Erythorbate
Mink Oil
Octyldodecyl Stearoyl Stearate
Sodium Lauryl Sulfosuccinate

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, Senior Scientific Analyst/Writer, CIR
Jinqiu Zhu, PhD, DABT, ERT, DCST, CIR Toxicologist
Date: September 22, 2022
Subject: Response to additional comments on Clays submitted by WVE

Enclosed are comments received from Women's Voices for the Earth (WVE), dated September 14, 2022, in which four studies on genotoxicity and cytotoxicity of Kaolin have been submitted (named *WVEcomments_Clays_Wave3_092022* in the pdf). WVE drew attention to the potential application of Kaolin in cosmetic formulations that might cause inhalation exposure, such as in a powder form or delivering by airbrush devices.

Regarding the health concerns associated with inhalation exposure when cosmetic ingredients are used with airbrush delivery systems, CIR staff has responded to WVE's comments multiple times. For more detail, please check previous meeting materials (pdf page 4 – 7 at https://www.cir-safety.org/sites/default/files/Supplement_Wave3_062022.pdf). An in-depth discussion is included in the Panel's respiratory exposure resource document (available at https://www.cir-safety.org/sites/default/files/report_InhalationDocument_122021.pdf).

Please note the following statements/discussions are already present in the Clays report:

> Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. (see pdf page 99 at https://www.cir-safety.org/sites/default/files/Clays_0.pdf)
>
> Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients, and without consumer habits and practices data or particle size data related to this use technology, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems. (see pdf page 100 at https://www.cir-safety.org/sites/default/files/Clays_0.pdf)
>
> The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system. (see pdf page 113 at https://www.cir-safety.org/sites/default/files/Clays_0.pdf)
>
> Moreover, clay ingredients are used in cosmetic formulations that could possibly be inhaled; for example, Bentonite is reported to be used at 0.9% in spray suntan products and Kaolin is reported to be used at up to 15% in face powders. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from

cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace. (see pdf page 99 at https://www.cir-safety.org/sites/default/files/Clays_0.pdf)

With the exception for Attapulgite and Bentonite, the limited data available from inhalation studies, including acute, chronic, and carcinogenicity data, suggest little potential for respiratory effects at relevant doses for the remaining naturally sourced clay ingredients. The Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which the ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients were also included in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings) (see pdf page 113 at https://www.cir-safety.org/sites/default/files/Clays_0.pdf)

In addition, major findings of the four studies submitted in WVE's current comments are summarized below for the Panel's consideration:

- In Kawanishi et al. 2020 study, three types of cells, including primary normal human diploid epidermal keratinocytes and fibroblasts, as well as Chinese hamster ovary (CHO) AA8 cells, were exposed to Kaolin particles (4.8 μm and 200 nm) in the dose range of 0.2 to 200 μg/mL. Six hours treatment resulted in micronucleus (MN) induction at a dose-dependent manner. The frequencies of micronucleated cells increased 3- to 4-fold at 200 μg/mL in all cells. Fine particles of Kaolin showed higher genotoxic potency than coarse particles, while no significant difference was detected among three cell types (i.e., the cell type did not affect the MN frequencies). In addition, results from comet assay indicated Kaolin particles treatment promoted DNA damage in a dose-dependent manner.

- In Totsuka et al. 2009 study, increased MN frequencies at a dose-dependent manner were observed in human lung cancer A549 cells after treatment by Kaolin nano/microparticles (particle distribution with a range of 5.1 to 4846.9 nm) at concentrations from 0.02 to 200 μg/mL. A six-hour treatment of 200 μg/mL Kaolin particles caused over 4-fold increased frequency of micronucleated cells. DNA damage, measured by comet assay, was induced in the lungs of C57BL/6J mice intratracheally administered with Kaolin particles at a dose of 0.2 mg/mouse but not 0.05 mg/mouse. In addition, increased *gpt* and Spi^- mutant frequencies were observed in the lungs of *gpt* delta transgenic mice. Mutation spectra analysis demonstrated more than 60% of G:C to C:G transversion occurred in the *gpt* genes.

- In Kato et al. 2017 study, two types of Kaolin (Kaolin-S with smooth, sphere-shaped crystals, and Kaolin-P with clusters of thin pseudohexagonal plates) were intratracheally administered to male ICR mice at 0.05 and 0.2 mg/mouse. The most abundant sizes of Kaolin-S at doses of 0.5 and 2.0 mg/mL were 827.4 ± 186.2 and 1390.1 ± 226.3 nm, respectively, while those of Kaolin-P were 700.0 ± 128.6 and 1488.3 ±83.7 nm, respectively. Comet assay on the lungs of mice showed that both types of Kaolin induced DNA damage, and Kaolin-P had higher DNA damaging potency than Kaolin-S. DNA damage was also examined in human lung cancer A549 cells by comet assay with formamidopyramidine-DNA glycosylase (FPG) treatment. The

tail intensity was significantly elevated in A549 cells (epithelial cells) co-cultured with macrophage-like RAW264 cells (immune cells), but elevation was not observed in a single-culture system (in A549 cells only). The results indicated that Kaolin may induce oxidative DNA damage in epithelial cells through activation of macrophages.

- In Shan et al. 2021 study, human bronchial epithelial (16HBE) cells were treated by nano-scale Kaolin at concentrations of 40 to 240 μg/mL (particle size data of Kaolin were not available, the authors stated the nano-scale Kaolin was purchased from a company to imitate Kaolin in atmospheric fine particles $PM_{2.5}$) Cytotoxicity assay results, measured by Cell counting kit-8 (CCK-8), showed the 16HBE cells have high viability after exposing to 40 μg/mL particle, but cell viability decreased significantly over the dose of 80 μg/mL particles. Lactate dehydrogenase (LDH) assay indicated Kaolin caused membrane disruption a at dose-dependent manner in the dose range of 40 to 240 μg/mL.

While WVE pointed out in their comments "The last study also found kaolin to be cytotoxic to human bronchial epithelial cells," please note such study (Shan et al. 2021) aimed at *examining toxic mechanisms of typical $PM_{2.5}$ (air pollution dominated) components at the cellular and subcellular levels*, and Kaolin was tested because *Kaolin mainly comes from air dust, and represents the typical soil source clay minerals in $PM_{2.5}$*. The author claimed that the finding of the study *is helpful to evaluate of the health hazard of air pollution*. No cosmetic use-related adverse effects were investigated or discussed in the study.

The Panel should consider whether the four studies submitted by WVE have sufficient value to be incorporated into the report. If so, whether additional caveats should be added with regard to potential application of Kaolin in cosmetic formulations that may be incidentally inhaled.

From: Alexandra Gorman Scranton <alexs@womensvoices.org>
Sent: Wednesday, September 14, 2022 6:11 PM
To: CIRINFO <cirinfo@cir-safety.org>
Subject: Additional comment from WVE on Kaolin (Clays Assessment)

To the CIR:

Please include the following studies which are currently missing from the safety assessment of clays.

These are studies finding kaolin to be genotoxic, and potentially carcinogenic. Of particular interest are the adverse impacts of kaolin on both human and rodent lung epithelial cells. The last study also found kaolin to be cytotoxic to human bronchial epithelial cells.

Thank you for your consideration of this science – particularly as it pertains to cosmetic ingredients containing Kaolin that may be inhaled, such as airbrush cosmetics and/or cosmetic powders.

1.
Kawanishi M, Yoneda R, Totsuka Y, Yagi T. Genotoxicity of micro- and nano-particles of kaolin in human primary dermal keratinocytes and fibroblasts. Genes Environ. 2020 Apr 16;42:16. doi: 10.1186/s41021-020-00155-1. PMID: 32322315; PMCID: PMC7164293.
Full text available here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164293/
Abstract
Introduction: Kaolin is a clay mineral with the chemical composition $Al_2Si_2O_5(OH)_4$. It is an important industrial material, **and is also used as a white cosmetic pigment**. We previously reported that fine particles of kaolin have genotoxic potency to Chinese hamster ovary CHO AA8 cells, and to the lungs of C57BL/6 J and ICR mice. In the present study, we evaluated the genotoxicity of different particle sizes of kaolin using primary normal human diploid epidermal keratinocytes and primary normal human diploid dermal fibroblasts, in addition to a CHO AA8 cell line.

Findings: After 6-h treatment with kaolin micro- and nano-particles of particle sizes 4.8 μm and 0.2 μm (200 nm), respectively, the frequencies of micronucleated cells increased in a dose-dependent manner. The frequency increased 3- to 4-fold by exposure to the particles at 200 μg/mL (i.e., 31.4 μg/cm^2) in all cells tested. Two-way ANOVA revealed a significant main effect of particle size, and the nano-particles tended to have a higher potency of micronucleus (MN) induction. However, the cell type did not significantly affect the MN frequencies. In addition, one-hour treatment with the kaolin particles increased DNA damage in a dose-dependent manner in a comet assay. The %tail DNA was increased 8- to 20-fold by exposure to the particles at 200 μg/mL, for all cells tested. The kaolin nano-particles had higher DNA-damaging potency than the micro-particles. Furthermore, treatment with kaolin particles dose-dependently increased the production of reactive oxygen species (ROS) in all cells. Again, we observed that kaolin nano-particles induced more ROS than the micro-particles in all cells.
Conclusion: Kaolin particles demonstrated genotoxicity in primary normal human diploid epidermal keratinocytes and fibroblasts as well as in CHO AA8 cells. Although no significant difference was observed among these three types of cells, fine particles of kaolin tended to have higher genotoxic potency than coarse particles. **Since studies on its genotoxicity to skin have been scarce, the findings of the present study could contribute to safety evaluations of kaolin particles when used as a white cosmetic pigment**.

2.
Totsuka Y, Higuchi T, Imai T, Nishikawa A, Nohmi T, Kato T, Masuda S, Kinae N, Hiyoshi K, Ogo S, Kawanishi M, Yagi T, Ichinose T, Fukumori N, Watanabe M, Sugimura T, Wakabayashi K.

Genotoxicity of nano/microparticles in in vitro micronuclei, in vivo comet and mutation assay systems. Part Fibre Toxicol. 2009 Sep 3;6:23. doi: 10.1186/1743-8977-6-23. PMID: 19725983; PMCID: PMC2745356.
Full text available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2745356/

Abstract

Background: Recently, manufactured nano/microparticles such as fullerenes (C60), carbon black (CB) and ceramic fiber are being widely used because of their desirable properties in industrial, medical and **cosmetic** fields. However, there are few data on these particles in mammalian mutagenesis and carcinogenesis. To examine genotoxic effects by C60, CB and kaolin, an in vitro micronuclei (MN) test was conducted with human lung cancer cell line, A549 cells. In addition, DNA damage and mutations were analyzed by in vivo assay systems using male C57BL/6J or gpt delta transgenic mice which were intratracheally instilled with single or multiple doses of 0.2 mg per animal of particles.

Results: In in vitro genotoxic analysis, increased MN frequencies were observed in A549 cells treated with C60, CB and kaolin in a dose-dependent manner. These three nano/microparticles **also induced DNA damage in the lungs** of C57BL/6J mice measured by comet assay. Moreover, single or multiple instillations of C60 and kaolin, increased either or both of gpt and Spi- mutant frequencies in the lungs of gpt delta transgenic mice. Mutation spectra analysis showed transversions were predominant, and more than 60% of the base substitutions occurred at G:C base pairs in the gpt genes. The G:C to C:G transversion was commonly increased by these particle instillations.

Additional language from the Conclusion of this paper:

"***This study demonstrated the genotoxicity of nano/microparticles widely used for industrial, cosmetic and medical fields****. In in vitro genotoxic analysis, increased MN frequencies were observed in A549 cells treated with C_{60}, CB and* ***kaolin*** *in a dose-dependent manner. On the other hand, these three particles also induced DNA damage in the lungs of C57BL/6J mice measured by comet assay. Furthermore, we found that C_{60} and* ***kaolin*** *demonstrated mutagenicity either or both of gpt and Spi- mutations in the gpt delta transgenic mice systems."*

This paper also referenced another paper (Bunn, 1993) which investigated the carcinogenicity of kaolin. It states:

"*In 1993, W. B. Bunn 3rd et al. reported that increased incidences of lung tumors and mesotheliomas were observed in long-term inhalation studies of rats and hamsters treated with micro-sized refractory ceramic fibres containing* ***kaolin*** *as the main component* [16]."

{16} Bunn WB, 3rd, Bender JR, Hesterberg TW, Chase GR, Konzen JL. Recent studies of man-made vitreous fibers. Chronic animal inhalation studies. J Occup Med. 1993;35:101–113. doi: 10.1097/00043764-199302000-00009. https://pubmed.ncbi.nlm.nih.gov/8166769/

Kato T, Toyooka T, Ibuki Y, Masuda S, Watanabe M, Totsuka Y. Effect of physicochemical character differences on the genotoxic potency of kaolin. Genes Environ. 2017 May 1;39:12. doi: 10.1186/s41021-017-0075-y. PMID: 28469735; PMCID: PMC5410691.
Full text available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5410691/

Abstract

Background: Kaolin is white clay mineral with the chemical composition Al2Si2O5(OH)4, and many varieties of kaolins having different crystal structures are utilized in industrial, **cosmetic** and

medical fields. To evaluate the effect of physicochemical character differences on the genotoxicity of kaolin, two types of kaolin, kaolin-S with smooth, sphere-shaped crystals, and kaolin-P with clusters of thin pseudohexagonal plates, were used in the study.

Results: ICR mice were intratracheally instilled with the kaolins (0.05 and 0.2 mg/mouse), and comet assay was performed on their lungs. **Both kaolins showed DNA damage in the lungs of the mice**, however the DNA damaging potency was much higher with kaolin-P than that with kaolin-S. In order to clarify the mechanisms for the different genotoxic potency, we examined the incorporation rate and ROS generation of these two types of kaolin in alveolar epithelial A549 and macrophage-like RAW264 cells, using flow cytometric (FCM) analysis. Kaolin-P showed a higher incorporation rate into the mammalian cells and ROS generation than that of kaolin-S. Especially, RAW264 cells aggressively incorporated kaolins, and generated ROS, whereas almost no ROS generation was observed in A549 cells. In addition, inflammatory cytokines were quantified, using the ELISA method, to understand further genotoxic potency differences of kaolins. **Concentrations of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the media were increased by exposure to both kaolins**, but in the case of kaolin-P, these inflammatory cytokines were significantly elevated. Based on these findings, differences of genotoxic potency may contribute to incorporation rates into immune cells. Furthermore, it is likely that immune cells and epithelial cells might closely interact with each other for the appearance of genotoxocity in vivo. In order to clarify the interaction between epithelial and immune cells, A549 and RAW264 were co-cultured and RAW264 cells only were exposed to kaolins, then subsequently A549 was applied to FCM analysis and comet assay. DNA damage observed in the A549 cells markedly increased in the presence of kaolin-exposed RAW264 cells compared to the single culture.

Conclusion: From these observations, it is suggested that mechanisms of kaolin genotoxicity against epithelial cells are through the activation of macrophage cells. Therefore, it is thought that interactions between epithelial and immune cells would be very important for evaluation of the genotoxicity of fine particulate matter. We also showed here that co-culture models of epithelial and immune cells could be used as suitable models for evaluation of lung genotoxicity of fine particulate matter, including nanomaterials, as in vivo mimicking systems.

Shan X, Liu L, Li G, Xu K, Liu B, Jiang W. $PM_{2.5}$ and the typical components cause organelle damage, apoptosis and necrosis: Role of reactive oxygen species. Sci Total Environ. 2021 Aug 15;782:146785. doi: 10.1016/j.scitotenv.2021.146785. Epub 2021 Mar 27. PMID: 33838376. https://pubmed.ncbi.nlm.nih.gov/33838376/

Abstract

In this research, the organelle damage, apoptosis and necrosis induced by PM2.5, BC and **Kaolin were studied using human bronchial epithelial (16HBE) cells**. PM2.5, BC and **Kaolin all induce cell death, LDH release and excess intracellular ROS generation**. For the organelle injuries, Kaolin and high-dose PM2.5 (240 μg/mL) cause lysosomal acidification, but BC causes lysosomal alkalization (lysosomal membrane permeabilization, LMP). BC and Kaolin cause the loss of mitochondrial membrane potential (MMP), while PM2.5 does not. For the cell death mode, PM2.5 causes both apoptosis and necrosis. However only necrosis has been detected in the BC and Kaolin treated groups, indicating the more severe cellular insult. Excess ROS generation is involved in the organelle damage and cell death. ROS contributes to the BC-induced LMP and necrosis, but does not significantly affect the Kaolin-induced MMP loss and necrosis. Therefore, the BC component in

PM2.5 may cause cytotoxicity via ROS-dependent pathways, the Kaolin component may damage cells via ROS-independent mechanisms such as strong interaction. The PM2.5-induced apoptosis and necrosis can be partially mitigated after the removal of ROS, indicating the existence of both the ROS-dependent and ROS-independent mechanisms due to the complicated PM2.5 components. BC represents the anthropogenic source component in PM2.5, while Kaolin represents the natural source component. Our results provide knowledge on the toxic mechanisms of typical PM2.5 components at the cellular and subcellular levels.

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