Safety Assessment of Diatomaceous Earth as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: August 20, 2021
Panel Meeting Date: September 13-14, 2021

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.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., 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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Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst
Date: August 20, 2021
Subject: Safety Assessment of Diatomaceous Earth as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Diatomaceous Earth as Used in Cosmetics. (It is identified as *dearth092021rep* in the pdf document.) The Scientific Literature Review (SLR) of this ingredient was issued by CIR on April 30, 2021. This ingredient is reported to function in cosmetics as an abrasive, absorbent, anticaking agent, bulking agent, and opacifying agent.

According to 2021 VCRP survey data (*dearth 092021fda*), Diatomaceous Earth is used in a total of 116 formulations. Of these reported uses, the majority are in leave-on products and nearly a quarter (25) are in rinse-off paste masks (mud packs). The results of the concentration of use survey conducted by the Council in 2019 indicate that Diatomaceous Earth is used at up to 5% in face and neck skin care preparations, up to 20% in hair tonics and dressings, and up to 62.2% in rinse-off products (paste masks) (*dearth092021data1*).

In addition to concentration of use survey data, the Council provided human dermal irritation, sensitization, and phototoxicity data (*dearth092021data2* and *dearth092021data3*). Comments provided by the Council on the SLR have been addressed (*dearth092021pcpc*). CIR has also received comments on the SLR from the International Diatomite Producers Association (IDPA), which have been included in this report package for consideration (*dearth092021IDPA*).

Additional supporting documents for this report package include a flow chart (*dearth092021flow*), report history (*dearth092021hist*), a search strategy (*dearth092021strat*), and a data profile (*dearth092021prof*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

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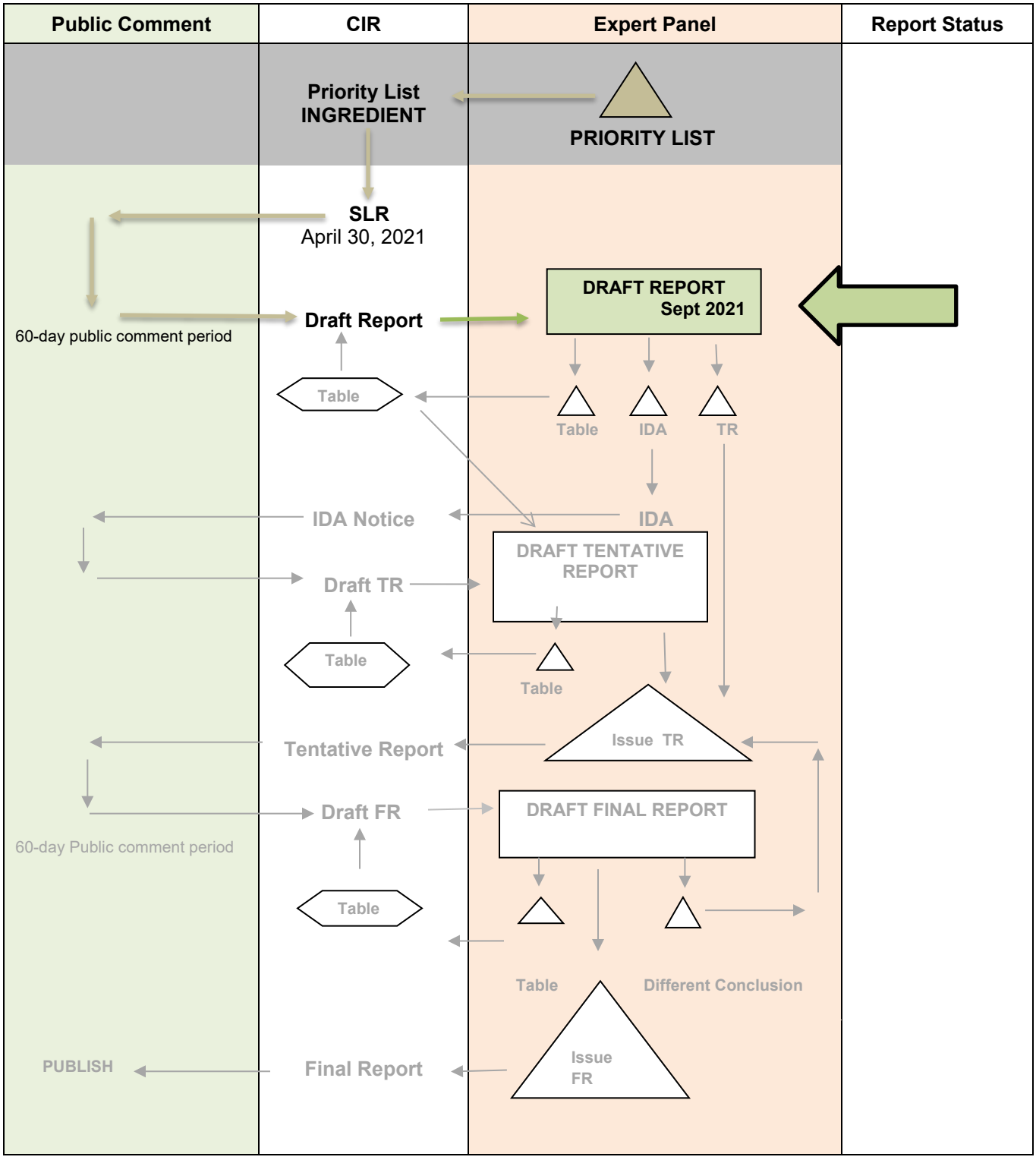
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Diatomaceous Earth

MEETING September 2021



Diatomaceous Earth History

April 30, 2021 – Scientific Literature Review issued.

Diatomaceous Earth Data Profile* – September 2021 – Christina Burnett																																
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies				
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports			
Diatomaceous Earth	X	X	X			X		X	X		X	X			X			X	X		X		X	X	X	X	X	X				X

* "X" indicates that data were available in a category for the ingredient

Diatomaceous Earth

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Diatomaceous Earth	61790-53-2; 68855-54-9	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	

Search Strategy

PubMed
Diatomaceous Earth = 938 hits, 73 relevant

Diatomaceous Earth toxicity = 75 hits, 30 relevant

Diatomaceous Earth cosmetics = 20 hits, 6 relevant

Diatomaceous Earth dermal = 0 hits

Diatomaceous Earth sensitization – 3 hits, 1 relevant

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

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ABBREVIATIONS

BAL	bronchoalveolar lavage
CIR	Cosmetic Ingredient Review
CHO	Chinese hamster ovary
Council	Personal Care Products Council
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
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
TG	test guideline
TWA	time weighted average
UICC	Union for International Cancer Control
US	United States
VCRP	Voluntary Cosmetic Registration Program

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] 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.[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

The methods described below are general to the processing of commercial forms of Diatomaceous Earth. It is unknown if these apply to cosmetic ingredient manufacturing.

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]

Composition and Impurities

The composition of Diatomaceous Earth varies depending on where it is mined and how it is processed.[11] Silica content in Diatomaceous Earth can vary between 83% to 96%.[3,11-14] 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.[3,11,13,14] Diatomaceous Earth usually contains 0.1% to 4% quartz.[3]

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.[3,15]

A supplier has reported that a product containing 100% Diatomaceous Earth has < 1% respirable crystalline silica.[16] Another product containing 9-11% Diatomaceous Earth was reported to have < 0.11% respirable crystalline silica.

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. 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 by product category.

According to 2021 VCRP survey data, Diatomaceous Earth is used in a total of 116 formulations (Table 2).[17] Of these reported uses, the majority are in leave-on products and nearly a quarter (25) are in rinse-off paste masks (mud packs). The results of the concentration of use survey conducted by the Council in 2019 indicate that Diatomaceous Earth is used at up to 5% in face and neck skin care preparations, up to 20% in hair tonics and dressings, and up to 62.2% in rinse-off products (paste masks).[18]

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 both eye shadows and bath soaps and detergents at up to 0.2%.[18] It is also reported to be used in products which maybe incidentally ingested, such as dentifrices and other oral hygiene products (concentrations not reported).[17] Additionally, Diatomaceous Earth is reported to be used in cosmetic sprays and powders, and could possibly be inhaled; for example, it is reported to be used in "other" fragrance products at up to 0.1% and in face powders (concentration not reported).[18] In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles < 10 µm compared with pump sprays.[19,20] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[21,22] 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.[23-25]

Diatomaceous Earth is not restricted from use in any way under the rules governing cosmetic products in the European Union.[26]

Non-Cosmetic Use

Diatomaceous Earth has uses in food and beverages, including anticaking material foodstuffs and clarifier in wine and beer.[27] 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.[28] Diatomaceous Earth is also GRAS as a substance migrating to food from paper and paperboard products (21CFR§182.90). Diatomaceous Earth is approved for use as a coating (21CFR§175.300), polymer (21 CFR§177.1680; §177.2260; §177.2410), and as a component of paper and paperboard (21CFR 176.170) and adjuvants (21CFR§178.3297) in indirect food additives. 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 (21 CFR§573.340).

The use of Diatomaceous Earth as a drug carrier is being investigated.[29,30] Diatomaceous Earth is an approved inactive ingredient in approved drug products, including capsules and tablets taken orally and in topical soaps.[31]

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.[9,10,15,27] It is also widely used in pesticide formulations.[10,12,15,27,32,33]

TOXICOKINETIC STUDIES

In a 90-d dietary study, groups of 15 male and 15 female weanling Wistar 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).[34] At study end, the livers, kidneys, and spleens of the rats were analyzed for residual silica. In both the treated male and female rats, residual silica values in the organs were comparable with the controls.

No further 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.

Repeated Dose Toxicity Studies

Repeated dose oral and inhalation studies summarized here are described in Table 3. 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.[5,34] 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) at up to 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.[13] 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.[35] No fibrosis was observed in mongrel dogs exposed to up to 5 mppcf flux-calcined Diatomaceous Earth for up to 2.5 yr.[13]

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 4. 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).[14] In studies with Syrian hamster embryo (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.[36-38] 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.[39] 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.[40] 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 $\leq$ 3.0 µm in diameter; 72% silica and 28% calcium silicates) in 0.5 ml physiological saline. [41] 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. Macrophage numbers, 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 5. 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 was not irritating in healthy volunteers at 100% or in volunteers with sensitive skin in a product at 9% - 11%.[42,43] 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 was not sensitizing in a human repeated insult patch test (HRIPT), nor was it phototoxic in a human single application study. [44,45]

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.

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 in a mill, 6 in an office) was reported to have a history of a recurrent peptic ulcer, pleurisy, and bronchopneumonia, with frequent attacks of bronchitis.[46] 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 STUDIES

Occupational exposure are described in Table 6. 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.[47-52] 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).[53] 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 2021 VCRP survey data, Diatomaceous Earth is used in a total of 116 formulations. Of these reported uses, the majority are in leave-on products and nearly a quarter (25) are in rinse-off paste masks (mud packs). The results of the concentration of use survey conducted by the Council in 2019 indicate that Diatomaceous Earth is used at up to 20% in hair tonics and dressings, up to 5% in face and neck skin care preparations, and up to 62.2% in rinse-off products (paste masks). Diatomaceous Earth is reported to be used in cosmetic sprays and powders, and could possibly be inhaled; for examples, it is reported to be used in "other" fragrance products at up to 0.1% and 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 an 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 was not irritating in healthy volunteers at 100% or in 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 was not sensitizing in a HRIPT, nor was it phototoxic in a human single application study. Flux-calcined Diatomaceous Earth was not an ocular irritant in an in vitro 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

To be determined.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	Powder	5
Color	White or beige	5
	Calcined = pink to light brown or light yellow to light orange	6
	Flux-calcined = white to pink or light brown	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. Frequency (2021)[17] and concentration (2019)[18] of use according to duration and exposure

	# of Uses	Max Conc of Use (%)
Totals*	**116**	**0.01-62.2**
Duration of Use		
Leave-On	72	0.01-20
Rinse-Off	44	0.2-62.2
Diluted for (Bath) Use	NR	NR
Exposure Type		
Eye Area	8	0.2
Incidental Ingestion	17	NR
Incidental Inhalation-Spray	5[a]; 8[b]	0.1; 20[b]
Incidental Inhalation-Powder	10; 5[a]	0.7-5[c]
Dermal Contact	82	0.1-62.2
Deodorant (underarm)	7[b]	NR
Hair - Non-Coloring	1	20
Hair-Coloring	NR	NR
Nail	16	0.01
Mucous Membrane	20	0.2
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.

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders

NR – not reported

Table 3. 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	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	[34]
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 3. 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.	[13]

Table 3. 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	[13]
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.	[35]

Table 3. 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.	[13]

Table 4. 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	[14]
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	[14]
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	[14]
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	[36]

Table 4. 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	[37]
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	[37]
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	[38]
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	[38]

Table 5. 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	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	[42]
Product containing 9% - 11% Diatomaceous Earth (Diatomaceous Earth contained < 0.11% respirable crystalline silica)	Amount not reported; undiluted	11 subjects with sensitive skin	Acute 24-h skin tolerance patch test; occluded; no further details	Not irritating	[43]
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)	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	[44]
PHOTOTOXICITY					
HUMAN					
Product containing 9% - 11% Diatomaceous Earth (Diatomaceous Earth contained < 0.11% respirable crystalline silica)	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	[45]

Table 6. Occupational 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	47
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	48
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	49
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	50

Table 6. Occupational 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	51
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.	52

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2021 FDA VCRP Raw Data

DIATOMACEOUS EARTH	Eye Shadow	8
DIATOMACEOUS EARTH	Tonics, Dressings, and Other Hair Grooming Aids	1
DIATOMACEOUS EARTH	Blushers (all types)	6
DIATOMACEOUS EARTH	Face Powders	10
DIATOMACEOUS EARTH	Lipstick	6
DIATOMACEOUS EARTH	Basecoats and Undercoats	1
DIATOMACEOUS EARTH	Nail Polish and Enamel	15
DIATOMACEOUS EARTH	Dentifrices	9
DIATOMACEOUS EARTH	Other Oral Hygiene Products	2
DIATOMACEOUS EARTH	Bath Soaps and Detergents	1
DIATOMACEOUS EARTH	Deodorants (underarm)	7
DIATOMACEOUS EARTH	Other Personal Cleanliness Products	2
DIATOMACEOUS EARTH	Cleansing	5
DIATOMACEOUS EARTH	Face and Neck (exc shave)	4
DIATOMACEOUS EARTH	Body and Hand (exc shave)	1
DIATOMACEOUS EARTH	Moisturizing	6
DIATOMACEOUS EARTH	Night	1
DIATOMACEOUS EARTH	Paste Masks (mud packs)	25
DIATOMACEOUS EARTH	Other Skin Care Preps	6

Concentration of Use by FDA Product Category – Diatomaceous Earth

Product Category	Maximum Concentration of Use
Eye shadows	0.2%
Other fragrance preparations	0.1%
Tonics, dressings and other hair grooming aids	20%
Foundations	2%
Nail polish and enamel	0.01%
Bath soaps and detergents	0.2%
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	2.5-5%
Face and neck products Not spray	 0.7-5%
Body and hand products Not spray	 3%
Paste masks and mud packs	16.4-62.2%

Information collected in 2018

Table prepared January 17, 2019

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: June 25, 2021

SUBJECT: Diatomaceous Earth

Biotechmarine. 2021. Diatomaceous Earth Product Information.

Bioethic. 2002. Study Summary: Study of acute skin tolerance of a cosmetic product after a 48-hour single patch-test (Micro Algues 80 - Diatomaceous Earth).

Groupe Dermscan. 2004. Assessment of the Sensitizing Potential of a Cosmetic Product: Final Clinical Security test under dermatological control (product LCA13049-13P379-1 was 10% Phycocorail which contains 9-11% Diatomaceous Earth).

Palmer Research. 1995. Evaluation du potentiel phototoxique apres application et exposition uniques sur 10 volontaires (Phycocorail) (English translation using Google translate provided).

Palmer Research. 2003. Study summary report: Determination of the irritation potential of a cosmetic products on human subject: 24-hours single occlusive patch test (Phycocorail tested at 100%).

Biotechmarine - June 2021

Diatomaceous Earth Product Information

Micro Algues 80 is 100% Diatomacous Earth that contains <1% respirable crystalline silica.
Recommended use concentrations in cosmetics: 0.5-10%

Phycocorail contains 9-11% Diatomaceous Earth (<0.11% respirable crystalline silica)
Recommended use concentration in cosmetics: 0.5 to 5%

STUDY SUMMARY

STUDY OF ACUTE SKIN TOLERANCE OF A COSMETIC PRODUCT AFTER A 48-HOUR SINGLE PATCH-TEST

- **Assessed product** : MICRO ALGUES 80 102 063
  code ID-02/155
- **Promoter** : BIOETHIC
- **Objective of study** : Determination of the acute skin tolerance of the cosmetic product by application over a 48 hour period.
- **Investigator** : Doctor Pascale DENIS
- **Location of the study** : Institut Dermatologique d'Aquitaine,
  Centre Montesquieu – 1, allée J. Rostand, 33651 MARTILLAC
- **Dates of the study** : From 03/12/2002 to 03/14/2002
- **Methodology** : Open and non-comparative study.

  ✓ ***Time, dose, and application conditions:*** Single application of 0.02 mg of the studied product, **pure**, on the external face of the arm, maintained for 48 hours in contact with the skin, with the help of an occlusive patch (Finn-Chambers).

  ✓ ***Assessment methods:***
  The clinical quotation is made 30 minutes after the patch removal and takes in account the erythema, the papules, the vesicles and the blisters. According to their intensity, the quotation is spread out from 0 to 4. The total sum of the scores, divided by the number of volunteers, define the average irritation index (A.I.I.) which allows to classify arbitrarily the product into « non irritant, slightly irritant, moderately irritant, very irritant and severely irritant. ».

- **Included subjects** : 10 volunteers, whom mean age is 32.3, have been included.
- **Results** : The average irritation index is 0.
- **Conclusion** : Non irritant

The product MICRO ALGUES 80 102 063, code ID-02/155, applied pure can be considered as <u>non irritant</u> after an application for 48 consecutive hours on 10 volunteers.

Tableau 1:
Caractéristiques des volontaires

N° d'inclusion	Initiales	Sexe	Age (ans)	Poids (kg)	Taille (cm)	Type de peau	Traitements en cours
1	DU-CA	F	25	62	162	normale	Cycleane 30
2	SE-KA	F	19	56	164	normale	-
3	PE-AU	F	20	52	172	normale	Mercillon
4	ZI-EV	F	36	56	168	mixte	-
5	TA-PA	F	52	67	164	normale	-
6	CA-JA	F	39	57	158	normale	-
7	CA-CA	F	32	62	158	sèche	Ginkor
8	CR-CH	F	22	51	158	normale	Diane 35
9	WA-AN	F	42	61	163	normale	-
10	BO-RO	M	36	69	170	normale	-
Moyenne			**32,3**	**59,3**	**163,7**		

Tableau 2 : Réactions cutanées du produit
MICRO ALGUES 80 102 063 code ID-02/155

N° d'inclusion	Initiales	Réactions sur le site produit ID-02/155	Score individuel du produit ID-02/155	Réactions sur le site témoin
1	DU-CA	-	0	-
2	SE-KA	-	0	-
3	PE-AU	-	0	-
4	ZI-EV	-	0	-
5	TA-PA	-	0	-
6	CA-JA	-	0	-
7	CA-CA	-	0	-
8	CR-CH	-	0	-
9	WA-AN	-	0	-
10	BO-RO	-	0	-
I.I.M. Classement			**0 non irritant**	

Abréviations :

- E = Erythème
- O = Oedème
- P = Papules
- V = Vésicules
- B = Bulles

Cotations :

- 0 = Pas d'érythème
- 0,5 = Erythème à peine visible
- 1 =Erythème léger avec présence ou non d'œdème
- 2 = Erythème modéré, œdème avec présence ou non de papules
- 3 = Erythème modéré, œdème avec présence de papules
- 4 = Erythème important, œdème, vésicules ou bulles

EVALUATION DU POTENTIEL PHOTOTOXIQUE APRES APPLICATION ET EXPOSITION UNIQUES SUR 10 VOLONTAIRES

Société : **SECMA Biotechnologies Marines**
B.P. 65
22260 PONTRIEUX

Produit : **PHYCOCORAIL 5.05.061**

Panel : **HRL # 95-515T (3)**

Arbanats, Décembre 1995

SOCIÉTÉ DE CONSEIL–EXPERTISE PHARMACEUTIQUE & COSMÉTOLOGIQUE

18 , RUE DE COULON - B.P. 15 - 33640 ARBANATS - **TÉL : 56 67 33 02** – FAX : 56 67 05 60
S.A.R.L. AU CAPITAL DE 120.000 FRANCS – APE 731Z – SIRET 384 324 141 00017

Je, soussigné, **Dominique SABOUREAU**, Docteur en Pharmacie, gérant de la Société **PALMER Research** (sis : 18, rue de Coulon ; B.P. 15 ; 33640 ARBANATS), atteste que le produit :

PHYCOCORAIL 5.05.061

a été confié à la Société HARRISON Research Laboratoires, INC (sis HRL : 2497 Vauxhall Road - Union, NJ 07083), afin d'évaluer la réponse phototoxique de ce produit chez l'homme, après application unique d'un pansement occlusif contenant le produit et exposition à des rayons ultraviolets sur 10 volontaires (10 ayant suivi le protocole jusqu'à la fin de l'essai).

Cette étude a été réalisée conformément :

- Au protocole HRL Standard, protocole # 500 T - Phototoxicity test (PT),

- Aux procédures en vigueur dans ce laboratoire,

- Dans le respect des réglementations internationales visant à la protection des personnes dans la recherche biomédicale.

- A fait l'objet d'un rapport référence **HRL Panel # 95-515T (TM#3)**

Elle a été réalisée selon le schéma expérimental suivant :

METHODOLOGIE

❶ *Volontaires inclus*

10 volontaires de type caucasien et de sexe féminin, sans affection cutanée ni antécédent médical empêchant l'application topique de substances, et âgées de 19 à 58 ans.

❷ *Traitements*

1 application d'un patch adhésif occlusif (Professional Medical Products # 4022) contenant environ 0,2 ml du produit tel que au niveau de chaque avant-bras, après nettoyage de la surface de la peau. Après application durant 24 heures, un avant-bras est irradié aux UV-A (four F4OBL avec tubes fluorescents et longueur d'onde entre 320 et 400 nm). L'autre avant-bras ne reçoit pas d'irradiation et est protégé de la lumière du jour pendant la durée du test.
Lecture immédiatement, 24 et 48 heures après irradiation, aux deux sites d'application des patchs (avant-bras irradié et non-irradié).

❸ *Appréciation des réactions*

Le produit testé est considéré comme phototoxique si, après exposition à la lumière UV-A, on observe immédiatement une réaction érythémateuse à type de papule, ou un érythème intense avec oedème au bout de 24 à 48 heures après application.

L'appréciation est donnée par un score :

0 = pas de réaction, +/- douteux
1 = érythème minime
2 = léger érythème modéré
4 = érythème avec papules ou vésicules
E = oedème.
C = changement du site testé

RESULTATS

Dans les conditions expérimentales retenues, aucune réaction cutanée n'a été enregistrée tant sur le site irradié/produit que sur le site témoin irradié sans produit.
Seul un très léger érythème passager a été noté chez 1 sujet sur le site produit non irradié.

CONCLUSION

Dans ces conditions, le produit :

PHYCOCORAIL 5.05.061

n'induit pas de réponse phototoxique de contact au niveau de la peau chez le volontaire sain.

La tolérance cutanée aiguë après application du produit sous pansement occlusif pendant 24 heures peut être jugée bonne, seul un sujet ayant présenté un léger érythème passager.

Fait à Arbanats, le 4 décembre 1995.

Dominique SABOUREAU,
Docteur en Pharmacie.

Google Translate (June 25, 2021) – Translation of Palmer Research 1995 Phototoxicity study of Phycocorail (contains 9-11% Diatomaceous Earth)

Phycocorail was tested undiluted in this study.

Methods

Volunteers

10 volunteers of Caucasian type and female, without skin disease or medical history preventing the topical application of substances, and approved from 19 to 58 years old

Treatments

1 application of an occlusive adhesive patch containing approximately 0.2 ml of the product on each forearm, after cleaning the skin surface. After application for 24 hours, a forearm is irradiated with UV-A (4 F4OBL with fluorescent tubes and wavelength between 320 and 400 nm). The other arm does not receive irradiation and is protected from daylight for the duration of the test.

Read immediately, 24 and 48 hours after irradiation, at the two patch application sites (irradiated and nonirradiated forearm).

Appreciation of Reactions

The product tested is considered to be phototoxic if, after exposure to UV-A light, an erythematous papule-like reaction is immediately observed, or an intense erythema with edema within 24 to 48 hours after application.

change of test site appreciation is given by a score

0 = no reaction, +/- doubtful
1 = minimal erythema
2 = mild moderate erythema
4 = erythema with papules or vesicles
E = edema
C = change of test site

Results

In the experimental conditions retained, no skin reaction was recorded either on the irradiated / product site or on the control site irradiated without product. Only a very slight transient erythema was noted in 1 subject on the non-irradiated product site.

Conclusion

Under these conditions, the product: PHYCOCORAIL does not induce a contact phototoxic response on the skin in healthy volunteers. Acute skin tolerance after application of the product under an occlusive dressing for 24 hours can be considered good, only one subject having presented a slight transient erythema.

STUDY SUMMARY REPORT

Sponsor : SECMA BIOTECHNOLOGIES MARINES **Address :** **ZI-BP65** **22260 PONTRIEUX** **FRANCE**	**Product : PHYCOCORAIL 209338** **PALMER Research code** *: PA-03/0063*

DETERMINATION OF THE IRRITATION POTENTIAL OF A COSMETIC PRODUCT ON HUMAN SUBJECT: 24-HOURS SINGLE OCCLUSIVE PATCH-TEST

Study date:	The study took place from January 29th to January 31st, 2003.	
Study place(s):	PALMER Research 13 rue Le Chatelier 42100 ST ETIENNE	
Objective(s):	Determination of the acute skin tolerance of a cosmetic product by application under occlusive patch over a 24-hours period.	
Methodology:	Open Study.	Number of subjects : 11 with sensitive skin.
Included criteria:	Skin without any dermatological lesion, non allergic volunteer.	• Length of application: 24 hours • Condition of use : pure
Evaluation criteria:	Calculation of an acute irritation index : $M.I.I = \frac{\text{total cutaneous reactions score (erythema + oedema)}}{\text{number of volunteers}}$ Skin responses are scored from 0 to 3.	
Analysis:	Classification of the product according to its M.I.I: if $M.I.I < 0.20$ Non-irritant if $0.20 \leq M.I.I < 0.50$ Slightly irritant if $0.50 \leq M.I.I < 1$ Moderately irritant if $M.I.I \geq 1$ Irritant	
Conclusion:	The irritation index of the product **PHYCOCORAIL 209338** is equal to **0** at the 30-minutes and at the 24-hours readings. It is thus classified as **non-irritant** to human skin.	
Dr Florence DURAFOUR **Dermatologist**		

4 - RESULTATS

Les résultats individuels des lectures à chaque temps expérimental sont regroupés dans le tableau ci-dessous.

PHYCOCORAIL 209338
(patch test 24 heures occlusif – pur)

SUJETS				LECTURES									
				Lecture 30 min après enlèvement du patch occlusif					Lecture 24 heures après enlèvement du patch occlusif				
N°	Identification	Age et sexe (1)	Type de peau	Témoin		Produit à l'essai		Modification de structure	Témoin		Produit à l'essai		Modification de structure
				E	O	E	O		E	O	E	O	
14S05	BRI Ma	44 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
15S05	SMA Ch	43 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
16S05	VER Ca	28 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
17S05	BLA Br	41 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
18S05	CON Je	19 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
19S05	LAS Jo	60 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
20S05	WIL Co	30 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
21S05	SEB Ha	20 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
22S05	MON Au	21 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
23S05	PAT Ni	18 ans / M	Sensible	0	0	0	0	-	0	0	0	0	-
25S05	MIG Co	32 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-

I.I.M	**0**	**0**
Résultats	**non irritant**	**non irritant**

(1) : M = masculin
F = féminin

EVALUATION DU POTENTIEL SENSIBILISANT D'UN PRODUIT COSMETIQUE : TEST CLINIQUE FINAL DE SECURITE SOUS CONTRÔLE DERMATOLOGIQUE

ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SECURITY TEST UNDER DERMATOLOGICAL CONTROL

Rapport / *Report*:	**14E0050**
Référence etude / *Study reference:*	**DN-1334**
Produit / *Product* :	**LCA13049 - 13P3793-1** formulation containing 10% Phycocorail, which contains 9-11% Diatomaceous Earth
Promoteur / *Sponsor* :	**SEPPIC** **127, chemin de la POUDRERIE** **BP 228** **81100 CASTRES cedex** **FRANCE**
C.R.O.	**DERMSCAN** Domaine Scientifique de la Doua 56, Boulevard Niels Bohr **69623 VILLEURBANNE Cedex - FRANCE**
Moniteur de l'étude / *Study Monitor*	**LISKIN - Dr. Bogdan WICHROWSKI** IMMEUBLE FONTENAY AFFAIRES 91, rue Boucicaut **92260 FONTENAY-AUX-ROSES - FRANCE**
Investigateur / *Investigator*	**PROCOS - Dr Marlena NOWAKOWSKA**

***Lyon*, 10/04/2014**

RAPPORT / *REPORT*

REFERENCE ETUDE / *STUDY REFERENCE*	**DN-1334/14E0050**
PRODUIT / *PRODUCT*	**«LCA13049 - 13P3793-1»**
NOMBRE DE SUJETS / *NUMBER OF SUBJECTS*	**100**
PROMOTEUR / *C.R.O*	**Groupe DERMSCAN**
MONITEUR / *MONITOR*	**Mr Bogdan WICHROWSKI** **LISKIN** **IMMEUBLE FONTENAY AFFAIRES** **91, rue Boucicaut** **92260 FONTENAY-AUX-ROSES** **FRANCE** **☎ : 33 (0)9 50 27 08 28** **🖷 : 33 (0)1 49 73 66 80**
INVESTIGATEUR / *INVESTIGATOR*	**Dr Marlena NOWAKOWSKA,** **Médecin Dermatologue / *Dermatologist***

Document comportant 28 pages / 28 pages document

SOMMAIRE / INDEX

RESUME DE L'ETUDE - *STUDY SUMMARY*

TITRE : TEST CLINIQUE FINAL DE SECURITE : ETUDE DU POUVOIR SENSIBILISANT D'UN PRODUIT, SELON LA METHODE DE MARZULLI-MAIBACH SUR 100 SUJETS PENDANT 6 SEMAINES.

TITLE : CLINICAL FINAL SECURITY TEST: SENSITIZING POTENTIAL STUDY OF A PRODUCT ACCORDING TO MARZULLI-MAIBACH METHOD, ON 100 SUBJECTS DURING 6 WEEKS.

PRODUIT / PRODUCT: LCA13049 - 13P3793-1

REALISATION DE L'ETUDE : L'étude a été réalisée et les valeurs numériques saisies par l'Unité Clinique PROCOS, localisée en Pologne ; ul. Slowackiego 27/33 lok. 33/34 ; 01-592 Varsovie.
STUDY IMPLEMENTATION: The study was carried out and all test values recorded by the Clinical Unit PROCOS, localized in Poland, ul. Slowackiego 27/33 lok. 33/34; 01-592 Warsaw.

INVESTIGATEUR / INVESTIGATOR: Dr Marlena NOWAKOWSKA
MONITEUR DE L'ETUDE / MONITOR: Dr ing. Bogdan WICHROWSKI

PROTOCOLE : TEST DE MAXIMALISATION SELON MARZULLI-MAIBACH
PROTOCOL: SENSITIZATION TEST ACCORDING TO MARZULLI-MAIBACH METHOD.

BUT DE L'ETUDE : Evaluer sous contrôle dermatologique le potentiel irritant et sensibilisant d'un produit dans les conditions prévues par le promoteur de l'étude.
AIM OF THE STUDY: To evaluate the sensitizing potential of a product under dermatological control and under the conditions defined by study's sponsor.

SUJETS : 100 volontaires à peau normale correspondant aux critères d'inclusion et de non-inclusion déterminés par le Groupe DERMSCAN.
SUBJECTS: 100 healthy volunteers with normal skin corresponding to the inclusion and non-inclusion criteria defined by the DERMSCAN Group.

PERIODE DE L'ETUDE / STUDY DATE: 24/02/14 - 04/04/14 / *February 24th, 2014 to April 4th, 2014*

PLAN EXPERIMENTAL : **Etude monocentrique en simple aveugle.**
STUDY DESIGN *Monocentric and simple blind study.*

PRINCIPAUX PARAMETRES DE TOLERANCE :

- Potentiel irritant (phase d'induction)
  Erythème, œdème, sécheresse, vésicules évalués par le dermatologue selon un score de 0 à 3
- Potentiel sensibilisant (phase de révélation)
  Réaction évaluée par le dermatologue selon un score de 0 à 3 établis par l'ICDRG (International Contact Dermatitis Research Group)

MAIN TOLERANCE PARAMETERS:

- *Irritation potential (Induction Phase)*
  Erythema, edema, desquamation, vesicles rated from 0 to 3 by the dermatologist
- *Sensitizing potential (Challenge Phase)*
  Reaction rated from 0 to 3 by the dermatologist according to ICDRG (International Contact Dermatitis Research Group)

RESULTATS - *RESULTS:*

Dénomination du produit - *Product name*	POTENTIEL IRRITANT *IRRITATION POTENTIAL*	POTENTIEL SENSIBILISANT *SENSITIZING POTENTIAL*
LCA13049 - 13P3793-1	non irritant *non-irritating*	Aucune réaction de type allergique *No allergic reaction*

CONCLUSION :
Dans les conditions de cette étude, le produit «LCA13049 - 13P3793-1» s'est avéré non irritant et non sensibilisant.

CONCLUSION :
Under these study conditions, the product «LCA13049 - 13P3793-1» can be considered non-irritating and non-sensitizing.

1. ASSURANCE QUALITE / *QUALITY ASSURANCE*

L'étude a été réalisée selon les règles des Bonnes Pratiques Cliniques définies par les ICH Topic E6 "Note for Guidance and good clinical practice" (CPMP/ICH/135/95), par la Déclaration d'Helsinki (1964, WMA) et ses mises à jours successives), par la CEE (Directives n° 91/507 et III 3976/88 EN du 11/07/1990) et par le Ministère de la Santé de la République Française.

Il est de la responsabilité de l'industriel, fabricant du produit testé, de justifier qu'aucune substance constituant ce produit n'est sensibilisante.

L'étude a été menée selon les Procédures Opératoires Standards et selon le protocole de l'étude défini par le promoteur.
Les cahiers d'observation et les journaux de suivi ont été vérifiés ainsi que l'exactitude des données.

L'authenticité et la véracité des données expérimentales recueillies ont été confirmées par les personnes ayant participé à l'étude (ANNEXE II).

The described study has been conducted according to the Good Clinical Practice defined by the ICH Topic E6 "Note for Guidance and good clinical practice" (CPMP/ICH/135/95), the Helsinki Declaration (1964, WMA) and its succesives updates, the EEC (Directives n° 91/507 and III 3976/88 of 11/07/1990) and to the Ministry of Health of the French Republic.

The first evaluation of sensitization risks for all ingredients depends on the responsibility of the tested product manufacturer.

The study has been conducted according to Standard Operating Procedures and to the study protocol defined by the sponsor. All study events recorded during the study are reported.

Controls on data veracity and conformity with the protocol have been performed and confirmed by persons participating in the study (APPENDIX II).

2. CERTIFICAT DE CONFORMITE / *CERTIFICATE OF CONFORMITY*

A ma connaissance, l'étude DN-1334/14E0050 a été conduite en accord avec l'**«Assurance qualité»** précitée.
I am aware that the study DN-1334/14E0050 *has been conducted according to the «**Quality Assurance**» described before.*

Il ne s'est pas produit d'événement susceptible d'affecter la qualité ou l'intégrité des données.
There was no event which may have affected the quality or integrity of the data.

p/o

Dr ing. B WICHROWSKI
Moniteur / Monitor

10/04/2014
date

3. METHODOLOGIE / *METHOD*

3.1 PRODUIT A L'ETUDE / *STUDY PRODUCT*

Le produit fourni par le Groupe DERMSCAN, présentait les caractéristiques suivantes :
The product supplied by Group DERMSCAN, had the following characteristics :

Dénomination du produit - *Product name*	Aspect du produit Product aspect	Code du produit *Product code*
LCA13049 - 13P3793-1	**Liquide beige opaque** *Opaque beige liquid*	EB

Le produit a été réceptionné le 17/02/2014.
The product was receipted on February 17th, 2014.

3.2 METHODES CLINIQUES / CLINICAL METHODS

3.2.1 Objectif de l'étude / Aim of the study

Evaluer le pouvoir irritant et sensibilisant du produit par la méthode de Marzulli-Maibach.
To evaluate irritating and the sensitizing potential of a product by Marzulli-Maibach method.

3.2.2 Plan expérimental / *Experimental design*

L'étude a été réalisée en ouvert.
This was an open study.

3.2.3 Sujets de l'étude / *Study subjects*

Critères d'inclusion

- **Volontaire sain d'origine caucasienne**
- **Age compris entre 18 et 70 ans**
- **Phototype II, III ou IV**
- **Personne ne présentant ni cicatrice, ni tatouage, ni tache pigmentaire d'aucune sorte, ni pilosité trop importante, ni lésion dermatologique, ni traces d'un maillot de bain au niveau du dos**
- **Personne ayant donné par écrit son consentement libre, éclairé et exprès**
- **Sujet coopérant, averti de la nécessité et de la durée des contrôles permettant d'espérer une parfaite adhésion au protocole mis en place par le Groupe DERMSCAN.**

Inclusion criteria

- *Healthy volunteer of Caucasian origin*
- *Age between 18 and 70*
- *Phototype II, III or IV*
- *Volunteer without scars, active dermal lesions, tattoos, any pigmentary marks, excessive pilosity and uneven skin tones of the areas of the back to be tested.*
- *Subjects having given their informed, written consent*
- *Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the DERMSCAN Group could have been expected.*

Critères d'exclusion

- Femme enceinte ou qui allaite
- Exposition au soleil ou aux U.V. depuis 15 jours avant le début et pendant l'étude et /ou ayant reçu des photopatch-tests depuis moins de 2 mois
- Peau hyper irritable ou pathologie cutanée,
- Allergique ou sensibilité connues au sparadrap et /ou aux produits cosmétiques
- Pathologie cutanée, cicatrices, grains de beauté, tache de rousseur ou toute anomalie sur la zone d'expérience et/ou présentant une lucite
- Maladie grave ou évolutive
- Sujet suivant un traitement médicamenteux topique ou systémique :
  - anti-inflammatoires et/ou antihistaminiques pendant la semaine qui précède et durant l'étude
  - substances photosensibilisantes et /ou phototoxiques depuis moins d'un mois et pendant l'étude
  - immunosuppresseurs et /ou corticoïdes pendant les 4 semaines qui précédent et durant l'étude
  - rétinoïdes pendant les 6 mois précédant l'étude et durant l'étude
- Troubles dus à l'absorption excessive d'alcool ou de substances toxiques.

Non-inclusion criteria

- *Pregnant or nursing women*
- *Sun exposure or UV exposure 15 days before or during the study and/or photopatch-tests from less than 2 months*
- *Hyperirritable skin or cutaneous pathology*
- *Known allergies or sensitivities to adhesive plaster and/or cosmetics products*
- *History of abnormal responses to sunlight or presence of active dermal lesions. Scars, beauty spots, freckle or any abnormality, on the back*
- *History of cancer or other important disease*
- *Volunteers undergoing a topical or systemic treatment:*
  - *anti-inflammatories and/or anti-histamines during the previous week and during the study*
  - *photo-allergic and/or phototoxic substances from less than 1 month and during the study*
  - *immuno-suppressors and/or corticoids during the four previous weeks and during the study*
  - *retinoids during the six previous months and during the study*
- *Excessive use of alcohol, tobacco and toxic substances.*

Inclusion

100 sujets volontaires ont été choisis en accord avec les critères d'inclusion et les critères d'exclusion, et 100 sujets ont réalisé la totalité de l'étude. Le tableau suivant regroupe les informations concernant la participation à l'étude de tous les sujets sélectionnés.

100 healthy volunteers were selected according to the inclusion and the non-inclusion criteria, and 100 subjects completed study. The table below presents the information concerning all the included volunteers.

	Non inclus *Non included*	Inclus *Included*	Arrêt en cours d'étude *Drop out*	Perdus de vue *Untraceable*
Nombre de sujets *Number of subjects*	0	100	0	0

Caractéristiques des sujets / *Subjects characteristics*

Le tableau récapitulatif ci-dessous présente une synthèse des observations concernant uniquement les volontaires inclus dans l'analyse des données.
The summary table below presents a synthesis of the observations concerning exclusively the volunteers taken into account for data analysis

Nombre de Volontaires *Number of subjects*	Sexe *Sex*	Age (moy±SEM) *Age (mean±SEM)*	Phototype	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
				avant l'étude *Before the study*	pendant l'étude *During the study*
100	86 F 14 M	44 ± 1	II : 100 III : 0 IV : 0	cf. Tableaux en ANNEXE II *cf. Tables in the APPENDIX II*	

3.3 MATERIEL / MATERIAL

Les patch-tests utilisés sont les FINN CHAMBERS ON SCANPOR® qui assurent une bonne occlusion.

The patch-tests used are FINN CHAMBERS ON SCANPOR® which ensures a good occlusion

4 APPLICATION DU PRODUIT / *PRODUCT APPLICATION*

Zones d'application	Zones scapulaires : homolatérale (zone d'induction) et controlatérale (zone de révélation)
Quantité et concentration appliquée	25 µl Pur
Fréquence	Phase d'induction : 3 fois par semaine pendant 48 heures Phase de révélation : 1 fois pendant 48 heures
Durée	Phase d'induction : 3 semaines Phase de latence : 2 semaines Phase de révélation : 1 semaine
Conditions d'application	Avant application, la peau a été préalablement nettoyée et séchée. Le produit «LCA13049 - 13P3793-1» a été déposé dans un patch occlusif (avec papier filtre), et appliqué sur le dos du volontaire. Un patch ne contenant aucun produit a été appliqué dans les mêmes conditions et a servi de témoin non traité. Durant toute la phase d'induction, la zone homolatérale n'a pas été mouillée. Les volontaires se sont douchés le dimanche après le retrait des patchs en faisant attention à ne pas mettre de produit détergent sur les sites. Lors de la Phase de Révélation, aucun lavage ni aucune application de quelconque produit n'ont été effectués sur la zone controlatérale.

Application area	*Scapular zones: homolateral (induction zone) and controlateral (challenge zone)*
Quantity and Concentration applied	*25 µl* *Pure*
Frequency	*Induction Phase: 3 times a week during 48 hours* *Challenge Phase: once during 48 hours*
Contact time	*Induction Phase: 3 weeks* *Rest Phase: 2 weeks* *Challenge Phase: 1 week*
Application conditions	*Before application, the skin was cleaned and dried. The product «LCA13049 - 13P3793-1» was applied in an occlusive patch with filter paper and applied to the volunteer's back.* *The patch containing no product was applied under the same conditions to serve as a non-treated control.* *During the whole induction phase, the homolateral zone was not wet. Volunteers took a shower on Sunday, after patches removing, and paid attention not to put a detergent product on all tested zones. During all the challenge phase, no washing and no product application took place on the controlateral zone.*

5 DEROULEMENT DE L'ETUDE / *STUDY SCHEDULE*

Phase d'induction - trois semaines (S1, S2, S3)
Induction phase – 3 weeks (W1, W2, W3)

S1 / *W1:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J1 *D1*	J2 *D2*	J3 *D3*	J4 *D4*	J5 *D5*	J6 *D6*	J7 *D7*
Application du produit *Product application*	↓		↓		↓		

S2 / *W2:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J8 *D8*	J9 *D9*	J10 *D10*	J11 *D11*	J12 *D12*	J13 *D13*	J14 *D14*
Application du produit *Product application*	↓		↓		↓		

S3 / *W3:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J15 *D15*	J16 *D16*	J17 *D17*	J18 *D18*	J19 *D19*	J20 *D20*	J21 *D21*
Application du produit *Product application*	↓		↓		↓		

Phase de latence - deux semaines (S4, S5)
***Rest Phase** - 2 weeks (W4, W5)*

Pas de lecture – *No reading*

S4/ *W4* :

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J22 *D22*	J23 D23	J24 *D24*	J25 *D25*	J26 *D26*	J27 D27	J28 *D28*

S5 / *W5:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J29 *D29*	J30 *D30*	J31 D31	J32 *D32*	J33 *D33*	J34 *D34*	J35 *D35*

Phase de révélation (double challenge test) - une semaine (S6)
***Challenge Phase** - 1 week (W6)*

S6 / *W6* :

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*
Jour d'étude *Study day*	J36 D36	J37 D37	J38 D38	J39 D39	J40 D40
Application du produit *Product application*	↓				
Jour d'étude *Study day*			L		L

6 CRITERES D'EVALUATION / *ASSESSMENT CRITERIA*

6.1 CRITERES CLINIQUES CONCERNANT LE POTENTIEL IRRITANT (PHASE D'INDUCTION) *CLINICAL CRITERIA REGARDING THE IRRITATING POTENTIAL (INDUCTION PHASE)*

Après chaque application, le patch est enlevé et la lecture est effectuée 30 minutes plus tard pour éliminer l'effet de pression, d'occlusion et d'arrachement dû au matériel.
Le test est négatif si la peau garde un aspect normal.
Les quatre critères suivants sont évalués par le dermatologue selon une cotation de 0 à 3 :

After each application, the patch is removed and the clinical examination is performed by the investigator 30 minutes later in order to eliminate the pressure and the occlusion effects
The result of examination is negative if the skin looks normal
The clinical examination is made on the back using the following criteria and scale (Quotation 0 to 3):

Score	Quotation	CRITERES : description *CRITERIA : description*			
		ERYTHEME *ERYTHEMA*	OEDEME *EDEMA*	SECHERESSE *DRYNESS*	VESICULES *VESICLES*
0	absent	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*
1	Léger *slight*	Coloration rosée discrète de toute la surface testée ou bien visible sur une partie de la surface testée *Discreet pink coloration of the whole tested area or rather visible on part of the tested area*	Plus palpable que visible *More palpable than visible*	Desquamation fine discrète, aspect dépoli *Discreet thin desquamation, tarnished aspect*	Vésicules plus palpables que visibles *More palpables than visible vesicles*
2	Net *obvious*	Erythème net couvrant toute la surface testée *Marked erythema covering the whole tested area*	Œdème visible *Visible edema*	Desquamation visible, aspect écailleux *Visible desquamation flaky aspect*	Vésicules visibles *Visible vesicles*
3	Important *important*	Erythème intense couvrant toute la surface testée ou érythème diffusant en dehors de la surface testée *Severe erythema covering the whole tested area or erythema diffusing beyond the tested area*	Pouvant déborder de la surface testée *Edema diffusing beyond the tested area*	Desquamation importante, fissuration *Important desquamation, cracking*	Vésicules débordant de la zone testée ou bulles *Vesicles diffusing beyond the tested area or blisters*

6.2 CRITERES CLINIQUES CONCERNANT LE POTENTIEL SENSIBILISANT (PHASE DE REVELATION)

CLINICAL CRITERIA REGARDING THE SENSITIZING POTENTIAL (CHALLENGE PHASE)

Les réactions allergiques ont été évaluées selon l'échelle suivante :
The allergic reactions were evaluated according to the following scale:

Critère - *Criterion*	Cotation ICDRG* *ICDRG (*)Quotation*	Cotation "notée" *Numeric score Quotation*
Absence de réaction *No reaction*	0	0
Réaction douteuse Doubtful reaction	?	?
Erythème et œdème *Erythema and edema*	+	1
Erythème, œdème et vésicules *Erythema, edema and vesicles*	++	2
Réaction forte avec présence de bulles ou d'ulcérations post-bulbeuses *Severe reaction with blisters*	+++	3

* (International Contact Dermatitis Research Group)

6.3 MODE D'EVALUATION / *ASSESSMENT METHOD*

6.3.1 Pouvoir irritant - Phase d'induction / *Irritating potential - Induction Phase*

A l'issue des 8 lectures de la phase d'induction, le score moyen de chaque volontaire a été calculé en additionnant les scores obtenus à chacune des lectures et en divisant cette somme par le nombre effectif de lectures (une lecture n'était pas prise en compte s'il y avait réaction au témoin ou irritation globale).

Le pouvoir irritant du produit a été évalué lors de la phase d'induction, en faisant la moyenne des réactions survenues.
Le pouvoir irritant du produit a été déterminé selon la formule suivante :

At the conclusion of the 8 readings of the induction phase, the average score of every volunteer was calculated by adding the scores obtained for each of the readings and by dividing this sum by the actual number of readings (a reading was not taken into account if there was reaction of the control or global irritation).
The irritating power of the product was estimated, by calculating the mean of the reactions observed.
The irritating power of the product was determined according to the following formula

$$\text{Score moyen} = \frac{[(\sum \text{scores J1...J19 / nb de lectures}) \text{ vol1} + ... + (\sum \text{scores J1...J19 / nb de lectures}) \text{ volN}[}{\text{nb de volontaires (N)}}$$

$$\textit{Average score} = \frac{[(\sum \text{scores D1...D19 / nb of readings}) \text{ vol1} + ... + (\sum \text{scores D1...D19 / nb of readings}) \text{ volN}]}{\textit{nb of volunteers (N)}}$$

Score moyen *Average score*	Pouvoir irritant *Irritating Potential*
0,000 – 0,080	Non irritant *Non-irritating*
0,081 – 0,160	Très légèrement irritant *Very slightly-irritating*
0,161 – 0,560	Légèrement irritant *Slightly-irritating*
0,561 – 1,000	Modérément irritant *Moderately-irritating*
1,001 – 1,600	Fortement irritant *Strongly-irritating*
> 1,600	Très fortement irritant *Very strongly-irritating*

6.3.2 Pouvoir sensibilisant - Phase de révélation
Sensitizing potential - Challenge Phase

Une réaction allergique éventuelle au cours des Phases d'Induction ou de Révélation était notée de 0 à 3 selon les critères de l'ICDRG (International Contact Dermatitis Research Group) – voir le tableau en paragraphe 6.2.
Lors de la révélation, une lecture sera faite 30 minutes après enlèvement des patch-tests puis 48h plus tard

The possible allergic reaction, during the Induction or Challenge Phase, was rated from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group) – see the table paragraph 6.2.
During the Challenge Phase, the reading took place 30 minutes after patch-tests removal and 48 hours later.

Le pouvoir sensibilisant du produit a été évalué lors des lectures à J38 et J40 (phase de révélation) en fonction des critères suivants : réaction ++ (2) ou +++ (3) en l'absence de phénomène d'irritation surajouté.

The sensitizing potential of the product was assessed by the readings on D38 and D40 (Challenge Phase) according to the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon. (3) in the absence of added irritation phenomenon.

La survenue d'un seul cas de sensibilisation active **(score supérieur ou égale à ++ (2))** du côté controlatérale conduit à la conclusion : « Produit potentiellement sensibilisant ».

*The presence of only one case of active sensitizing **(upper or equal score in ++ (2))** on controlateral side leads to the conclusion "Potentially sensitive product".*

7 ARRET PREMATURE / *PREMATURE STUDY TERMINATION*

Les sujets avaient le droit de sortir de l'essai à tout moment pour quelle que raison que ce soit.

The subjects had the right to leave the study at any time whatever the reason.

L'arrêt prématuré peut être du à des multiples raisons :
- non respect du calendrier des visites par le sujet
- événements indésirables (incluant les maladies intercurrentes)
- violations et déviations au protocole
- sorties après retrait du consentement du sujet.

The premature study termination could be due to multiple reasons:
- *non-compliance with the visits schedule,*
- *adverse events (including intercurrent diseases),*
- *protocol non-adherence/departures from protocol,*
- *withdrawal of subject consent*

Le médecin investigateur peut interrompre l'essai en cours, soit sur certains sujets, soit sur l'ensemble du panel, notamment, si le produit entraine des réactions cutanées importantes ou anormales, ou s'il juge que la poursuite de l'essai peut nuire à la santé du ou des sujets concernés.

The doctor investigator can interrupt the essay either on certain subjects or on the the whole panel, if the product induces important or abnormal cutaneous reactions or if he considers that the continuation of the essay can damage health of one or several concerned subjects.

8 AMENDEMENTS AU PROTOCOLE / *PROTOCOL AMENDMENT*

Néant / *None.*

9 RESULTATS / *RESULTS*

9.1 POUVOIR IRRITANT: PHASE D'INDUCTION / *IRRITATING POTENTIAL: INDUCTION PHASE*

Le TABLEAU DES LECTURES durant la phase d'induction est présenté en ANNEXE III.
The TABLE OF READINGS regarding the Induction Phase is presented in the APPENDIX III.

Ces lectures effectuées 30 minutes après le retrait des patch-tests ont montré les résultats suivants :
The readings done 30 minutes after having removed the patch-tests showed the following results:

Produit *Product*	score	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*		Conclusion
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
EB	T+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	non irritant *non-irritating* (IRR = 0.000)
	0 :	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	
	1 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

T+ = Témoin positif / *Positive control*
VM = valeur manquante / *missing value*
% = % of subjects / *% of subjects*
IRR = irritation globale / *global irritation*
n = nombre de sujet / *number of subjects*

Dans les conditions de cette étude, le produit «LCA13049 - 13P3793-1» a montré un score inférieur à 0,080.
Il peut donc être considéré comme non irritant.

Under these study conditions, the product «LCA13049 - 13P3793-1» showed a score lower than 0.080.
It can thus be considered as non-irritating.

9.2 POTENTIEL SENSIBILISANT : PHASE DE REVELATION
SENSITIZING POTENTIAL: CHALLENGE PHASE

Le TABLEAU DES LECTURES durant la phase de révélation est présenté en ANNEXE IV.
Les lectures effectuées 30 minutes et 48 heures après le retrait des patch-tests de révélation ont donné les résultats suivants :

The TABLE OF READING regarding the Challenge Phase is presented in APPENDIX IV.
These reading made 30 minutes and 48 hours after having removed the patch-tests showed the following results

Code Produit : EB *Product Code : EB*	Zones	score	Jour de lecture *Day of the reading*				Résultat global *Global result*
			J38 / *D38*		J40 / *D40*		
			n	%	n	%	
LCA13049 - 13P3793-1	Lectures zone homolatérale *Homolateral zone readings*	T+ :	0	0	0	0	non sensibilisant *non-sensitizing*
		0 :	100	100	100	100	
		? :	0	0	0	0	
		1 :	0	0	0	0	
		2 :	0	0	0	0	
		3 :	0	0	0	0	
	Lectures zone controlatérale *Controlateral Zone readings*	T+ :	0	0	0	0	
		0 :	100	100	100	100	
		? :	0	0	0	0	
		1 :	0	0	0	0	
		2 :	0	0	0	0	
		3 :	0	0	0	0	

EB = LCA13049 - 13P3793-1

T+ = **Témoin positif** / Positive control
IRR = **irritation globale** / global irritation
VM = **valeur manquante** / missing value
n = **nombre de sujet** / *number of subjects*
% = **% of subjects** / *% of subjects*

Dans les conditions de cette étude, aucune réaction ++ (2) ou +++ (3) ont été constatées. Le produit «LCA13049 - 13P3793-1» peut donc être considéré comme non sensibilisant.

Under these study conditions no reaction ++ (2) nor +++ (3) were observed, so the product «LCA13049 - 13P3793-1» can be considered non-sensitizing.

10 CONCLUSION

Dans les conditions de cette étude, le produit «LCA13049 - 13P3793-1» s'est avéré non irritant et non sensibilisant.

Under these study conditions, the product «LCA13049 - 13P3793-1» can be considered non-irritating and non-sensitizing.

ANNEXE / *APPENDIX*

ANNEXE I / *APPENDIX I*

FEUILLE D'AUTHENTIFICATION DES RESULTATS

AUTHENTIFICATION PAGE

PROCOS

KARTA AUTENTYCZNOŚCI REZULTATÓW
FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

Według posiadanych przeze mnie informacji, badanie Nr :
A ma connaissance l'étude N° :
I am aware that the study N° :

DN – 1334

było przeprowadzone zgodnie PROTOKOŁEM oraz KARTĄ PARAMETRÓW TESTU.
a été conduite en accord avec le PROTOCOLE et la FICHE DES PARAMETRES D'ETUDE.
has been conducted according to the PROTOCOL and to the STUDY PARAMETERS PAGE.

Mgr inż. Barbara WAŁEJKO Odpowiedzialna za badania *Responsable d'unité* Unit head	B. Wałejko podpis / *signature*	*04/04/2014* data / *date*
Dr Marlena NOWAKOWSKA Lekarz dermatolog *Dermatologue* Dermatologist	Nowakowska podpis / *signature*	*04/04/2014* data / *date*
Mgr Magdalena KUREK Odpowiedzialna za jakość *Responsable qualité* Responsible for quality control	M. Kurek podpis / *signature*	*04/04/2014* data / *date*
Barbara SZULC Asystentka *Assistante* Medical	B. Szulc podpis / *signature*	*04/04/2014* data / *date*

ANNEXE II / *APPENDIX II*

CARACTERISTIQUES DES VOLONTAIRES

SUBJECTS CHARACTERISTICS

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *during the study*
1	[redacted]	26	F	II	N	-	-
2	[redacted]	56	F	II	N	-	-
3	[redacted]	20	F	II	N	-	-
4	[redacted]	52	F	II	N	-	-
5	[redacted]	63	F	II	N	-	-
6	[redacted]	53	F	II	N	-	-
7	[redacted]	24	F	II	N	-	-
8	[redacted]	63	F	II	N	-	-
9	[redacted]	42	F	II	N	-	-
10	[redacted]	34	F	II	N	-	-
11	[redacted]	36	F	II	N	-	-
12	[redacted]	47	F	II	N	-	-
13	[redacted]	52	F	II	N	-	-
14	[redacted]	50	F	II	N	-	-
15	[redacted]	36	F	II	N	-	-
16	[redacted]	53	F	II	N	-	-
17	[redacted]	52	F	II	N	-	-
18	[redacted]	40	F	II	N	-	-
19	[redacted]	54	F	II	N	-	-
20	[redacted]	52	F	II	N	-	-
21	[redacted]	50	F	II	N	-	-
22	[redacted]	20	F	II	N	-	-
23	[redacted]	58	F	II	N	-	-
24	[redacted]	56	F	II	N	-	-
25	[redacted]	37	F	II	N	-	-
26	[redacted]	22	F	II	N	-	-
27	[redacted]	54	F	II	N	-	-
28	[redacted]	49	F	II	N	-	-
29	[redacted]	33	F	II	N	-	-
30	[redacted]	50	F	II	N	-	-
31	[redacted]	28	M	II	N	-	-
32	[redacted]	58	F	II	N	-	-
33	[redacted]	41	F	II	N	-	-
34	[redacted]	64	F	II	N	-	-
35	[redacted]	50	F	II	N	-	-
36	[redacted]	27	F	II	N	-	-
37	[redacted]	28	F	II	N	-	-
38	[redacted]	50	F	II	N	-	-
39	[redacted]	57	F	II	N	-	-
40	[redacted]	54	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *during the study*
41	[redacted]	40	F	II	N	-	-
42	[redacted]	50	F	II	N	-	-
43	[redacted]	63	F	II	N	-	-
44	[redacted]	49	F	II	N	-	-
45	[redacted]	29	F	II	N	-	-
46	[redacted]	31	F	II	N	-	-
47	[redacted]	46	F	II	N	-	-
48	[redacted]	37	F	II	N	-	-
49	[redacted]	29	F	II	N	-	-
50	[redacted]	44	M	II	N	-	-
51	[redacted]	49	M	II	N	-	-
52	[redacted]	57	F	II	N	-	-
53	[redacted]	53	M	II	N	-	-
54	[redacted]	60	F	II	N	-	-
55	[redacted]	21	F	II	N	-	-
56	[redacted]	51	M	II	N	-	-
57	[redacted]	27	F	II	N	-	-
58	[redacted]	34	M	II	N	-	-
59	[redacted]	23	M	II	N	-	-
60	[redacted]	55	M	II	N	-	-
61	[redacted]	61	F	II	N	-	-
62	[redacted]	48	F	II	N	-	-
63	[redacted]	55	F	II	N	-	-
64	[redacted]	47	F	II	N	-	-
65	[redacted]	46	F	II	N	-	-
66	[redacted]	23	F	II	N	-	-
67	[redacted]	35	F	II	N	-	-
68	[redacted]	52	F	II	N	-	-
69	[redacted]	64	F	II	N	-	-
70	[redacted]	52	F	II	N	-	-
71	[redacted]	37	F	II	N	-	-
72	[redacted]	55	M	II	N	-	-
73	[redacted]	36	F	II	N	-	-
74	[redacted]	27	F	II	N	-	-
75	[redacted]	42	F	II	N	-	-
76	[redacted]	28	F	II	N	-	-
77	[redacted]	40	F	II	N	-	-
78	[redacted]	60	M	II	N	-	-
79	[redacted]	24	F	II	N	-	-
80	[redacted]	41	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *during the study*
81	[redacted]	41	F	II	N	-	-
82	[redacted]	37	F	II	N	-	-
83	[redacted]	51	F	II	N	-	-
84	[redacted]	34	M	II	N	-	-
85	[redacted]	48	F	II	N	-	-
86	[redacted]	58	F	II	N	-	-
87	[redacted]	41	F	II	N	-	-
88	[redacted]	53	F	II	N	-	-
89	[redacted]	49	F	II	N	-	-
90	[redacted]	34	M	II	N	-	-
91	[redacted]	57	F	II	N	-	-
92	[redacted]	29	M	II	N	-	-
93	[redacted]	43	F	II	N	-	-
94	[redacted]	33	F	II	N	-	-
95	[redacted]	32	F	II	N	-	-
96	[redacted]	53	F	II	N	-	-
97	[redacted]	20	M	II	N	-	-
98	[redacted]	52	F	II	N	-	-
99	[redacted]	52	F	II	N	-	-
100	[redacted]	48	F	II	N	-	-

N : Normale / *normal*

ANNEXE III / *APPENDIX III*

TABLEAUX DES LECTURES - PHASE D'INDUCTION

TABLES OF THE READINGS – INDUCTION PHASE

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / *control*
EB = LCA13049 - 13P3793-1

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / control
EB = LCA13049 - 13P3793-1

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB	T	EB
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / control
EB = LCA13049 - 13P3793-1

ANNEXE IV / *APPENDIX IV*

TABLEAUX DES LECTURES - PHASE DE REVELATION

TABLES OF THE READINGS – CHALLENGE PHASE

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	EB	T	EB	T	EB	T	EB
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0

T = témoin / control
EB = LCA13049 - 13P3793-1

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	EB	T	EB	T	EB	T	EB
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0

T = témoin / control
EB = LCA13049 - 13P3793-1

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	EB	T	EB	T	EB	T	EB
81	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0

T = témoin / control
EB = LCA13049 - 13P3793-1

IDPA

International Diatomite Producers Association

June 29, 2021

Bart Heldreth, Ph.D.
Executive Director
Cosmetic Ingredient Review
Suite 1200
1620 L Street, NW
Washington, DC 20036

Re: Comments on the Scientific Literature Review (Safety Assessment of Diatomaceous Earth as Used in Cosmetics)

Submitted via E-Mail: cirinfo@cir-safety.org

Dear Dr. Heldreth:

The International Diatomite Producers Association (IDPA) is a trade association representing major manufacturers of diatomaceous earth products worldwide. Founded in 1987, IDPA is committed to the safe use of diatomaceous earth products and to advancing research and maintaining a dialogue with industry, regulatory agencies and the scientific community in support of the safety of our employees and the communities we serve. The Cosmetic Ingredient Review (CIR) has made available for public comment a draft Scientific Literature Review (SLR) on diatomaceous earth (DE) as used in cosmetics. IDPA is pleased to submit the following comments to CIR on its draft SLR on DE. IDPA respectfully requests that a copy of these comments be shared with members of the Expert Panel for Cosmetic Ingredient Safety (Expert Panel), liaison representatives and CIR staff, as appropriate, so that all may better understand the suggested approach of IDPA on CIR's consideration of DE as used in cosmetics.

While you may not be familiar with DE, you undoubtedly are familiar with products that incorporate it or processes to which it is beneficially applied. The largest use of DE is as a filter aid. DE is used to filter beer, wine and other liquors, vegetable oil, syrup and sugar, pharmaceuticals, motor oil and swimming pool water. As a functional filler, DE is used in paint, rubber and plastic formulations. Other uses include use as an anti-blocking agent in plastic film, as an anti-caking agent for fertilizer, as a soil amendment, as a thermal insulating material, as a catalyst carrier, in polishes and abrasives, as pesticide and fertilizer carriers, in insulating brick and as an absorbent. DE is used as a natural grade insecticide and in the separation of fluid chemicals. Natural DE also is used in cosmetics and personal care products.

To our knowledge, the main applications of natural DE in cosmetics include three product types: bar soaps, antiperspirant deodorants and dry shampoos. The natural DE used in these applications ranges between two and 10 percent. Additionally, natural DE is used as a filter aid for filtration of active ingredients, such as fragrance formulations and oil separation, which also may be used in cosmetic formulations. However, natural DE does not remain in these filtered active ingredients and, consequently, should not contribute to the presence of DE in these cosmetic products where natural DE is not intentionally added.

Diatomaceous earth is a naturally occurring mineral, composed of nearly pure sedimentary deposits of the microscopic skeletal remains, or frustules, of unicellular algae known as diatoms. DE is highly siliceous and consists primarily of amorphous opaline silica. We understand from the SLR that CIR recently concluded that synthetically manufactured amorphous silica and hydrated silica are safe in present practices of use and concentration when formulated to be non-irritating.[1] IDPA member companies would like to see a similar conclusion reached with respect to natural DE.

DE is commercially available in three grades: natural, calcined and flux-calcined. All grades of DE are produced from naturally occurring amorphous silica mined ore. To produce natural DE an amorphous silica ore is minimally crushed, dried, milled and air classified to produce desired particle sizes. The other two grades of DE are further thermally processed by calcining or flux-calcining. This additional thermal processing produces fundamental changes in the composition of the opaline silica frustule. Calcination and flux-calcination dehydrate the amorphous silica and initiate its conversion to crystalline cristobalite. Thermal processing also reduces the surface area of the diatoms by altering their physical form and thereby imparts certain desirable properties for a variety of commercial uses. However, the formation of crystalline silica produced by calcining and flux-calcining natural DE likely renders these grades inappropriate for use in cosmetics. Consequently, IDPA would like the SLR on DE to be revised to focus exclusively on natural DE as the only form of DE IDPA deems appropriate for use in cosmetics.

To that end, IDPA would like to work with the CIR to address the characteristics of natural DE and its use in cosmetics with the ultimate goal of having the Expert Panel conclude that natural DE is safe in present practices of use and concentration when formulated to be non-irritating.

In reviewing the draft SLR, IDPA notes that there are many references to calcined and flux-calcined DE. IDPA recommends that discussion of these two grades be deleted from the draft SLR as inappropriate for consideration. Instead, IDPA proposes to work with CIR staff to assemble the information and data the Expert Panel typically would require to conduct a safety assessment of natural DE. Some of this information and data may not be readily available, as the use of natural DE in cosmetics represents but a small part of production or sales of DE for other uses. However, IDPA and its member companies are willing to assemble, and potentially generate, the necessary information and data.

In the draft SLR, under "Information Sought," CIR staff seek additional information specific to the cosmetic use of DE:

- Method of manufacturing and/or source data for Diatomaceous Earth as used in cosmetics
- Mean and range particle size (especially in spray and powder formulations)
- Composition (including degree and % of crystallinity) and impurities data
- Dermal penetration data, and if absorbed, DART studies
- Inhalation toxicity data
- Dermal irritation and sensitization studies at maximum concentrations of use

[1] *Amended Safety Assessment of Synthetically-Manufactured Amorphous Silica and Hydrated Silica as Used in Cosmetics*, published by the Cosmetic Ingredient Review (2019).

SLR at [9].

IDPA currently is not in a position to provide all the requested information or data. However, as an article of good faith, it would like to submit some information and reference some data that can help refocus the current draft SLR on natural DE, to the exclusion of calcined or flux-calcined DE.

The first submission is the chapter on "Diatomite" from *Industrial Minerals & Rocks – Commodities, Markets, and Uses*, published by the Society for Mining, Metallurgy, and Exploration, Inc.[2] (Attached.) Generally regarded as a standard reference on industrial minerals and their properties and uses, IDPA believes that much can be gleaned from the chapter to begin addressing the additional information specific to the cosmetic use of DE identified above.

Other sources of information and data are the specific medical tests or examinations identified in Department of Health and Human Services (DHHS) National Institute for Occupational Safety and Health (NIOSH) Publication Number 2005-110[3], which addresses specific medical tests or examinations published in the literature for OSHA-regulated substances, specifically addressing Silica, amorphous, diatomaceous earth CAS No: 61790-53-2:

NOTE:

(1) Efficacy of Medical Tests has not been evaluated.
(2) NIOSH references include diagnostic, screening, and other tests.
(3) OSHA mandated medical tests, if any, are provided in **BOLD** on a yellow background.
(4) This HTML page was created from DHHS (NIOSH) Publication No. 2005-110pdf icon. December 2004.
(5) If a medical test/examination contains multiple references, each is listed separately.

Silica, amorphous, diatomaceous earth

Editor(s) /Author(s)	**Specific Medical Test(s) or Examination(s)**	**Reference(s)**
US DHHS PHS CDC NIOSH and US DOL OSHA.	Chest X-ray	*NIOSH/OSHA Occupational Health Guidelines for Chemical Hazards DHHS (NIOSH) Pub No. 81-123; 88-118; Suppls. I-IV. 1981-1995.*
Proctor NH, Hughes JP.	Chest X-ray	*Chemical Hazards of the Workplace.* JB Lippincott Company. 1978.
US DHHS PHS CDC NIOSH and US DOL OSHA.	Pulmonary Function Tests • Forced Vital Capacity • Forced Expiratory Volume (1 sec)	*NIOSH/OSHA Occupational Health Guidelines for Chemical Hazards DHHS (NIOSH) Pub No. 81-123; 88-118; Suppls. I-IV. 1981-1995.*

[2] *Industrial Minerals & Rocks – Commodities, Markets, and Uses*, 7th Edition, edited by Jessica Elzea Kogel, et al., published by the Society for Mining, Metallurgy, and Exploration, Inc. (2006).

[3] National Institute for Occupational Safety and Health (NIOSH). *Publication No. 2005-110*: *Specific Medical Tests or Examinations Published in the Literature for OSHA-Regulated Substances*. Department of Health and Human Services (DHHS). https://www.cdc.gov/niosh/docs/2005-110/nmed0204.html (2005). Accessed 6/10/2021.

Proctor NH, Hughes JP.	Pulmonary Function Tests • Forced Vital Capacity • Forced Expiratory Volume (1 sec)	*Chemical Hazards of the Workplace.* JB Lippincott Company. 1978.
Proctor NH, Hughes JP.	Radiography	*Chemical Hazards of the Workplace.* JB Lippincott Company. 1978.
Proctor NH, Hughes JP.	Sputum Cytology	*Chemical Hazards of the Workplace.* JB Lippincott Company. 1978.
Proctor NH, Hughes JP.	Tuberculin Skin Test	*Chemical Hazards of the Workplace.* JB Lippincott Company. 1978.

IPDA also notes that the International Agency for Research on Cancer (IARC) concluded that amorphous silica is not classifiable as to its carcinogenicity to humans (Group 3) based on inadequate evidence in humans for the carcinogenicity of amorphous silica and inadequate evidence in experimental animals for the carcinogenicity of uncalcined diatomaceous earth.[4]

IDPA looks forward to continuing the dialogue on the safety of natural DE as used in cosmetics initiated by the SLR and IDPA's comments. Please do not hesitate to contact me with regard to suggestions you may have as to how IDPA and its member companies can best continue this exchange of views, information and data on the relevant science.

Respectfully submitted,

Mark G. Ellis
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International Diatomite Producers Association
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(703) 927-7665 (Cell)
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IDPA Member Companies:

Chemviron, a Kuraray company
Dicalite Management Group, Inc.
EP Minerals, LLC, a U.S. Silica company
Imerys Performance Minerals
Showa Chemical Industry Company, Ltd.

[4] International Agency for Research on Cancer (IARC). (1997). *Silica, Some Silicates, Coal Dust and para-Aramid Fibrils*. Vol. 68. Lyon, France: World Health Organization.

Attachment: Chapter on "Diatomite", *Industrial Minerals & Rocks – Commodities, Markets, and Uses*, 7th Edition, edited by Jessica Elzea Kogel, et al., published by the Society for Mining, Metallurgy, and Exploration, Inc. (2006).

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: July 21, 2021

SUBJECT: Scientific Literature Review: Safety Assessment of Diatomaceous Earth as Used in Cosmetics (release date April 30, 2021)

The Personal Care Products Council respectfully submits the following comments on the scientific literature review, Safety Assessment of Diatomaceous Earth as Used in Cosmetics.

Repeated Dose – This section states "In 13-wk dietary studies…" but only one 13-week dietary study in rats is described. In the text, please explain what is meant by the 5 + 50 mppcf exposure protocol.

Summary – Please correct: "but not malignant tumors were observed"

Table 3, reference 5, 28-day study - What about the lymph nodes were increased?

Table 3, reference 12 – Please explain the 5 + 50 exposure more clearly. How many hours/day was the 50 mppcf exposure?

Table 3, reference 33 – The daily exposure duration is not clear. It states "for 24 h/day" but also states "7 to 8 h/d".

Table 3, reference 12 – Please correct: "intro-alveolar" and "similar those in the 2 yr group" [needs "to"]

Table 4, reference 35 – If uncalcined Diatomaceous Earth was not active, how could the heated Diatomaceous Earth be "less active"?