Safety Assessment of Diatomaceous Earth as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (May 27, 2022) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. Previous Panel member involved in this assessment: Lisa A. Peterson, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

BAL	bronchoalveolar lavage
CIR	Cosmetic Ingredient Review
CHO	Chinese hamster ovary
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DART	developmental and reproductive toxicity
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
ECHA	European Chemicals Agency
FDA	Food and Drug Administration
GRAS	generally recognized as safe
HET-CAM	chorioallantoic membrane of a fertilized hen's egg
HRIPT	human repeated insult patch test
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life or health
ILO	Intentional Labor Office
LLNA	local lymph node assay
mppcf	million particles per cubic foot
NIOSH	National Institute for Occupational Safety and Health
NMRD	non-malignant respiratory disease
NOAEC	no-observable-adverse-effect-concentration
NR	not reported/none reported
OECD	Organization for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
Panel	Expert Panel for Cosmetic Ingredient Safety
PEL	permissible exposure limit
ppm	parts per million
REL	recommended exposure limit
SCOGS	Select Committee on GRAS Substances
SHE	Syrian hamster embryos
SI	stimulation index
SMR	standardized mortality ratio
SWeRF	size-weighted relevant fine fraction
TG	test guideline
TWA	time weighted average
UICC	Union for International Cancer Control
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Diatomaceous Earth as used in cosmetic formulations. It is reported to function as an abrasive, absorbent, anticaking agent, bulking agent, and opacifying agent in cosmetic products. The Panel reviewed all relevant data, and concluded that Diatomaceous Earth is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of Diatomaceous Earth as used in cosmetic formulations. Diatomaceous Earth is reported to function as an abrasive, absorbent, anticaking agent, bulking agent, and opacifying agent in cosmetics, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*).[1]

The Expert Panel for Cosmetic Ingredient Safety (Panel) has reviewed related ingredients. In a report that was finalized in 2019, the Panel concluded that synthetically-manufactured amorphous silica and hydrated silica are safe in the present practices of use and concentration when formulated to be non-irritating.[2] Diatomaceous Earth is considered a natural amorphous form of silica. Synthetically-manufactured amorphous silica and hydrated silica are neither part of this safety assessment, nor are data from those reports included in this assessment; however, the reports on these ingredients are available on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on Diatomaceous Earth included in this safety assessment were obtained from assessments by the International Agency for Research on Cancer (IARC)[3] and the Agency for Toxic Substances and Disease Registry (ATSDR),[4] as well as from robust summaries of data submitted to the European Chemical Agency (ECHA; listed as Kieselguhr)[5] by companies as part of the REACH chemical registration process. These data summaries are available on the IARC, ATSDR, and ECHA websites, respectively, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition

Diatomaceous Earth (CAS No.61790-53-2 or 68855-54-9) is defined by the *Dictionary* as a mineral material consisting chiefly of the siliceous frustules and fragments of various species of diatoms, which may or may not be calcined.[1] [A frustule is the cell wall of a diatom]. Natural calcined and uncalcined forms are associated with the CAS No. 61790-53-2, and the flux-calcined form is associated with the CAS No. 68855-54-9.[3,6] The "calcined" form is processed Diatomaceous Earth that is heated to 800 - 1000 ºC to eliminate organic and carbonaceous material.[7] The "flux-calcined" form is Diatomaceous Earth that is heated with the addition of sodium carbonate as a fluxing agent that results in a coarser material. Diatomaceous Earth is considered a natural amorphous form of silica.[3,8]

Diatomaceous Earth is a polymorph of silica, or silicon dioxide.[3,4] Silica may exist in amorphous or crystalline structures. While both forms are made up of silicon-oxygen tetrahedra, crystalline silica is determined by a regular, repeating arrangement of the silicon and oxygen tetrahedra, while the arrangement of bonds in amorphous silica is highly disordered and randomly linked. Silica can be sourced naturally as a mineral, biogenically through diatoms, or it can be synthetically produced. Natural and biogenic forms of amorphous silica include opal, Diatomaceous Earth, silicates and volcanic glass; while natural forms of crystalline silica include quartz, cristobalite, flint, and sandstone.

Chemical Properties

Available chemical properties for Diatomaceous Earth are provided in Table 1. Particle size distributions for Diatomaceous Earth (flux-calcined) for coarse, medium, and fine-grade materials were 59.5%, 81.6%, and 99.6% less than 90 µm, respectively, and 4.56%, 14.7%, and 58.7% less than 10 µm, respectively (Table 2).[5] Diatomaceous Earth has an infinite variety of shapes, due to its origins in the living matter (diatoms) from which it formed.[3]

Method of Manufacture

Diatomaceous Earth is obtained by strip mining, commonly from the western portion of the United States (US).[9] Diatomaceous Earth is also mined in western Canada, France, Denmark, Spain, Iceland, Romania, the Czech Republic, Algeria, Kenya, Morocco, Japan, South Korea, China, Australia, New Zealand, Mexico, Peru, Argentina, Costa Rica, Chile, Brazil, Colombia, and Peru.[10] Following extraction from a mine, the raw material is crushed, dried, ground, purified and alimented.[7] The resulting material may be used as-is (natural or milled product), or can be further process by heating (800 - 1000 ºC) in one of two ways to produce a "calcined" product or a "flux-calcined" product.[7,10] After heating, the material is then cooled and further ground before packaging. In commercial products, a large proportion of the amorphous silica in Diatomaceous Earth is converted into a crystalline form (cristobalite, up to 40% to 60%) during thermal processing.[3,10]

The International Diatomite Producers Association and a supplier have reported that natural and flux-calcined Diatomaceous Earth are used in the formulation of cosmetic products.[11,12] The flux-calcined material is produced through the following steps: harvesting, calcination, milling, sieving, quality control, packaging, and quality control. [11]

Composition and Impurities

The composition of Diatomaceous Earth varies depending on where it is mined and how it is processed.[13] Silica content in Diatomaceous Earth can vary between 68% to 96%.[3,10,13-16] Other components may include aluminum (III) oxide (~4 - 7%), iron (III) oxide (~1 - 4%), titanium (IV) oxide; ions of calcium, magnesium, sodium, and potassium; and phosphates.[3,10,13,15,16] Many elements are present in trace amounts, and co-deposited and secondary minerals can include clays, quartz, gypsum, mica, calcite, feldspars, salt, pyrite, sulfur, manganese nodules, and phosphates.[10] Diatomaceous Earth usually contains 0.1% to 4% quartz.[3] Chert and volcanic ash can be abundant constituents of the sediment, and common biogenic constituents include the siliceous remains of sponges, silicoflagellates, radiolaria, carbonized fossil leaves, and fossilized fish bones.[10] Chemical and mineral impurities can affect the properties of the final Diatomaceous Earth product, including pH, solubles present, density, and abrasiveness: commercial uses can be adversely affected unless contaminants can be removed or made insoluble through processing.

Crystalline silica content of Diatomaceous Earth is dependent on the degree of exposure to high temperatures and pressures; surface chemistry of an individual Diatomaceous Earth sample may vary, depending upon production method and degree of hydration.[4] The crystalline silica content of uncalcined Diatomaceous Earth is 0.1% to 4.0%. Cristobalite content of straight-calcined flux products is between 10% to 20%, and between 40% to 60% in flux-calcined products.[3,17]

A supplier has reported that a product containing 100% Diatomaceous Earth has < 1% respirable crystalline silica.[18] Another product containing 9-11% Diatomaceous Earth was reported to have < 0.11% respirable crystalline silica. This product also contained 57% - 61% *Lithothamnion calcareum* powder, 29% - 31% mannitol, and 0.7% - 1.5% zinc sulfate.[19] This supplier has reported that flux-calcined Diatomaceous Earth is used in the finished products at concentrations below 10% and has a respirable crystalline silica content of < 1% (cristobalite) based on the size-weighted relevant fine fraction (SWeRF) method of analysis.[11]

According to international standards for food additives, Diatomaceous Earth should not contain more than 10 mg/kg arsenic or lead.[6]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. The cosmetic product categories named in the VCRP database indicate the intended uses of cosmetic ingredients, and are identified in 21 CFR§720. Data are submitted by the cosmetic industry in response to a survey conducted by the Personal Care Products Council (Council) of maximum reported use concentrations, also by product category. Neither the categories provided by the VCRP, nor those provided by the Council survey, include a designation for use via airbrush application. Airbrush devices, alone, are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients used in airbrush devices are within the jurisdiction of the FDA. As airbrush technology use for cosmetics has neither been evaluated by the CPSC, nor the use of cosmetic ingredients in airbrush technology by the FDA, no US regulatory authority has evaluated the safety of this delivery methodology for cosmetic ingredients. Moreover, no consumer habits and practices data are available to evaluate the risks associated with this use type.

According to 2022 VCRP survey data, Diatomaceous Earth is used in a total of 135 formulations (Table 3).[20] Of these reported uses, the majority are in leave-on products, with over a third of the uses (50) reported to be in nail products. Twenty-five uses are reported to be in rinse-off paste masks (mud packs). While uses were reported in a number of categories in the VCRP, the results of the concentration of use survey conducted by the Council in 2021 reported uses for Diatomaceous Earth in only 3 categories: at 0.001% in hair dyes and colors, up to 0.01% in nail polish and enamel, and at 2% in rinse-off products (paste masks).[21]

Diatomaceous Earth may be used in products that can come into contact with the eyes or mucous membranes; for example, it is reported to be used in eye shadow, eye lotion, bath soaps and detergents, and other personal cleanliness products (concentrations not reported).[20,21] It is also reported to be used in products which maybe incidentally ingested, such as lipsticks and dentifrices (concentrations not reported). Additionally, Diatomaceous Earth is reported to be used in face powders (concentration not reported), and could possibly be inhaled. 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 cosmetics would be deposited in the nasopharyngeal and tracheobronchial regions 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.

Additionally, although products containing this ingredient may be marketed for use with airbrush technology, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of this ingredient (and without consumer habits and practices data related to this use technology), the data are insufficient to evaluate the safety thereof in airbrush applications.

Diatomaceous Earth is not restricted from use in any way under the rules governing cosmetic products in the European Union.[22]

Non-Cosmetic Use

Diatomaceous Earth has uses in food and beverages, including anticaking material foodstuffs and clarifier in wine and beer.[23] In 1979, the Select Committee on GRAS (generally recognized as safe) Substances (SCOGS) opined that Diatomaceous Earth is GRAS as a filtering aid in such food and beverages as apple cider, beer, beet and cane sugar, vinegar, and wine in natural, calcined, or flux-calcined forms.[24] Diatomaceous Earth is also GRAS as a substance migrating to food from paper and paperboard products (21CFR§182.90). Diatomaceous Earth is approved as an indirect food additive with use as a polymer (21CFR§177.2410), as a component of paper and paperboard (21CFR176.170), and as a colorant for polymers (21CFR§178.3297). It is an approved food additive in animal feed with the restrictions that it cannot contain more than 15 ppm lead, 20 ppm arsenic, and 600 ppm fluorine (21CFR§573.340).

The use of Diatomaceous Earth as a drug carrier is being investigated.[25,26] Diatomaceous Earth is an approved inactive ingredient in approved drug products, including capsules and tablets taken orally and in topical soaps.[27]

Diatomaceous Earth is used in refractory and insultation bricks, filtration media, fertilizers, abrasives, insulation materials, lubricants, paints, rubbers, absorbents, bulking agents, and as carriers for catalysts.[9,10,17,23] It is also widely used in pesticide formulations.[10,14,17,23,28,29]

TOXICOKINETIC STUDIES

No toxicokinetic studies were discovered in the published literature, and no unpublished data were submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

In an oral study in accordance with Organization for Economic Co-operation and Development (OECD) test guideline (TG) 401, female Wistar rats received 300 mg/kg (1 rat) or 2000 mg/kg (5 rats) flux-calcined Diatomaceous Earth in arachis oil by gavage.[5] The purity of the test material was not stated. Clinical observations were made at 0.5, 1, 2, and 4 h post-dosing, and then daily for 14 d. Morbidity and mortality were checked twice daily and body weights were recorded on days 0, 7, and 14. No mortalities were observed at either dose level. No signs of systemic toxicity were observed at 300 mg/kg; however, at 2000 mg/kg, clinical signs of toxicity included hunched posture in all animals and ataxia in one animal. All animals had expected body weights gains, and no abnormalities were observed at necropsy. The LD_{50} for Diatomaceous Earth in this study was greater than 2000 mg/kg.

Inhalation

In a dust aerosol study in accordance with OECD TG 403, 5 male and 5 female Wistar rats received 2.7 mg/l flux-calcined Diatomaceous Earth (100%; target particle size 1 to 4 µm).[5] The rats were exposed to the test material nose-only for 24 h. Clinical observations were made during exposure, immediately after exposure, and 1 h after exposure, and then once daily for 14 d. Body weights were recorded on test days 1 (before exposure), 2, 4, 8, and 15 (before necropsy). No mortalities were observed. Clinical signs of toxicity included moderately-ruffled fur in all animals on test day 1 that persisted until day 2, and slight nose scabbing on day 1 in all animals. Marginal to slight body weight loss was noted in all males and 4 females on day 1 and 2 but returned to expected gains thereafter. No abnormalities were observed at necropsy. The LC_{50} for Diatomaceous Earth in this study was greater than 2.7 mg/l.

Short-Term, Subchronic, and Chronic Dose Toxicity Studies

Repeated dose oral and inhalation studies summarized here are described in Table 4. In 13-wk dietary studies, rats that received up to 5% natural or flux-calcined Diatomaceous Earth did not exhibit effects outside of increased body weight gains starting at 3% natural Diatomaceous Earth in one study.[5,30]

In inhalation studies, a no-observable-adverse-effect-concentration (NOAEC) could not be determined in a 28-d inhalation rat study of 100% pure flux-calcined Diatomaceous Earth (particle size range 1 to 3 µm) tested at 0, 0.044, 0.207, or 0.7 mg/l.[5] In a 2-yr rat inhalation study of a flux-calcined Diatomaceous Earth at up to 5 million particles per cubic foot (mppcf) per day plus 50 mppcf for 1 h three times per week (5 + 50 mppcf), no fibrosis was observed.[15] Perivascular and peribronchiolar localization of dust-laden macrophages were observed in both the 2 and 5 mppcf dose groups, and nodular lesions and reactions of the nodes were greater in the 5 mppcf dose group. A similar study of the same test material in guinea pigs also found no fibrosis after 1.5 yr, and a light increase in intra-alveolar macrophages with peribronchiolar localization in the 5 mppcf group. In another guinea pig study of unheated and heated Diatomaceous Earth (particle size range ~0.45 µm to > 10 µm), no fibrosis was noted during observations made at 2-3 mo intervals until study end at 2 yr.[31] No fibrosis was observed in mongrel dogs exposed to up to 5 mppcf flux-calcined Diatomaceous Earth for up to 2.5 yr.[15]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

No DART studies were discovered in the published literature, and no unpublished data were submitted.

GENOTOXICITY STUDIES

In vitro genotoxicity studies summarized here are described in Table 5. Diatomaceous Earth (100% pure flux-calcined) was not mutagenic in an Ames test (up to 5000 µg/plate) or a mouse lymphoma cell gene mutation test (up to 40 µg/ml), and was not clastogenic in a human lymphocyte chromosome aberration test (up to 40 µg/ml).[5] Abnormal cell proliferation, colony-forming efficiency, and nuclei formation was observed in Chinese hamster ovary (CHO) cells in assays with unprocessed and flux-calcined Diatomaceous Earth (1.3 µm and 2.1 µm, respectively; concentrations tested not reported).[16] In studies with Syrian hamster embryo (SHE) cells treated with high temperature calcined and flux-calcined Diatomaceous Earth, concentration-dependent increases in cell division aberrations and cell transformations were observed; the induction of transforming potency was correlated with the amount of hydroxyl radicals generated.[32-34] Cell transformation was decreased or not observed in SHE cells exposed to uncalcined Diatomaceous Earth samples where the likelihood of radical generation was decreased or non-existent.

CARCINOGENICITY STUDIES

The International Agency for Research on Cancer (IARC) has determined that "there is *inadequate evidence* in experimental animals for the carcinogenicity of uncalcined Diatomaceous Earth." Overall, amorphous silica is not classifiable as to its carcinogenicity to humans (Group 3).[3]

Oral

In a feeding study, a group of 30 weanling Sprague-Dawley rats (sex not reported) received 20 mg/d Diatomaceous Earth (particle size not reported) mixed with cottage cheese at a concentration of 5 mg/g cheese.[35] The rats also received commercial rat chow and filtered tap-water ad libitum. A control group of 27 rats was only fed commercial rat chow. The animals were observed for their life span (mean survival following the start of treatment for treated rats was 840 d, and for control rats was 690 d). Complete gross and microscopic thoracic and abdominal necropsies were performed on each animal upon expiration, with special attention given to the gastrointestinal tract. During the course of the study, 5 malignant tumors (1 salivary gland carcinoma, 1 skin carcinoma, 2 sarcomas of the uterus, and 1peritoneal mesothelioma) and 13 benign tumors (9 mammary fibroadenomas, 1adrenal pheochromocytoma, and 3 pancreatic adenomas) were observed in the treated animals. The control group had 3 carcinomas (1 each in the lung, forestomach and ovary) and 5 mammary fibroadenomas. The authors determined that the difference in tumor incidence between treated and control rats was not statistically significant ($0.25 < p < 0.5$, χ^2-test).

Subcutaneous

A group of 36 female Marsh mice, 3-mo-old, received a subcutaneous injection of 20 mg Diatomaceous Earth (uncalcined, particle size, 3 - 9 µm, with some crystalline materia1 of larger size) suspended as a 10% slurry in isotonic saline (volume unspecified).[3] Another group of 36 female littermates received an injection of 0.2 ml saline only. The numbers of mice still alive at 19 mo were 19/36 in the treated group and 20/36 in the control group. The treated group showed an extensive reactive granulomatous and fibroplastic reaction at the site of injection, but no malignant tumors. No further details were available.

Intraperitoneal

In another study by the same researchers, a group of 29 female Marsh mice, 3-mo-old, received an intraperitoneal injection of 20 mg Diatomaceous Earth suspended as a 10% slurry in isotonic saline.[3] A group of 32 female littermates received an injection of the same volume of saline only (volume unspecified). The numbers of mice still alive at 19 mo were 1l/29 in the treated group and 19/32 in the control group. Lymphosarcomas at the injection site in the abdominal cavity were reported in 6/17 treated animals and 1/20 controls (p = 0.02; method of statistical analysis unspecified). No further details were available.

OTHER RELEVANT STUDIES

Pulmonary Response

The following summaries demonstrate the physiological changes to the pulmonary system when Diatomaceous Earth enters the lung. In an intratracheal study, groups of 6 male Sprague-Dawley rats received a single instillation of Diatomaceous Earth (90% amorphous silica; particle size < 7 µm) suspended in isotonic saline.[36] Rats that underwent bronchoalveolar lavage (BAL) examinations received 10 mg/animal, and rats that underwent lung biochemical examinations received 15 mg/animal. Determinations in the BAL and phospholipids in the lung tissue were determined after 15, 60, and 180 d and 90, 180, and 360 d, respectively. Acute/subacute inflammation was observed that gradually became moderate after 60 d. No further details provided.

In another intratracheal study, groups of Hartley-Duncan guinea pigs (sex not specified) received a single instillation of 25 mg flux-calcined Diatomaceous Earth (particles ≤ 3.0 µm in diameter; 72% silica and 28% calcium silicates) in 0.5 ml physiological saline.[37] A control group of 2 animals received 0.5 ml saline only. After 2 or 4 h, 1, 2, 3, 4, 5, 6, or 7 d, and 5, 6, or 15 mo, 2 animals/time period were killed and lungs were dissected. No signs of infection nor significant individual variation in response within time period were observed. Pronounced neutrophil invasion of the bronchioles was observed by 4 h post-exposure, which remained well developed through 1 d post-exposure. The number of macrophages and neutrophils in the alveoli increased through 1 d post-exposure and remained greater than control values through 7 d post-exposure. The number of macrophages, many of which contained Diatomaceous Earth, remained elevated for the duration of the experiment. Phagocytosis of the particles was mainly performed by the macrophages, with some participation by the neutrophils. Many of the reactive macrophages in the groups longer than 2-h post-exposure had various types of pathological alterations. Some particles were found in type I epithelial cells. Edematous changes were observed in some type I epithelial cells, and proliferation of type II epithelial cells was observed in some alveoli, especially near the respiratory bronchiole. Mild, diffuse fibrosis was observed starting at 6 mo post-exposure and persisted at 15 mo post-exposure.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation, sensitization, and phototoxicity studies summarized here are described in Table 6. Diatomaceous Earth (flux-calcined, up to 100% pure) was considered non-corrosive and non-irritating in EpiSkin™ reconstituted human epidermis model tests.[5] In acute skin tolerance patch tests, Diatomaceous Earth (flux-calcined) was not irritating in 10 healthy volunteers at 100% or in 11 volunteers with sensitive skin in a product at 9% - 11%.[38,39] Diatomaceous Earth was not sensitizing in a local lymph node assay (LLNA) at up to 10%.[5] A cosmetic product containing 9% - 11% Diatomaceous Earth (soda ash flux-calcined) was not sensitizing in a human repeated insult patch test (HRIPT) of 100 healthy subjects when tested at a 10% dilution, nor was it phototoxic in a human single application study in 10 healthy female subjects when tested neat.[40,41]

OCULAR IRRITATION STUDIES

In Vitro

The ocular irritation potential of Diatomaceous Earth (flux-calcined, purity not reported) was assessed in a SkinEthic™ reconstituted human corneal epithelium model test.[5] The test material was used as supplied, and 30 mg was applied to the tissue cultures. Triplicate cultures were exposed for 10 min, and then examined after 3 h. Viability of the tissues following exposure to the test material was 99.1% and the qualitative evaluation of the tissue following exposure indicated it was viable. The positive and negative controls yielded expected results. Based on the results of the study, the test material was considered non-irritating.

In another in vitro study, the ocular irritation potential of a formulations containing 9% - 11% Diatomaceous Earth (soda ash flux-calcined) was assessed using the chorioallantoic membrane of a fertilized hen's egg (HET-CAM test).[42] The material was tested at 2%, 5%, and 10% w/v dilutions in water. Approximately 0.3 ml of the sample was spread over membrane and rinsed with 5 ml of demineralized water after 20 s. The test material was non-irritating at the 2% and 5% dilution, but moderately irritating at the 10% dilution. The 10% dilution had low solubility and rapid sedimentation; however, the results were reproducible between eggs and were considered relevant.

Animal

The ocular irritation potential of Diatomaceous Earth (flux-calcined, purity not reported) was assessed in 2 New Zealand White rabbits (sex not reported) in accordance with OECD TG 405.[5] The undiluted test material was instilled at a volume of 0.1 ml in the right eye of the animals. The left eye was left untreated as a control. After instillation, the rabbits were observed for 72 h. No corneal effects were reported. Iridial inflammation was reported in one animal at 1 and 24 h post-instillation. Moderate conjunctival irritation was noted in both animals at 1 and 24 h post-instillation, and up to 48 h post-instillation in 1 animal. Both animals had recovered by 72 h post-instillation. The test material was considered to be non-irritating to the eye in this study.

CLINICAL STUDIES

Case Report

A 51-yr-old male employed in the Diatomaceous Earth industry for 26 yr (20 yr in a mill, 6 yr in an office) was reported to have a history of a recurrent peptic ulcer, pleurisy, and bronchopneumonia, with frequent attacks of bronchitis.[43] The patient was a nonsmoker. An electrocardiogram indicated right ventricular hypertrophy. The patient had a 4-yr history of intermittent palpitation, severe exertional moderate paroxysmal dyspnea, and orthopnea. He also complained of wheezing and hoarseness, with productive cough, until a year and a half before presentation. Cough, but not dyspnea, was relieved by bronchodilator aerosols. At physical examination, no apparent distress or cyanosis were noted; however, slight clubbing of the fingers was observed. Rales were detected over most of the chest except in infraclavicular areas anteriorly. Resonance was diminished over the upper lung fields posteriorly, and on the left anteriorly. Chest films were interpreted as consistent with far-advanced coalescent pneumoconiosis. The patient died 5 yr after the chest films were made, reportedly due to heart failure from cor pulmonale.

OCCUPATIONAL EXPOSURE STUDIES

Occupational exposure studies are described in Table 7. Occupational exposure studies indicate a risk of pneumoconiosis in Diatomaceous Earth mine and mill workers, which can be mitigated with dust control measures and personal protective equipment.[44-49] Studies were of quarry and mill workers in the western US and exposures were to raw, calcined, or flux-calcined Diatomaceous Earth.

OCCUPATIONAL EXPOSURE LIMITS

Occupational exposure to Diatomaceous Earth, and the quartz and amorphous silica dust it contains, can occur during mining, the calcination process, and through handing the calcined product in end-use industries as a filtration agent, mineral charge, refractory, abrasive, carrier, or adsorbent.[3] The National Institute for Occupational Safety and Health (NIOSH) time weighted average (TWA) for recommended exposure limits (REL) for Diatomaceous Earth (also characterized as amorphous silica) is 6 mg/m^3, and the Occupational Safety and Health Administration (OSHA) TWA permissible exposure limit (PEL) is 20 mppcf (80 mg/m^3/% silicon dioxide).[50] The immediately dangerous to life or health (IDLH) value is 3000 mg/m^3.

SUMMARY

Diatomaceous Earth is reported to function as an abrasive, absorbent, anticaking agent, bulking agent, and opacifying agent in cosmetics. The “calcined” form is processed Diatomaceous Earth that is heated to 800 - 1000 ºC to eliminate organic and carbonaceous material. The “flux-calcined” form is Diatomaceous Earth that is heated with the addition of sodium carbonate as a fluxing agent that results in a coarser material). Diatomaceous Earth is considered a natural amorphous form of silica.

The composition of Diatomaceous Earth varies depending on where it is mined and how it is processed. Silica content in Diatomaceous Earth can vary between 83% to 96%. Crystalline silica content of Diatomaceous Earth is dependent on the degree of exposure to high temperatures and pressures; surface chemistry of an individual Diatomaceous Earth sample may vary, depending upon production method and degree of hydration. The crystalline silica content of uncalcined Diatomaceous Earth is 0.1% to 4.0%. Cristobalite content of straight-calcined flux products is between 10% to 20%, and between 40% to 60% in flux-calcined products.

According to 2022 VCRP survey data, Diatomaceous Earth is used in a total of 135 formulations. Of these reported uses, the majority are in leave-on products with over a third of the uses (49) reported to be in nail polish and enamel. Twenty-five uses are reported to be in rinse-off paste masks (mud packs). The results of the concentration of use survey conducted by the Council in 2021 indicate that Diatomaceous Earth is used at 0.001% in hair dyes and colors, up to 0.01% in nail polish and enamel, and at 2% in rinse-off products (paste masks). Diatomaceous Earth is reported to be used in cosmetic powders, and could possibly be inhaled; for examples, it is reported to be used in face powders (concentration not reported).

In a 90-d dietary study, male and female rats were fed a diet containing 5% Diatomaceous Earth. (Estimated intake ranged from about 12 g/kg bw/d at the start of the experiment to about 5 g/kg at the end of the experiment.) Residual silica values in the organs of treated rats were comparable with the controls.

In oral rat studies with flux-calcined Diatomaceous Earth, the LD_{50} was greater than 2000 mg/kg. The LC_{50} was greater than 2.7 mg/l in a 24 h inhalation rat study of flux-calcined Diatomaceous Earth.

In 13-wk dietary studies, rats that received up to 5% natural or flux calcined Diatomaceous Earth did not exhibit adverse effects outside of increased body weight gains in one study. An NOAEC could not be determined in a 28-d inhalation rat study of 100% pure flux-calcined Diatomaceous Earth (particle size range 1 to 3 µm) at up to 0.7 mg/l. In a 2-yr rat inhalation study of a flux-calcined Diatomaceous Earth at up to 5 mppcf, no fibrosis was observed. Perivascular and peribronchiolar localization of dust-laden macrophages were observed in the 2 and 5 mppcf dose groups and nodular lesions and reactions of the nodes was greater in the 5 mppcf dose group. A similar study of the same test material in guinea pigs also found no fibrosis after 1.5 yr and a light increase in intra-alveolar macrophages with peribronchiolar localization in the 5

mppcf group. In another guinea pig study of unheated and heated Diatomaceous Earth (particle size range ~0.45 µm to > 10 µm), no fibrosis was noted during observations made at 2-3 mo intervals until study end at 2 yr. No fibrosis was observed in mongrel dogs exposed to up to 5 mppcf flux-calcined Diatomaceous Earth for up to 2.5 yr.

Diatomaceous Earth (100% pure flux-calcined) was not mutagenic in an Ames test (up to 5000 µg/plate) or a mouse lymphoma cell gene mutation test (up to 40 µg/ml); and was not clastogenic in a human lymphocyte chromosome aberration test (up to 40 µg/ml). Abnormal cell proliferation, colony-forming efficiency, and nuclei formation was observed in CHO cells in assays with unprocessed and flux-calcined Diatomaceous Earth (1.3 µm and 2.1 µm, respectively; concentrations tested not reported). In studies with SHE cells, high temperature calcined and flux-calcined Diatomaceous Earth had increased cell division aberrations and cell transformations in a concentration-dependent manner; the induction of transforming potency was correlated with the amount of hydroxyl radicals generated. Cell transformation was decreased or not observed in SHE cells exposed to uncalcined Diatomaceous Earth samples where the likelihood of radical generation was decreased or non-existent.

IARC has determined that there is inadequate evidence in experimental animals for the carcinogenicity of uncalcined Diatomaceous Earth. In an oral feeding study in Sprague-Dawley rats that received 20 mg/d Diatomaceous Earth in cottage cheese, there was no statistically significant difference in cancer incidence between treated and control rats. A subcutaneous study in mice found uncalcined Diatomaceous Earth led to extensive reactive granulomatous and fibroplastic reactions at the injection site, but no malignant tumors were observed. The same research group performed an intraperitoneal study in mice and found lymphosarcomas at the injection site in the abdominal activity.

In an intratracheal rat study of Diatomaceous Earth that was 90% amorphous silica, acute/subacute inflammation was observed that gradually became moderate after 60 d. Guinea pigs that received a single 25 mg intratracheal instillation had mild, diffuse fibrosis observed starting 6 mo after exposure that persisted to 15 mo.

Diatomaceous Earth (flux-calcined, up to 100% pure) was considered non-corrosive and non-irritating in EpiSkin™ reconstituted human epidermis model tests. In acute skin tolerance patch tests, Diatomaceous Earth (flux-calcined) was not irritating in 10 healthy volunteers at 100% or in 11 volunteers with sensitive skin in a product at 9% - 11%. Diatomaceous Earth was not sensitizing in a LLNA at up to 10%. A cosmetic product containing 9% - 11% Diatomaceous Earth (soda ash flux-calcined) was not sensitizing in a HRIPT of 100 healthy subjected when tested at a 10% dilution, nor was it phototoxic in a human single application study in 10 healthy female subjects when tested neat.

In ocular studies, flux-calcined Diatomaceous Earth in a formulation at 9%-11% was non-irritating at 2% and 5% dilutions, but was moderately irritating at a 10% dilution. However, flux-calcined Diatomaceous Earth (tested neat) was not an ocular irritant in an in vitro reconstituted human corneal epithelium model test nor in a rabbit eye test.

A case report of a worker at a Diatomaceous Earth mill observed far-advanced coalescent pneumoconiosis. Occupational studies indicate a risk of pneumoconiosis in Diatomaceous Earth mine and mill workers, which can be mitigated with dust control measures and personal protective equipment. The TWA REL for Diatomaceous Earth set by NIOSH is 6 mg/m^3 and the TWA PEL set by OSHA is 20 mppcf (80 mg/m^3/% silicon dioxide). The IDLH value is 3000 mg/m^3.

No DART studies were discovered in the published literature, and no unpublished data were submitted.

DISCUSSION

The Panel reviewed the safety of Diatomaceous Earth as used in cosmetic formulations. The Panel concluded that the available data are sufficient for determining the safety of this ingredient as reportedly used in cosmetics.

Diatomaceous Earth is naturally-occurring and is a polymorph of silica, or silicon dioxide. Regardless of whether Diatomaceous Earth is unprocessed (natural) or heat-processed (calcined or flux-calcined), it can contain crystalline silica; crystalline silica is a known respiratory carcinogen. Diatomaceous Earth is reported to be used in face powders (concentration not reported), and could possibly be inhaled. The Panel noted that chronic inhalation studies of flux-calcined Diatomaceous Earth (which may comprise up to 60% crystalline silica) were negative for fibrosis or tumors in rats and guinea pigs. Additional data from acute and short-term inhalation studies suggest little potential for respiratory effects at relevant doses. Coupled with the small actual exposures in the breathing zone and the concentrations at which this ingredient is used (or expected to be used) in potentially inhaled products, concerns about use in products that may be incidentally inhaled were mitigated. (The Panel's initial concern that face masks may flake during drying, and those flakes could incidentally be inhaled, was mitigated once the Panel had a better understanding as to how those products work.) A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel expressed concern over the lack of DART studies for Diatomaceous Earth. However, the Panel noted that Diatomaceous Earth did not produce adverse effects in oral rodent studies, and is GRAS for uses in food and beverages. This information, coupled with noted lack of residual silica absorption in a 13-wk dietary study in rats, helped mitigate concern over the absence of DART data.

The Panel also expressed concern about the presence of heavy metals in Diatomaceous Earth. Although heavy metals may be present during mining, those should be readily avoidable/separable. Accordingly, the Panel stressed that the cosmetics industry should continue to use current good manufacturing processes (cGMPs) to limit impurities in cosmetic formulations.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Diatomaceous Earth is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	Powder	5
Color	White or beige Calcined = pink to light brown or light yellow to light orange Flux-calcined = white to pink or light brown	5 6 6
Density/Specific Gravity (g/ml @ 20 ºC)	2.36	5
Melting Point (ºC)	1710	4
Boiling Point (ºC)	2230	4
Water Solubility (mg/l @ 20 ºC & pH 3)	3.7	5

Table 2. Particle size distributions for flux-calcined Diatomaceous Earth[5]

	Volume % less than		
Diameter of particles (µm)	**Fine-Grade**	**Medium-Grade**	**Coarse-Grade**
1	3.81	1.89	0.68
1.5	6.81	3.09	1.18
2	9.63	3.89	1.56
3	15.7	5.12	2.09
4	22.3	6.28	2.49
6	35.3	8.76	3.18
10	58.7	14.7	4.56
20	90.3	32.2	9.59
28	95.9	43.7	15.1
40	98.4	56.4	24.4
50	99.1	64.1	32.1
75	99.5	76.7	50
90	99.6	81.6	59.5
250	99.996	97	96.2
600	100	99.95	99.98

Table 3. Frequency (2022)[20] and concentration (2021)[21] of use according to duration and exposure

	# of Uses	Max Conc of Use (%)
Totals*	**135**	**0.001-2**
Duration of Use		
Leave-On	92	0.0049-0.01
Rinse-Off	43	0.001-2
Diluted for (Bath) Use	NR	NR
Exposure Type		
Eye Area	2	NR
Incidental Ingestion	17	NR
Incidental Inhalation-Spray	9[a]; 8[b]	NR
Incidental Inhalation-Powder	5; 9[a]	NR
Dermal Contact	67	2
Deodorant (underarm)	3[b]	NR
Hair - Non-Coloring	1	NR
Hair-Coloring	NR	0.001
Nail	50	0.0049-0.01
Mucous Membrane	20	NR
Baby Products	NR	NR

*Because this ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[a] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[b] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
NR – not reported

Table 4. Repeated dose toxicity studies of Diatomaceous Earth

Test Material Dose/Concentration	Animals/Group	Study Duration	Vehicle	Protocol	Results	Reference
			ORAL			
0%, 1%, 3%, or 5% Diatomaceous Earth of freshwater origin, particle size range was 0.46 µm to 640 µm, with 90% smaller than 100 µm and 55% smaller than 12 µm	Groups of 15 male and 15 female Wistar rats	13-wk study	Dietary pellets	Body weights recorded weekly; at study end animals were killed and necropsied; livers, kidneys, and spleens of rats fed test material at 5% were analyzed for residual silica	Body weights of the 5% dose group were greater than the controls through the course of the study, with the maximum weight differential occurring at week 6; body weight gains in the 3% dose group were similar to those in the 5% group; body weight gains in the 1% dose group were similar to controls; histologic examination of organs of the 5% dose group were comparable to controls; residual silica values in the organs of the 5% dose group were comparable with the controls	30
1% and 5% natural Diatomaceous Earth and 5% flux-calcined Diatomaceous Earth as feed; 5% natural mixture contained 4.8% silica, 0.44% quartz, and no cristobalite; 1% natural mixture contained 1.2% silica, 0.24% quartz, and 0.26% cristobalite; 5% flux calcined mixture contained 5.1% silica, 0.43% quartz, and 1.70% cristobalite	Groups of 20 male and 20 female Sprague-Dawley rats	13-wk study	Dietary pellets	Study performed in accordance with OECD TG 408; control animals received plain diet;	No clinical signs of toxicity or mortalities observed; no effects observed in body weight; feed consumption, ophthalmological findings, hematological findings, clinical biochemistry findings, or urinalysis findings; no treatment-related effects were observed at necropsy	5
			INHALATION			
100% pure flux-calcined; 0, 0.018, 0.58, or 1.57 mg/l; target particle size range was 1 to 3 µm	5 male and 5 female Wistar rats/dose group	5-d range finding study	None described	Nose-only aerosol inhalation study; 6 h/exposure performed	No clinical signs of toxicity or mortalities observed; reduced feed consumption was observed in the high dose group; mean body weight loss was recorded in both male and female animals in the high dose group and a statistically significant reduced body weight gain was observed in male rats in the high dose group only when compared with controls; dose-dependent alveolar histiocytosis was observed in all dose groups; alveolitis was observed in one male in the mid-dose group and in all animals in the high-dose group as well as increased absolute and relative lung weights in the mid- and high-dose groups; microgranulomas were found in one male and female in the mid-dose group and in all animals in the high-dose group; the test material was observed in the alveoli in most of the high-dose group animals; a no-observable-effect-concentration (NOEC) could not be determined	5

Table 4. Repeated dose toxicity studies of Diatomaceous Earth

Test Material Dose/Concentration	Animals/Group	Study Duration	Vehicle	Protocol	Results	Reference
100% pure flux-calcined; 0, 0.044, 0.207, or 0.700 mg/l; target particle size range was 1 to 3 µm	20 male and 20 female Wistar rats/dose group	28-d study	Compressed air	Study performed in accordance with OECD TA 412; nose-only aerosol inhalation study; 6 h/exposure performed 5d/wk with a 9-wk recovery period	No clinical signs of toxicity or mortalities observed; a slight and transient effect on body weight gain occurred in the high dose group; dose-dependent increase in lung weights recorded at the end of treatment period that further increased at the end of the recovery period; lymph nodes were also increased in size at the end of the recovery period; increase in spleen, adrenal, and liver weights was observed in the high-dose group at the end of the recovery period; histiocytosis was observed in the alveoli with a dose-dependent increase in incidence and severity that progressed during the recovery period; test material was detected in the alveoli in the mid and high dose group animals at the end of the treatment period that persisted until the end of the recovery period; a NOAEC could not be determined	5
Flux-calcined Diatomaceous Earth (61% cristobalite); 0, 2, 5, 50, and 5+50 mppcf; mean particle size 0.7 µm	Male Wistar rats divided as follows in the 0, 2, 5, 50, and 5+50 mppcf dose groups: 47, 79, 82, 46, and 53 animals, respectively	2- yr study	None described	Rats exposed to test material in exposure chambers for 6 h/d, 5 d/wk for up to 2 yr except in the 50 mppcf (1 h, 3 times/wk) and the 5+50 mppcf (daily 5 mppcf exposure plus 50 mppcf 3 times/wk for 1 h each) dose groups; rats killed at 6 mo, 1 yr, 1.5 yr, and 2 yr.	Terminal body weights at 1 yr and 1.5 yr in treated groups were comparable to controls except for in the rats exposed to 5+50 mppcf, which were below the control and 5 mppcf group; tissues studied other than the lungs had no test material-related changes. At 6 mo, rats in 2 and 5 mppcf dose groups had scattered macrophages and occasional giant cell within alveolar spaces; there was no significant septal reaction; pulmonary hilar lymph nodes only slightly enlarged and contained small clusters of macrophages in medullary portions; 5+50 mppcf group had slightly enhanced cellular reaction, when compared to the 5 mppcf group, and macrophages were noted to accumulate around bronchioles. At 1 yr, an increased macrophagic infiltration of perivascular and peribronchiolar areas were observed in the 2 and 5 mppcf groups; reactions were dose dependent; in 5+50 mppcf, macrophagic cells accumulated in a nodular fashion and reticular condensation was evident in lung parenchyma and hilar nodes. At 1.5 yr, no definite parenchymal or lymph node fibrosis was observed. At 2 yr, perivascular and peribronchiolar localization of dust-laden macrophages was observed in the 2 and 5 mppcf dose groups; nodular lesions and reaction of the nodes was greater in the 5 mppcf dose group; no fibrosis evident.	15

Table 4. Repeated dose toxicity studies of Diatomaceous Earth

Test Material Dose/Concentration	Animals/Group	Study Duration	Vehicle	Protocol	Results	Reference
Flux-calcined Diatomaceous Earth (61% cristobalite); 0, 2, 5, 50, and 5+50 mppcf; mean particle size 0.7 µm	Male guinea pigs (strain not reported) divided as follows in the 0, 2, 5, 50, and 5+50 mppcf dose groups: 47, 57, 69, 20, and 20 animals, respectively	1.5-yr study	None described	Guinea pigs exposed to test material in exposure chambers for 6 h/d, 5 d/wk for up to 1.5 yr except in the 50 mppcf (1 h, 3 times/wk) and the 5+50 mppcf (50 mppcf for 3 d/wk plus daily 5 mppcf) dose groups; rats killed at 6 mo, 1 yr, and 1.5 yr	Terminal body weights at 1 yr and 1.5 yr were comparable to controls; tissues studied other than the lungs had no test material-related changes. At 6 mo, same as the findings for the rats above. At 1 yr, definite cellular reaction with large clusters of macrophages and multinucleated giant cells in alveolar spaces in the 5 mppcf group; macrophages observed to accumulate around bronchioles and alveolar ducts; hilar lymph nodes were markedly enlarged and medullary portions were packed with dust cells and interwoven reticulum fibers. At 1.5 yr, a slight increase in intra-alveolar macrophages with peribronchiolar localization was observed in the 5 mppcf group; alveolar septa were unaffected and no fibrosis evident	15
Diatomaceous Earth at 171 mppcf (natural, unheated), cristobalite at 167 mppcf (from heat-treated Diatomaceous Earth), or sodium silicate; particle size range ~0.45 µm to > 10 µm	Albino guinea pigs (sex and number/group not reported)	21-24 mo study	None described	Guinea pigs were placed in separate cubical dust rooms (512 ft^3) for 24 h/d until killed for examination; dust was generated within the room for 7 to 8 h/d, 5.5 d/wk for 21-24 mo; control animals kept in ambient air; pairs of animals selected at random were killed at 2-3 mo intervals and lung tissues were collected and analyzed for total silica content and total ash	In animals exposed to Diatomaceous Earth, fibrosis was only noted at 24 mo, and not at the same severity as in the cristobalite-exposed animals; in animals exposed to cristobalite, fibrosis first observed after 15 mo and was severe by 21 mo; no fibrosis observed in animals exposed to sodium silicate, but alveoli became heavily packed with phagocytic macrophages. Total silica content per lung increased linearly throughout at least 21 mo in each experiment, and total ash weight increased more rapidly than dust was accumulating. Cristobalite produced a greater increment in ash weight than Diatomaceous Earth and sodium silicate. Total amount of silica accumulated varied inversely with the degree of tissue damage occurring, even though atmospheric dust concentrations were comparable for the 3 silica types. Maximum total content of cristobalite reached only 68 mg/lung, while that of Diatomaceous Earth and sodium silicate was 120 mg/lung and 465/lung, respectively. Author noted that siliceous dust that produces cell damage may be cleared more effectively from the lung than innocuous dust.	31

Table 4. Repeated dose toxicity studies of Diatomaceous Earth

Test Material Dose/Concentration	Animals/Group	Study Duration	Vehicle	Protocol	Results	Reference
Flux-calcined Diatomaceous Earth (61% cristobalite); 0, 2, and 5 mppcf; mean particle size 0.7 µm	Male mongrel dogs divided as follows in the 0, 2, and 5 mppcf dose groups: 8, 16, and 17 animals, respectively	2.5-yr study	None described	Dogs exposed to test material in exposure chambers for 6 h/d, 5 d/wk for up to 30 mo; an unreported number of dogs were killed at 6 mo, 1 yr, 1.5 yr, 2 yr, and 2.5 yr. One dog in the control and each dose group was killed 10 mo after cessation of exposure to examine recovery	Terminal weights comparable or slightly greater than controls; no changes in hematology during the course of the study; tissues studied other than the lungs had no test material-related changes. At 6 mo, no reaction observed in the 2 mppcf group and minimal intra-alveolar macrophages observed in the 5 mppcf group; however, hilar nodes had greater macrophagic infiltration than rats and guinea pigs described above. At 1 yr, little to no changes observed. At 1.5 yr, clusters of dust cells in alveolar spaces adjacent to bronchioles observed in 5 mppcf group, with hilar lymph nodes enlarged and medulla replaced with hyalinized tissue. At 2 yr, slight perivascular and peribronchiolar localization of macrophages observed in 2 mppcf group that were definite nodules extending into bronchiolar lumina in the 5 mppcf group; hilar lymph nodes were enlarged and diffusely packed with macrophages; medulla had numerous nodules. At 2.5 yr, observations similar to those in the 2 yr group with no significant progression in reactions; no fibrosis evident. In the recovery animals, parenchymal and nodal changes did not increase compared to 2.5 yr group.	15

Table 5. In vitro genotoxicity studies of Diatomaceous Earth

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
0, 50, 150, 500, 1500, or 5000 µg/plate flux-calcined (100% pure)	polyethylene glycol 400	*Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100; *Escherichia coli* strain WP2 uvr A	Ames test in accordance with OECD TG 471, with and without metabolic activation	Not mutagenic	5
0, 2.5, 5, 10, 20, 30 or 40 µg/ml flux-calcined (100% pure)	R0 medium	Mouse lymphoma L5178Y cells	Mammalian cell gene mutation test in accordance with OECD TG 476, with and without S9 metabolic activation	Not mutagenic	5
0, 1.25, 2.5, 5, 10, 20, or 40 µg/ml flux-calcined (100% pure)	Minimal essential medium or dimethyl sulfoxide	Human lymphocytes	Mammalian chromosome aberration test in accordance with OECD TG 473, with and without S9 metabolic activation	Not clastogenic	5
Natural and flux-calcined Diatomaceous Earth (average diameters 1.3 µm and 2.1 µm, respectively) in addition to titanium dioxide, crocidolite, chrysotile, quartz, and cristobalite; concentration ranges not reported; crystalline silica content of the natural Diatomaceous Earth was 4% quartz and of the flux-calcined Diatomaceous Earth was 40% cristobalite and 2% quartz	Not reported	Cultured Chinese hamster ovary (CHO) cells	Cell proliferation assays; 100,000 cells seeded/dish and incubated for 1 d prior to exposure to test dust for 3 d; cells then harvested and counted	The ranking of toxicity as measured by the inhibition of cell proliferation was chrysotile > crocidolite > natural Diatomaceous Earth > flux-calcined Diatomaceous Earth > quartz > cristobalite > titanium dioxide; effective concentration-50% (EC_{50}) for natural Diatomaceous Earth was 3.6 µg/cm^2 and for flux-calcined Diatomaceous Earth was 10.8 µg/cm^2; responses were concentration-dependent; researchers found that the toxicity of the dusts did not correlate with crystalline silica content, surface area, composition, volume, particles/cm^2, or fibrous geometry; however, toxicity was closely associated with the number of particles/cm^2 culture surface that had one dimension > 7.5 µm; authors indicated that particle size impacted toxicity	16
Natural and flux calcined Diatomaceous Earth as described above	Not reported	Cultured CHO cells	Colony-forming efficiency assays; 200 cells seeded/dish and the test dusts added 24 h later; cultures then incubated for 5 d before being fixed and number of colonies containing > 20 cells was determined for each dish.	Similar ranking of toxicity observed as in the cell proliferation assay described above; colony formation was not as inhibited as cell proliferation; results were concentration-dependent	16
Natural and flux calcined Diatomaceous Earth as described above	Not reported	Cultured CHO cells	Abnormal nucleus induction assays; cultures prepared in the same manner as the above inhibition of cell proliferation assays, exposed for 2 d and then fixed; percentage of cells containing micronuclei and/or polynuclei was determined for each dish.	Similar qualitative, concentration-dependent results were observed as in the cell proliferation and colony-forming efficiency assays described above	16
Three different sourced uncalcined Diatomaceous Earth samples (96%-98% pure; 0.6% -1.4% iron impurities) and 2 calcined Diatomaceous Earth samples (~98% pure; 0.7% - 0.9% iron impurities); concentrations not well defined, but at least 3 concentrations per sample were tested starting at 2 µg/cm^2 and were up to approximately 40 µg/cm^2	Suspended in sterile tridistilled water; culture medium without serum and complete medium	Syrian hamster embryo (SHE) cells	Cell transformation assay; without metabolic activation	Morphological transformation of the uncalcined and calcined Diatomaceous Earth samples occurred in a dose-dependent manner; authors concluded that samples with fractured surfaces and/or iron-active sites were able to generate reactive oxygen species-induced SHE cell transformation	32

Table 5. In vitro genotoxicity studies of Diatomaceous Earth

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
Uncalcined Diatomaceous Earth (100% amorphous), Diatomaceous Earth heated to 900ºC (98.5% amorphous, 1% quartz, <0.5% cristobalite), Diatomaceous Earth heated to 1200ºC (51% amorphous, 1% quartz, 48% cristobalite), a generically heated flux-calcined Diatomaceous Earth (53% amorphous, 47% cristobalite), and the generically heated flux-calcined Diatomaceous Earth (42% amorphous, 58% cristobalite) depleted of particles greater than 10 µm; concentrations tested for each material were 4.5, 9, and 18 µg/cm^2 (also 36 µg/cm^2 for generically heated Diatomaceous Earth)	Culture medium	SHE cells	Cell division aberration assay; without metabolic activation	A concentration-dependent increase in abnormal mitoses frequency was observed with all dusts tested, except uncalcined Diatomaceous Earth at 4.5 and 9 µg/cm^2; Diatomaceous Earth heated to 900ºC and 1200ºC appeared "less active" than the uncalcined – the authors theorized this may be due to cytotoxic potential, which appeared "blunted" through heating	33
Uncalcined Diatomaceous Earth (100% amorphous), Diatomaceous Earth heated to 900ºC (98.5% amorphous, 1% quartz, <0.5% cristobalite), Diatomaceous Earth heated to 1200ºC (51% amorphous, 1% quartz, 48% cristobalite), a generically heated flux-heated Diatomaceous Earth (53% amorphous, 47% cristobalite), and the generically heated flux-calcined Diatomaceous Earth (42% amorphous, 58% cristobalite) depleted of particles greater than 10 µm; concentrations tested for each material were between1.9 and 30.4 µg/cm^2 (up to 60.8 µg/cm^2 for generically heated Diatomaceous Earth)	Culture medium	SHE cells	Cell transformation assay; without metabolic activation	Uncalcined Diatomaceous Earth did not induce morphological transformation while a concentration-dependent increase of the transformation frequency was induced by all other test materials; the heated samples exhibited a certain degree of transformation with the 1200ºC heated sample greater than the 900ºC (which was weakly active only above 15 µg/cm^2); transformation potential appears to be correlated with the ability to generate radicals	33
Uncalcined Diatomaceous Earth with 0.03% iron impurities and uncalcined Diatomaceous Earth depleted of iron; concentrations started at 3.5 µg/cm^2 and included up to 60 µg/cm^2	Not reported	SHE cells	Cell transformation assay, with and without antioxidants	Concentration-dependent increase in transformation frequency starting at 3.5 µg/cm^2 was observed in samples with iron, transforming potency was 1.8-fold less in samples depleted of iron; in presence of antioxidants, transformation frequencies were significantly decreased; authors concluded iron may generate reactive oxygen species that increase transforming potency	34
Uncalcined Diatomaceous Earth with 0.03% iron impurities and uncalcined Diatomaceous Earth depleted of iron; concentrations between 2.25 and 34 µg/cm^2	Not reported	SHE cells	Cell division aberration assay, with and without antioxidants	A significant concentration-dependent increase in frequency of abnormal mitoses was induced by sample with iron; mitotic spindle disturbances, mono- and multi-polar mitoses, and some chromosome lagging were most frequently observed; iron-depleted samples induced abnormal mitoses in a similar manner to the samples with iron; in presence of antioxidants, frequency of abnormal mitoses were significantly decreased	34

Table 6. Dermal irritation and sensitization studies of Diatomaceous Earth

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			IRRITATION		
			IN CHEMICO / IN VITRO STUDIES		
100% Diatomaceous Earth; flux-calcined	20 mg; undiluted	Reconstituted human epidermis samples	EpiSkin™ reconstituted human epidermis model test in accordance with OECD TG 431; duplicate tissues treated for 3, 60, and 240 min	Non-corrosive; relative mean viability after exposure to test material for 3, 60, and 240 min was 102.8%, 111.3%, and 114.1%, respectively; qualitative evaluation indicated tissue was viable at each time point following exposure to test material; positive and negative controls yielded expected results	5
Diatomaceous Earth; flux-calcined, purity not reported	Not reported	Reconstituted human epidermis samples	EpiSkin™ reconstituted human epidermis model test; tissues treated for 15 min before incubation for 42 h; no further details reported	Not irritating; relative mean viability after exposure to test material was 102.6%; qualitative evaluation indicated tissue was viable following exposure to the test material; positive and negative controls yielded expected results	5
			HUMAN		
100% Diatomaceous Earth; flux-calcined	0.02 mg; undiluted	10 subjects	Acute skin tolerance test; 48-h single patch test using Finn Chambers; occluded; test material applied to external face of the arm	Not irritating	38
Product containing 9% - 11% Diatomaceous Earth (Diatomaceous Earth contained < 0.11% respirable crystalline silica); soda ash flux-calcined	Amount not reported; undiluted	11 subjects with sensitive skin	Acute 24-h skin tolerance patch test; occluded; no further details	Not irritating	39
			SENSITIZATION		
			ANIMAL		
Diatomaceous Earth; flux-calcined, purity not reported	0%, 2.5%, 5%, or 10% in propylene glycol; 25 µl	Groups of 4 female CBA mice	LLNA; animals received test material daily on dorsum of each ear lobe for 3 consecutive days; positive control group received 90% phenylacetaldehyde in a solution of propylene glycol (final concentration 2.5% v/v)	Not sensitizing; all treated animals survived treatment; no clinical signs of toxicity observed in any test groups; stimulation indices (SI) for 2.5%, 5%, and 10% dose groups were 1.13, 0.97, and 0.99, respectively; SI of positive control was 18.43	5
			HUMAN		
Cosmetic formulation containing 0.9% - 1.1% Diatomaceous Earth (Diatomaceous Earth contained < 0.11% respirable crystalline silica); soda ash flux-calcined	25 µl; applied neat	100 healthy subjects with normal skin	HRIPT according to Marzulli-Maibach method; test material applied on back of subjects with Finn Chambers on Scanpor®; occluded; duplicate patches without test material applied to serve as control only during the induction phase; induction patches occurred 3 times a week for 3 wk and a 2-wk rest period occurred prior to the single challenge patch; patches were in place for 48 h	Not irritating and not sensitizing	40
			PHOTOTOXICITY		
			HUMAN		
Product containing 9% - 11% Diatomaceous Earth (Diatomaceous Earth contained < 0.11% respirable crystalline silica); soda ash flux-calcined	0.2 ml; undiluted	10 healthy female subjects	Phototoxicity study of single application of test material on each forearm; occluded for 24 h; one arm was irradiated with UV-A (4 F4OBL with fluorescent tubes; 320-400 nm), while the other arm served as control	Not phototoxic; no skin reactions observed on irradiated product site and control site without product; very slight transient erythema observed in 1 subject on non-irradiated product site	41

Table 7. Occupational exposure studies of Diatomaceous Earth

Diatomaceous Earth Composition	Study Population and Location	Time Frame Examined	Procedure/Parameters Measured/Limitations	Findings	Reference
Quarry dust was essentially amorphous silica with quartz content of crude Diatomaceous Earth being 2%; mill dust had high percentage of cristobalite	869 workers of 5 plants in California, Nevada, and Oregon	1953-1954	X-ray investigation	-9% of the workers had lung changes interpreted as pneumoconiosis and that an equal number had doubtful changes -prevalence of abnormal chest films especially high in employees in mills -exposure in quarries associated with a lower proportion of abnormal films; none of 25 employees who had worked there exclusively for over 5 yr had a positive film, but 40% showed doubtful linear nodular changes	44
Same as above	Follow-up study in 428 workers from one plant from the above study (state not specified); plant included a quarry and a mill	1974; including employees terminated between July 1, 1969 and July 1, 1974	X-ray investigation	-films interpreted as positive for pneumoconiosis (Union for International Cancer Control (UICC)/Cincinnati classification of 1/1) observed in 20 (4.7%) of the workers -another 6 films had a UICC/Cincinnati classification of 1/0 -of these 26, 14 were determined to have findings consistent with Diatomaceous Earth pneumoconiosis, and all but 2 of these 14 had been employed before 1953 -in 129 employees in the industry for 20 yr or more, 13 had positive films considered consistent with Diatomaceous Earth pneumoconiosis, of which 6 had negative films in 1953 -only 4 individuals had complicated or coalescent lesions: these workers had been mill workers employed 27- 46 yr -no massive coalescent lesions or distorting changes noted in the existing work force -researchers pointed out that this evidence agreed with earlier observations indicating that the risk of pneumoconiosis was relatively low in workers whose exposure was confined to crude Diatomaceous Earth, as compared with those exposed to calcined Diatomaceous Earth -researchers noted that strict occupational dust control measures and personal protective equipment led to the near elimination of new cases of Diatomaceous Earth pneumoconiosis	45
Raw material contained ~ 4% crystalline silica; calcined and fluxed-calcined material had 10-20% and 20-25% cristobalite, respectively	2570 white male Diatomaceous Earth mining and processing workers in California; at least 12 mo cumulative service	1942-1987	Mortality patterns analysis; mortality trends assessed in respect of an index of cumulative exposure to crystalline silica and crystalline silica index; workers with known potential occupational asbestos exposure excluded; cigarette smoking was a confounding factor	-all causes combined standardized mortality ratio (SMR) slightly increased when compared with rates among US white males (SMR 1.12: 628 observed) -increased risks from lung cancer (SMR 1.43; 59 observed) and non-malignant respiratory disease (NMRD; excluding infectious diseases and pneumonia; SMR 2.59, 56 observed) were main contributors to the observed excess -excess lung cancer also observed when rates were compared with local county rates instead of the US national rates -increasing gradients of risk detected for lung cancer and NMRD with both crystalline silica exposure indices -researchers stated smoking was not likely to account for all associations between dust exposure and lung cancer -prior to the 1950s, poor dust control measures likely largest contributors to lung cancer and NMRD; the absence of excess lung cancer in workers hired after 1960 and no deaths attributed to pneumoconiosis in workers hired after 1950 indicated exposure reductions were successful in reducing excess risks in workers	46
Same as above	2342 white male Diatomaceous Earth workers; a subset of the above California workers cohort (406 had been excluded due to potential inadequate exposure data or definitive asbestos exposure	1942-1987	Mortality patterns analysis as above; results not likely to be confounded by smoking or asbestos exposure	-mortality excesses detected for NMRD (SMR 2.01) and lung cancer (SMR 1.29) -mortality from NMRD rose sharply with cumulative exposure to respirable crystalline silica (mostly cristobalite), indicating a strong dose-response relationship for crystalline silica and NMRD mortality -while not as strong of a relationship, lung cancer results further support an etiologic role for crystalline silica	47

Table 7. Occupational exposure studies of Diatomaceous Earth

Diatomaceous Earth Composition	**Study Population and Location**	**Time Frame Examined**	**Procedure/Parameters Measured/Limitations**	**Findings**	**Reference**
Same as above	1809 white male Diatomaceous Earth workers; a subset of the above California workers cohort; workers had at least 1 yr of exposure to crystalline silica	1942-1987	X-ray investigation	-81 workers (4.5%) had opacities on chest radiographs -age-adjusted relative risk of opacities increased significantly with cumulative exposure to crystalline silica -risk of opacities for cumulative exposure to crystalline silica of 2.0 mg/m^3-yr was 1.1% when average crystalline silica exposure was < 0.50 mg/m^3, but was 3.7% when average crystalline silica exposure was > 0.50 mg/m^3	48
Same as above	759 white male Diatomaceous Earth workers; a subset of the above California workers cohort;	1942-1987	X-ray and spirometry investigation; chest radiographs interpreted by the International Labor Office (ILO) system; individual-based reconstructed exposure indices for total dust (largely Diatomaceous Earth) and cristobalite were used in performing regression analyses	-of 492 chest radiographs, 5% had ILO scores > 1/0 and 25% had score of 0/1 or higher -radiographic patterns were not typical of classic silicosis - regression analyses showed there was a relationship between both total cristobalite exposure and total dust exposure and the ILO score -differences observed in spirometric data according to radiographic ILO category, but the results were inconsistent and did not allow for determining if physiologic changes were associated with radiographic change or through confounding factors, such as smoking -researchers noted that recent exposure level may produce radiographic abnormalities, but a demonstrable physiologic effect may not be observed; this decrease in observed effects was noted to be due to modern dust control measures.	49

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