

United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet

<https://www.ams.usda.gov/rules-regulations/organic/petitioned-substances>

Document Type:

☒ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☐ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.



Andrea Holm
Materials Specialist
USDA/AMS/NOP, Standards Division
1400 Independence Ave. SW
Room 2642-So., Ag Stop 0268
Washington, DC 20250-0268

Subject: Petition to amend the National List

Dear Andrea,

We, Ironwood Clay Company Inc., are a manufacturer of skincare raw ingredients and finished products. We would like to have the raw ingredient with INCI names Sea Silt (common/trade name: Glacial Oceanic Clay Powder) to be added to the NOP National List. Below please find the information and supporting documents for your perusal.

Manufacturer information:

Ironwood Clay Company Inc.
Unit 450 - 3771 Jacombs Road, Richmond, British Columbia
Canada V6V 2L9

Section the petitioned substance to be included:

Nonagricultural (nonorganic) substances allowed in or on processed products labeled as "organic" or "made with organic (specified ingredients)" (§ 205.605)

Substance name:

INCI name: Sea Silt

Common/trade Name: Glacial Oceanic Clay Powder

Current use:

Sea Silt is used as an ingredient for cosmetics, skincare, and sunscreen products to exfoliate or moisturize skin.

Intended Activities and Application Rate:

Sea Silt is incorporated as a key ingredient in a wide range of cosmetic, skincare, and sunscreen formulations, including both rinse-off and leave-on products. These formulations, which may include cleansers, moisturizers, sunscreens, and other topical products, are eligible for organic certification under the USDA National Organic Program (NOP) once Sea Silt is approved for inclusion in the National List. The inclusion of Sea Silt in these products ensures that they meet the rigorous standards for organic ingredients, provided that the final product adheres to the USDA NOP's requirements for organic certification, including the sourcing, processing, and handling of all ingredients. This encompasses skincare and cosmetic products that are designed for use on the skin, such

as face creams, serums, masks, body lotions, and sun protection products, as well as rinse-off items like cleansers and exfoliating scrubs.

These products would qualify for organic certification if Sea Silt were to be added to the National List, contributing to their eligibility under organic certification standards.

Manufacturing process for Sea Silt (Glacial Oceanic Clay Powder):

The Sea Silt (Glacial Oceanic Clay) is mined, from intertidal glacial deposit on the Northern Coast of British Columbia, Canada, and transported to Ironwood Clay's manufacturing facility for storage and processing. The Sea Silt is screened and dried by our sub-contractor using enclosed concrete dryers. The heat source is natural gas burners running in flu lining under the concrete pad. The dried sea silt is moved into a hopper to grind into fine powder. Then, it is filled into bags for sale as cosmetic raw ingredient.

Supporting documents:

- A. Glacial oceanic clay powder process flow chart

Ancillary Substances:

There are no ancillary substances added to the Sea Silt.

Previous Reviews:

Sea Silt is listed under the cosmetic database as approved cosmetic ingredient in Canada, EU, and China. It is also approved as a natural ingredient by Cosmos/Ecocert. Moreover, it is rated "1" under EWG's Skin Deep database which means this ingredient has very low toxicity and safety concerns to be used in cosmetic and skincare products. Detail information on how Skin Deep works can be found from the link below.

https://www.ewg.org/skindeep/learn_more/about/

Supporting documents:

- B. Sea Silt listed in Canada cosmetic database
- C. Sea Silt listed in EU Cosing cosmetic database
- D. Sea Silt rating in EWG's Skin Deep database
- E. Conformity attestation COSMOS/Ecocert

Regulatory Authority:

Sea Silt is rated "1" under EWG's Skin Deep database which means this ingredient has very low toxicity and safety concerns to be used in cosmetic and skincare products. EWG (Environmental Working Group) uses nearly 60 databases and sources from government agencies [including U.S. Environmental Protection Agency (U.S. EPA) and FDA], industry panels, academic institutions, or other credible bodies to assess potential health hazards and data gaps for cosmetic ingredients. Individual toxicity, regulatory, and study availability data sources compiled can be found from the link below.

https://www.ewg.org/skindeep/learn_more/about/

Chemical Abstracts Service (CAS) Number and Product Labels:

CAS number 999999-99-4, Naturally occurring substances, is used for the petitioned substance Sea Silt.

Label:

GLACIAL OCEANIC CLAY POWDER
ICC-POWCLAY10K
10 KILOGRAMS
LOT# 11203B
PRODUCT OF CANADA

Physical and Chemical Properties:

The petitioned substance, Sea Silt, is a naturally occurring substance rich in over 50 minerals, including rare earth elements, and possesses a natural pH and ion exchange capacity. It is chemically stable under recommended handling conditions and does not react with substances commonly used in organic production. There is no data indicating it causes adverse chemical interactions with other organic ingredients. It is a non-hazardous substance and does not meet the criteria for classification as hazardous under WHMIS 2015 or GHS standards. It is compatible with standard cosmetic formulations and should not interfere with the properties of organic materials used in cosmetics or skincare products.

Sea Silt is not classified as toxic under normal usage conditions. It does not present significant hazards upon exposure via inhalation, skin contact, or ingestion. However, inhalation of dust may cause mild irritation, and ingestion of large amounts may lead to gastrointestinal irritation or blockage. It is biodegradable and does not accumulate in the environment, reducing its persistence and potential for long-term environmental impact. Its low toxicity profile and biodegradable nature support its use in organic production, aligning with sustainability goals. The substance is not listed as hazardous under environmental or regulatory standards in Canada or the U.S.

The extraction of Sea Silt from the oceanic deposits in Northern British Columbia is an environmentally responsible process, conducted under governmental approval. The process of mining does not involve harsh chemicals or industrial waste, thereby minimizing environmental pollution. As a naturally occurring substance, Sea Silt contributes minimal disruption to the ecosystem, and its manufacturing and use are consistent with sustainable practices. There is no indication of significant adverse effects on the environment from its production or use in organic cosmetic products. Its environmental footprint remains low due to its natural origin and minimal processing.

Sea Silt has mild effects on human health, with potential for mild irritation upon skin or eye contact. If ingested in small amounts, it may cause minor gastrointestinal discomfort. There is no evidence suggesting that it poses long-term health risks or that it is

carcinogenic, mutagenic, or reproductive toxic. The substance's primary application in personal care and cosmetic products aligns with safety standards for use in organic formulations. It is generally considered safe for use in topical applications when handled properly. There are no acute or chronic health effects reported under normal use.

While no specific data on the effects of Sea Silt on soil organisms, crops, or livestock is available, its use in organic production is considered safe. Its natural mineral composition suggests it would not adversely affect soil health or microbial activity, as it is primarily composed of minerals and rare earth elements that are commonly found in the earth's crust. Furthermore, Sea Silt is biodegradable and would not accumulate in soil or water systems, making it unlikely to have negative long-term effects on agriculture or wildlife. The substance's gentle nature and low toxicity support its inclusion in organic handling practices without posing harm to ecosystems or livestock.

In summary, Sea Silt is a safe, environmentally responsible substance with minimal toxicological concerns, no significant environmental impacts, and positive effects on human health. It offers a viable alternative to synthetic ingredients in organic cosmetic and skincare formulations, with the added benefit of being a naturally sourced substance.

Supporting documents:

- F. Elemental Analysis Clay Comparison Table
- G. Raw Clays Comparison Table
- H. Determination of Cutaneous Tolerance and Sensitization on Healthy Participants by Human Repeat Insult Patch Test (HRIPT)
- I. In Vitro evaluation of the eye irritation potential on corneal epithelium

Safety Information:

There is no substance report for the Sea Silt from the National Institute of Environmental Health Sciences (NIEHS). However, other safety references with similar or related substances can be found from the EWG link below.

https://www.ewg.org/skindeep/learn_more/about/

Supporting documents:

- J. Amended Safety Assessment of Naturally-Sourced Clays as Used in Cosmetics
- K. Glacial Oceanic Clay Powder SDS

Research Information:

Sea Silt (Glacial Oceanic Clay Powder) is reviewed and rated as "1" by EWG. References with similar or related substances can be found from the EWG link below.

https://www.ewg.org/skindeep/learn_more/about/

Petition Justification Statement:

Ironwood Clay Company Inc. has been harvesting Sea Silt since 1988 in a unique region of Northern Coast of British Columbia, Canada. The company has received approval and certification from the government of British Columbia to extract this sea silt. This sea silt is the result of erosion caused by the movement and melting of Canadian glaciers, eventually depositing as sediment in the ocean and becoming enriched with over 50 minerals, including more than 10 rare earth elements. This composition provides effective cosmetic benefits, with the sea silt offering a natural pH and ion exchange capacity that enhances its efficacy in skincare.

Sea Silt is necessary for use in organic handling because it provides a naturally occurring, minimally processed substance that supports the health and quality of skincare products. As a non-synthetic, naturally sourced substance, it offers a holistic alternative to more heavily processed or synthetic ingredients. Given its mineral-rich composition and its beneficial properties for the skin, it serves a unique role in the formulation of cosmetic and skincare products, with no comparable alternative on the National List for use in organic cosmetic formulations.

Currently, there are no similar nonsynthetic or synthetic substances listed on the National List that can serve as an effective substitute for Sea Silt. The use of Sea Silt in cosmetics and skincare products supports natural, organic handling standards by relying on a minimally processed, earth-derived substance, which aligns with the goals of organic certification. Alternative cultural methods, such as mineral-rich soil amendments or plant-based options, do not replicate the specific benefits and unique properties of Sea Silt, particularly its ability to balance pH and provide ion exchange, making it an essential ingredient.

The use of Sea Silt also offers several beneficial effects on both the environment and human health. By utilizing a product harvested from natural erosion processes and oceanic sedimentation, the company's practices contribute to sustainable resource extraction, with minimal impact on the surrounding ecosystem. Furthermore, Sea Silt's rich mineral composition offers many benefits to the skin, such as improving hydration, detoxification, and overall skin health, making it a valuable ingredient for health-conscious consumers. These benefits underscore the importance of including Sea Silt as a substance on the National Organic Program (NOP) list, as it provides both environmental and health-related advantages that cannot be fully replicated by other synthetic or nonsynthetic alternatives.

Supporting documents:

- L. Determination of the Efficacy and Tolerance of a Facial Mask
- M. Leaching of chemical elements from Canadian Natural Oceanic Clay
- N. The Effect of Ironwood Clay on Skin Fibroblasts and Immune Cells

- O. Effect of Glacial Oceanic Clay tested against French Green Clay on Collagen and Pro-inflammatory Protein Production
- P. Comparative study for the determination of In Vivo SPF (Sun Protection Factor) of two topical products according to FDA Method 21 CFR 201.327(i)
- Q. Mineral benefits in Glacial Oceanic Clay

Below table is a summary of the supporting documents attached for this petition.

Sub-titles	Number	Documents
Manufacturing process for Sea Silt (Glacial Oceanic Clay Powder)	A	Glacial oceanic clay powder process flow chart
Previous Reviews	B	Sea Silt listed in Canada cosmetic database
	C	Sea Silt listed in EU Cosing cosmetic database
	D	Sea Silt rating in EWG's Skin Deep database
	E	Conformity attestation COSMOS/Ecocert
Physical and Chemical Properties	F	Elemental Analysis Clay Comparison Table
	G	Raw Clays Comparison Table
	H	Determination of Cutaneous Tolerance and Sensitization on Healthy Participants by Human Repeat Insult Patch Test (HRIPT)
	I	In Vitro evaluation of the eye irritation potential on corneal epithelium
Safety Information	J	Amended Safety Assessment of Naturally-Sourced Clays as Used in Cosmetics
	K	Glacial Oceanic Clay Powder SDS
Petition Justification Statement	L	Determination of the Efficacy and Tolerance of a Facial Mask
	M	Leaching of chemical elements from Canadian Natural Oceanic Clay
	N	The Effect of Ironwood Clay on Skin Fibroblasts and Immune Cells
	O	Effect of Glacial Oceanic Clay tested against French Green Clay on Collagen and Pro-inflammatory Protein Production
	P	Comparative study for the determination of In Vivo SPF (Sun Protection Factor) of two topical products according to FDA Method 21 CFR 201.327(i)
	Q	Mineral benefits in Glacial Oceanic Clay

Please feel free to contact me if you have any questions or need further information.

Sincerely,



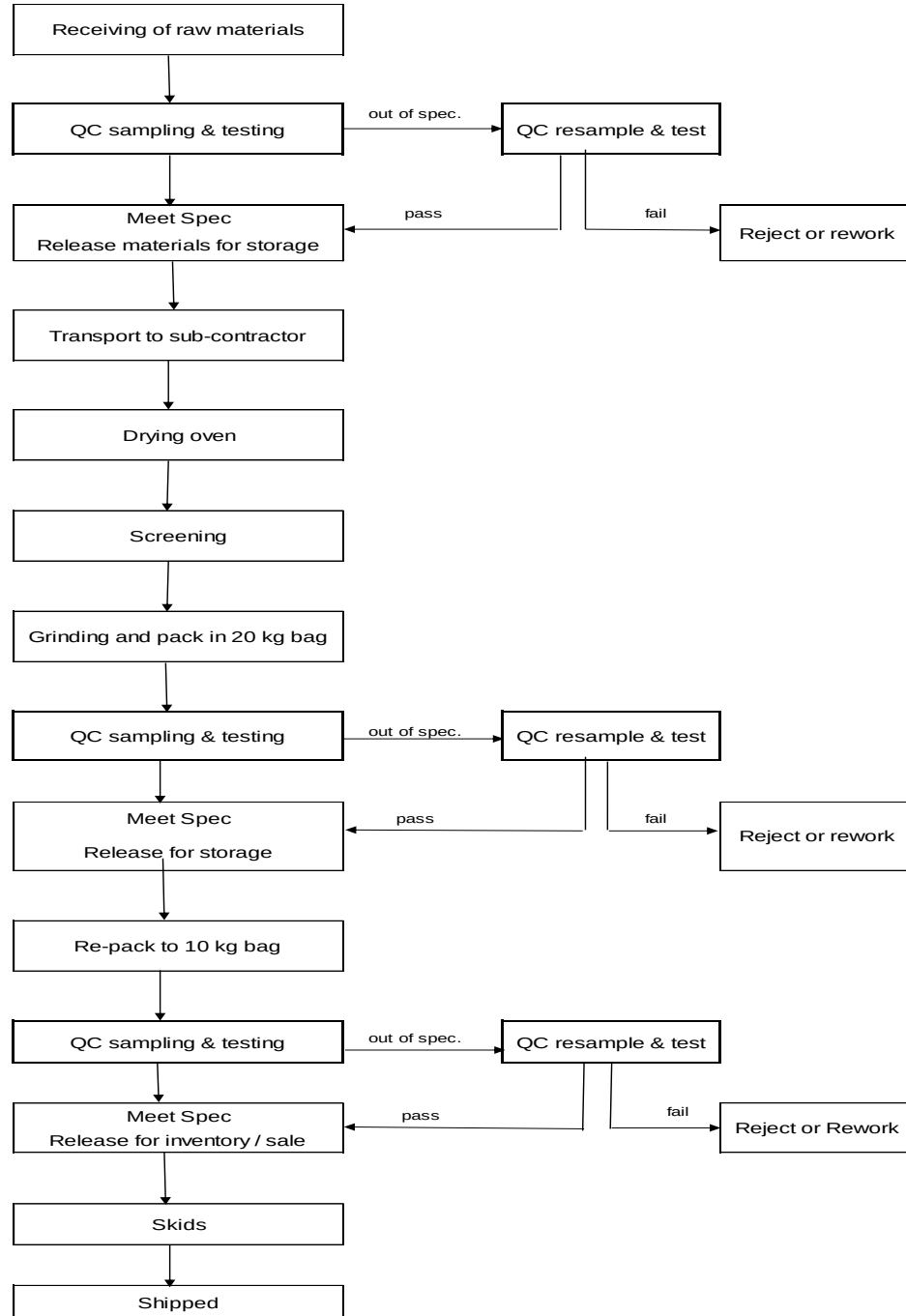
Pauly Yau
Technical Director

January 24, 2025

Date

Manufacturing Process Flow Chart

Product Name: Glacial Oceanic Clay Powder
Formula Number: CC-001



January 2025

Product Ingredients - Section 5

Enter one ingredient per line using the International Nomenclature of Cosmetic Ingredients (INCI) where possible. More information on INCI names can be found on the [Personal Care Products Council](#) website. Enter at least 3 characters in the INCI Name field, select the operator "Begins With" or "Contains" and click on the button "Find INCI Name". Select the ingredient contained in the notified product from the list.

An ingredient that has no INCI name or that is not found when using the "Find INCI Name" button must be entered using its chemical name in the field "Other Chemical Name". You must indicate all ingredients of this notified product. As per the *Cosmetic Regulations*, the complete list of ingredients and their concentration or concentration ranges must be provided. Submitting a non-INCI name may cause delays in processing your cosmetic notification.

After you have entered the ingredient name, click the "Search Ingredient" button to validate the ingredient. The status of the ingredient will appear in the "Search Result" field. Any condition that applies to the ingredient will appear in the "Condition of Use" field. If the status of the ingredient is "Unknown" or "Not found" check for spelling, punctuation errors, and extra spaces.

If entering the exact concentration, enter the concentration in the "Exact concentration" field. You may also select a range code from the drop-down list and the lower and upper concentrations will be automatically entered.

Check "May Contain" only if the ingredient is variable. It should not be selected for all ingredients.

Ingredient 1

*** INCI Name (required)**

Operator Begins With ▾

*** Other Chemical Name (required)**

May Contain ☐

*** Exact Concentration % (number between 0 and 100, format: 99 or 99.999999) (required)**

Range Code

Select Range Code ▾

*** Lower Concentration % (number between 0 and 100, format: 99 or 99.999999) (required)**

*** Upper Concentration % (number between 0 and 100, format: 99 or 99.999999) (required)**

Search Result

Condition of use

Total Lower Concentration (%):

0

Total Upper Concentration (%):

0

Documents and pictures - Section 6

Use this section to submit product labels, evidence of safety, or any other documents relevant to this product, if applicable. Refer to the Guidance Document [Guide for Cosmetic Notifications](#) for more information.

Documents

Select one or many documents

Number of attached documents: 0

Cumulative total size of attached documents is: 0 Mb (Maximum is: 70 Mb)

How to submit your Cosmetic Notification Form - Section 8

1. Click the "Proceed to Submit" button below.
2. The mandatory fields identified on the form and size limit of 70 Mb will be verified automatically.
3. You will see a pop up displaying a confirmation that your submission was successful along with the assigned Case Number and Submission Number.
4. Save the information entered on the online form on your computer once the Case Number and Submission Number are provided. See section on 'How to save' for more details.

Declaration

* ☐ I hereby declare that all the information I am submitting is, to the best of my knowledge, true, accurate, current, and complete. (required)

* ☐ I understand that providing false or incomplete information may result in the Cosmetic Notification Form not being processed, which can result in compliance and enforcement actions. (required)

* Signature (first name and last name) (required)

Date

2025-01-23

Proceed to submit

The Case Number and Submission Number are assigned by Health Canada when you submit the form.

Ingredient: SEA SILT

INCI Name	SEA SILT
Description	Sea Silt is sediment from the sea.
INN Name	
Ph. Eur. Name	
CAS #	
EC #	
Chemical/IUPAC Name	Maris Limus
Cosmetic Restriction	
Other Restriction(s)	
Functions	SKIN CONDITIONING
SCCS opinions	
Identified INGREDIENTS or substances e.g.	

KNOW YOUR ENVIRONMENT. PROTECT YOUR HEALTH.



EWG's Skin Deep®

Best



1

2

3

4

5

6

7

8

9

10

Worst

EWG
VERIFIED*[HOW WE DETERMINE SCORES](#)[LEARN MORE ABOUT EWG VERIFIED®](#)

1

Sea Silt

Data: None



SEARCH PRODUCTS

FIND PRODUCTS WITH THIS INGREDIENT

OTHER CONCERNS

SYNONYMS

- SEA SILT

This ingredient is not currently on

Unacceptable Lists.

Sea Silt is sediment from the sea.

Common concerns

See how this product scores for common concerns.

LOW	Cancer
LOW	Allergies & Immunotoxicity
LOW	Developmental and Reproductive Toxicity
LOW	Use Restrictions

Ingredient concerns

- CONCERNS
- DATA SOURCES

Products with this Ingredient +

Understanding scores

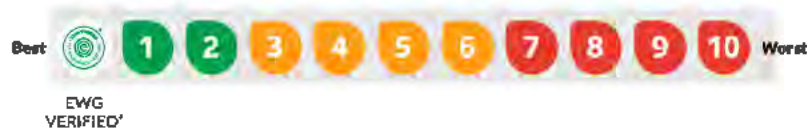
Cosmetics and personal care products are not required to be tested for safety before being allowed on the market. The Skin Deep® scoring system was designed to help the public understand whether a product is safe to use or whether it contains ingredients of concern.

Every product and ingredient in Skin Deep gets a two-part score – one for hazard and one for data availability. The safest products score well by both measures, with a low hazard rating and a fair or better data availability rating.

HOW WE DETERMINE SCORES

Hazard score

The Skin Deep ingredient hazard score, from 1 to 10, reflects known and suspected hazards linked to the ingredients. The EWG VERIFIED® mark means a product meets EWG’s strictest criteria for transparency and health.



WEIGHT-OF-EVIDENCE APPROACH

A product’s hazard score is not an average of the ingredients’ hazard scores. It is calculated using a weight-of-evidence approach that factors in all of the hazards or health impacts associated with the ingredients.

Data availability

The Skin Deep data availability rating reflects the number of scientific studies about the product or ingredient in the published scientific literature.



NONE

Ingredients have not been assessed.

LIMITED-FAIR

There is only a modest volume of research.

GOOD-ROBUST

Ingredients have been studied extensively.

KNOW YOUR ENVIRONMENT. PROTECT YOUR HEALTH.



EWG's Skin Deep®

Understanding Skin Deep® ratings

Every product and ingredient in Skin Deep® gets a **two-part score** – one for hazard and one for data availability.

Hazard Score



The Skin Deep ingredient hazard score, from 1 to 10, reflects known and suspected hazards linked to the ingredients.



A product's hazard score is not an average of the ingredients' hazard scores. It is calculated using a weight-of-evidence approach that factors in all of the hazards or health impacts associated with the ingredients. To see details of how our hazard scores are calculated, see the "Hazard Ratings" section of our **methodology**.

1.5k
Shares

Least Data	None	Limited	Fair	Good	Robust	Most Data
	Low Availability		Moderate Availability		High Availability	

The Skin Deep data availability rating reflects the number of scientific studies about the product or ingredient in the published scientific literature. Not all ingredients are equal when it comes to safety data. Some have been studied extensively. For others there is only a modest volume of research, and still others have not been assessed at all. The data availability rating indicates the scope of data on which EWG has based an ingredient and/or product score.

Overall Product Score

To calculate a product score, we review individual ingredient hazards and evaluate each product in relation to the rest of the products in the Skin Deep database. **The safest products score well by both measures, with a low hazard rating and a fair or better data availability rating.**

What does the EWG VERIFIED® mark mean?



The EWG VERIFIED® mark on a product indicates that the product meets EWG's strictest standards for transparency and health.

1.5k
Shares

EWG VERIFIED products cannot contain any ingredients on EWG's "Unacceptable" list, which includes ingredients with health, ecotoxicity and/or contamination concerns.

✓ Full transparency

EWG VERIFIED products must meet EWG's standards for ingredient disclosure on the label; products with the VERIFIED mark are made by manufacturers that have been fully transparent with EWG about that product's ingredients, including fragrance ingredients.

✓ Good manufacturing practices

EWG VERIFIED product manufacturers must develop and follow current good manufacturing practices to further ensure the safety of their products.



Ingredient Concerns

Cancer: Ingredients linked to cancer in government, industry or academic studies or assessments.

Developmental and reproductive toxicity: Ingredients linked to developmental and reproductive toxicity, a broad class of health effects that range from infertility and reproductive organ cancers to birth defects and developmental delays in children.

Allergies and immunotoxicity: Ingredients linked to harm to the immune system, a class of health problems that manifest as allergic reactions or an impaired capacity to fight disease and repair damaged tissue in the body.

Use restrictions: Ingredients restricted or prohibited for use in cosmetics, according to industry safety guidelines, government requirements or

1.5k
Shares

Endocrine disruption: Ingredients may affect the proper functioning of the body's hormone system.

Neurotoxicity: Ingredients linked to harm to the brain and nervous system, a class of health problems that can range from subtle developmental delays to chronic nerve degeneration diseases.

Organ system toxicity: Ingredients linked to toxicity of one or more biological systems in the body, for instance, cardiovascular, stomach and digestive track, or respiratory system, through laboratory studies or studies of people.

Biochemical or cellular level changes: Ingredients affect the body at a cellular or biochemical level, potentially leading to larger but poorly understood health implications.

Persistence and bioaccumulation: Ingredients resist normal breakdown in the environment; build up in wildlife, the food chain, and people; and linger in the body for years or even decades after exposure.

Ecotoxicity: Ingredients linked to toxicity of wildlife that may include fish, plants or other wild organisms.

Irritation: Ingredients linked to irritation of the skin, eyes or lungs.

Occupational hazards: Ingredients linked to hazards for workers exposed on the job, including immediate dangers from chemical handling, or longer-term health effects from routine occupational exposures.

Enhanced skin absorption: Ingredients may more readily absorb through the skin due to properties like penetration-enhancing abilities or small particle size, or by virtue of where they are applied on the body (on infant skin, or on lips or damaged skin).

Contamination concerns: Ingredients may be contaminated with toxic impurities, many of which are linked to cancer, according to government and cosmetic industry ingredient safety assessments or peer-reviewed studies.

1.5k
Shares

Conformity attestation

N°84068-20250102_1823



Ecocert Greenlife SAS hereby confirms that:

IRONWOOD CLAY COMPANY

450, 3771 JACOMBS ROAD, V6V 2L9, RICHMOND, British Columbia, Canada

has raw materials approved in accordance with standard requirements:

COSMOS v4 in force (including all sub-versions)



For the following products categories:

Derivatives of minerals, Dry extracts, Liquid extracts, Minerals, Mixtures of CPAI, PPAI and Minerals

PPAI : Physically Processed Agro-Ingredients / CPAI : Chemically Processed Agro-Ingredients / NNI : Non Natural Ingredients (Petrochemical origin) / PeMo : Petrochemical Moiety

Without animal origin : Raw material compliant to the complementary assessment « without animal origin » in force

The asterisk * is used to identify the commercial name of the raw materials concerned by the appendices II and/or V of the Cosmos-standard.

Attestation issued on 02 January 2025 and valid until 31 December 2025.

ECOCERT Greenlife S.A.S

BP 47 Lieudit Lamothe Ouest - 32600 L'Isle Jourdain

FRANCE Phone : 05 62 07 34 24 - ecocert.com

Capital 50 000 € - SIREN 509 534 095 RCS AUCH

<https://ap.ecocert.com/ecoproducts>

F028(GL)v04en

Emilie Cherhal, Ecoproducts Division Director



Accreditation by COFRAC # 5-0520 for Certification of Products and Services, scope available on www.cofrac.fr.

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Annex to the conformity attestation

N°84068-20250102_1823



IRONWOOD CLAY COMPANY

450, 3771 JACOMBS ROAD, V6V 2L9, RICHMOND, British Columbia, Canada

has raw materials approved in accordance with standard requirements:

COSMOS v4 in force (including all sub-versions)

Products covered:

DERIVATIVES OF MINERALS

> **Natural Oceanic Clay / Glacial Oceanic Clay / Natural Glacial Oceanic Clay / Canadian Oceanic Clay**
Sea Silt

Cleansing agent, Exfoliating

%PPAI : 0 %CPAI : 0 %NNI : 0 %PeMo : 0

> **Glacial Oceanic Clay Powder**
Sea Silt

Skin conditioning

%PPAI : 0 %CPAI : 0 %NNI : 0 %PeMo : 0

DRY EXTRACTS

> **Glacial Oceanic Mineral Water - ASP**
Sea Silt Extract (and) Populus Tremuloides Bark Extract

Skin conditioning

%PPAI : 1 %CPAI : 0 %NNI : 0 %PeMo : 0

LIQUID EXTRACTS

> **Glacial Oceanic Mineral Water - EC**
Sea Silt Extract (and) Benzyl Alcohol (and) Potassium Sorbate

Moisturising

%PPAI : 0.8 %CPAI : 0 %NNI : 0.2 %PeMo : 0

Attestation issued on 02 January 2025 and valid until 31 December 2025.

ECOCERT Greenlife S.A.S

BP 47 Lieudit Lamothe Ouest - 32600 L'Isle Jourdain

FRANCE Phone : 05 62 07 34 24 - ecocert.com

Capital 50 000 € - SIREN 509 534 095 RCS AUCH

<https://ap.ecocert.com/ecoproducts>

F028(GL)v04en



Accreditation by COFRAC # 5-0520 for Certification of Products and Services, scope available on www.cofrac.fr.

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Annex to the conformity attestation

N°84068-20250102_1823



IRONWOOD CLAY COMPANY

450, 3771 JACOMBS ROAD, V6V 2L9, RICHMOND, British Columbia, Canada

has raw materials approved in accordance with standard requirements:

COSMOS v4 in force (including all sub-versions)

MINERALS

> Glacial Oceanic Mineral Water / Glacial Mineral Water / Natural Oceanic Clay Mineral Water / Natural Glacial Oceanic Mineral Water

Sea Silt Extract

Moisturising

%PPAI : 0 %CPAI : 0 %NNI : 0 %PeMo : 0

> Glacial Oceanic Mineral Water - COS

Sea Silt Extract

Skin conditioning

%PPAI : 0 %CPAI : 0 %NNI : 0.52 %PeMo : 0

MIXTURES OF CPAI, PPAI AND MINERALS

> Natural Oceanic Clay - EC / Glacial Oceanic Clay - EC

Sea Silt (and) Benzyl Alcohol (and) Aqua (and) Potassium Sorbate

Skin conditioning

%PPAI : 0.8 %CPAI : 0 %NNI : 0.2 %PeMo : 0

> Glacial Oceanic Clay - MM

Sea Silt (and) Glyceryl Caprylate (and) Pentylene Glycol (and) Magnolia Officinalis Bark Extract

Skin conditioning

%PPAI : 0.14 %CPAI : 1.66 %NNI : 0 %PeMo : 0

Attestation issued on 02 January 2025 and valid until 31 December 2025.

ECOCERT Greenlife S.A.S

BP 47 Lieudit Lamothe Ouest - 32600 L'Isle Jourdain

FRANCE Phone : 05 62 07 34 24 - [ecocert.com](https://www.ecocert.com)

Capital 50 000 € - SIREN 509 534 095 RCS AUCH

<https://ap.ecocert.com/ecoproducts>

WARNING: The sole purpose of the present attestation is to allow the raw material(s) to be used in finished products to be certified as compliant to the standard specified in the first page. In no event this attestation should constitute proof of the actual certification of the conformity of the raw material(s) to this standard. In that context, the raw material(s) listed in this attestation must not be qualified and / or marketed as «organic» raw material(s) certified in accordance with the abovementioned standard. The approval of the raw material(s) listed in the present attestation is personally addressed to the above-mentioned beneficiary. It is the beneficiary's liability to ensure that its own customers are aware of the requirements and prohibitions defined in the terms and conditions and governing any reference to and use of the approval of the raw material(s) and that they abide by it.

F028(GL)v04en



Accreditation by COFRAC # 5-0520 for Certification of Products and Services, scope available on www.cofrac.fr.

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ELEMENTAL ANALYSIS CLAY COMPARISON TABLE

	Beneficial Elements									Heavy Metals			
	Calcium	Copper	Iron	Potassium	Magnesium	Sodium	Phosphore	Selenium	Zinc	Arsenic	Cadmium	Lead	Antimony
	%	ppm	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Dead Sea Mineral Mud	14.7	17.1	1.84	0.74	2.62	1.28	1000	1.9	53	3.6	0.75	4.9	0.13
Australian Pink	0.06	4.4	8.28	0.04	0.09	0.1	70	0.4	5	7.2	0.01	11.6	7.5
Rhassoul Clay	4.02	10.5	0.76	0.43	11.45	0.35	170	3.5	28	2.5	0.07	6.1	0.12
French Green Clay	5.68	19	2.34	1.1	0.84	0.04	460	0.6	64	5.6	0.2	17.2	0.13
Bentonite	3.88	0.21	1.41	4.56	0.79	0.13		0.388	2.09	5	5	5	
Glacial Oceanic Clay	2.36	48.1	3.4	1.21	1.49	1.99	629.7	<0.5	61.6	2	<0.1	2.16	<0.1
Red : Highest amount Blue : 2nd highest Glacial Oceanic Clay is in the top 2 for highest amount of beneficial minerals.										Green : lowest amount Glacial Oceanic Clay has the lowest amount of heavy metals.			

Raw Clays Comparison Table								
	Glacial Oceanic Clay	Dead Sea Mud	Kaolin Clay	Bentonite	Pink Clay	French Green Clay	Charcoal	Marrocan Rhassoul Clay
Country of Origin	Unique to the region of Northern Bristih Columbia where glacier meets the Pacific Ocean	Dead Sea, Israel, Palestine and Jordan	Mined in many countries including United States, Uzbekistan, Czech Republic, Germany and Korea.	Mined in more than 40 countries around the wrld	Mined in France and Australia	Mostly from France, China and United States.	Manufactured anywhere	Marocco
Form	Wet	Wet	powder	powder	Powder	Powder	Powder	Powder
Process	Harvested from British Columbia lagun. Pure and processed w/out chemicals or irradiation	Harvested from the Dead Sea	Most domestic clay is mined by open-pit methods using various types of equipment, including draglines, power shovels, front-end loaders, backhoes, scraper-loaders, and shale planers.				Charcoal is a manufactured ingredient, it is the carbon residue left over after slow heating high-carbon-content materials like wood, coconut shells or even sugar to remove most of the water they	Mined by open pit method
Benefits	I. This clay is a product of erosion caused by the movement and melting of Canadian glaciers. It eventually deposits as a sediment in the ocean and becomes enriched with beneficial minerals for effective cosmetic benefits. The osmotic power of the clay pulls moisture up from deep inner tissues to hydrate the surface of the skin, and also cleans the skin without dehydrating it. II. High spreadability without addition of solvent. III. Cooling	High sodium content, can be irratating, requires processing to remove excessive sodium. Can be affected by population and industries.	Most commonly used clay with minimal skin functions. Higher microbial content, requires irradiation.	Can be drying		Can be drying	The activated charcoal removes everything from your face, which means it strips your skin off all the natural oils present on the outer surface.	
Percentages in Formulas	1 to 99% Easy to disperse	Visible particles	1 to 50%	1 to 50%			1 to 10%	1 to 50%

Determination of Cutaneous Tolerance and Sensitization on Healthy Participants by Human Repeat Insult Patch Test (HRIPT)

Final Report Study N°18J-0305-12

Study Sponsor:

Ironwood Clay

Represented by Dr. Patricia Truter –Director R&D

450 -3771 Jacombs Road,

Richmond, BC, V6V 2L9

Canada

Tel : 604-279-9498

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Study Investigator:



Represented by Marie-Laure Oula, Laboratory Director

5475, rue Paré, Suite 206,

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Canada

Tel: (514) 343-0001

Email: mloula@evalulab.com

***This final report was prepared by a clinical testing laboratory with a quality management system
registered to ISO 9001.***

This report is composed of 11 pages including appendices (2 pages).

May 4th, 2018

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SUMMARY

Human Repeat Insult Patch Test *STUDY 18J-0305-12*

Sponsor Code: EA

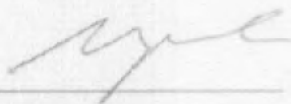
Test: Determination of the cutaneous tolerance (irritation potential) and sensitization to a facial mask tested on fifty (50) healthy participants by HRIPT.

Product tested: Raw unpreserved Glacial Oceanic Clay
Customer Lot#: BN 4461
Evalulab Lot#: 180301.EA.02
s

Date: April 23rd, 2018

Results: Under the conditions of the test procedure referenced herein, the test product referenced above has produced no signs of cutaneous irritation or intolerance and no signs of sensitization.
Hence, the test product may be considered as non-irritant and hypo-allergenic.

Signatures:



Doctor Michel Journet, M.D.
Dermatologist F.R.C.P (C)



Marie-Laure Oula, M. Sc.
Investigator, Evalulab Inc.



Marilou Nadeau, B. Sc.
Quality Assurance Director, Evalulab Inc.

STUDY OBJECTIVE

To carry out a "Human Repeat Insult Patch Test" (HRIPT).

This test determines the cutaneous irritation (contact dermatitis) and sensitization (contact allergy) potential of a topical product applied to the skin of 50 healthy participants. It consists of repetitively applying 10 patches during an induction period of 3 weeks, followed by a rest period and a challenge period.

PROTOCOL

1. Ethics Committee

This standard procedure and associated documents were reviewed and approved prior to the commencement of the study, by an Ethics Committee (an independent organization whose responsibility is to ensure the protection of the rights, safety and well-being of the subjects participating in the study).

2. Duration

The study took place between March 5th and April 11th, 2018.

3. Investigation Site

Evalulab Inc. located at 5475 rue Paré, Suite 206, Mont-Royal, Quebec, H4P 1P7, Canada.

4. Personnel

This study was conducted by Evalulab Inc., represented by Rébecca Padey, M.A. – Laboratory Assistant, under the supervision of Marie-Laure Oula, M. Sc. – Investigator and under the control of Doctor Michel Journet, MD Dermatologist.

5. Test Product

Upon reception, the test product was registered in the "Receptions Book" and assigned a code, followed by its storage at ambient humidity and temperature in its original container (as received) in an area allocated for this purpose.

2 units x 100g were received.

Description of the product tested:

Product name: Raw unpreserved Glacial Oceanic Clay

Category: Facial Mask

Client lot #: BN 4461

Evalulab lot #: 180301.EA.02

Application: The product was used without dilution and was generously applied on the entire surface of the patch. The total area of application is about 2.25 cm².

Description of control used:

Pure Vaseline USP

6. Material

The patches used in this study were TruMed® semi-occlusive, cotton "BBA149-129 Absorbent" with "3M 1530 Tape" adhesive backing.

7. Quality Assurance

Good Clinical Practice (GCP) is defined by the totality of the pronouncements put in place for ensuring the quality and authenticity of the trials and the obtained data on one hand and the respect for the ethics on the other.

The data obtained for each participant is recorded in individual Case Report Forms. The data entry is made in black ink. In case of errors or omissions, the initial entry is crossed out and initialed by the investigator.

All recorded data is validated by the investigator, who assumes responsibility for the quality of the work presented and verifies that all gathered data is in agreement with the protocol.

The records obtained during the study will be kept by Evalulab Inc. for a period of 2 years.

8. Adverse Events or Severe Adverse Events

An "Adverse Event" is defined as any noxious and unintended response observed in a participant testing a product that does not necessarily have to have a causal relationship with the test product or the treatment in question.

The risks for adverse events associated with this test may vary amongst the participants. Participants may be subject to a rash (intense redness), cracking, exfoliation effect, dryness, or even pain if the test product is strongly irritant or if the participant is particularly sensitive to the product. Participants may also develop an allergic reaction to the test products or to their components.

The term "Severe Adverse Event" refers to all medical manifestations, related or not to the test products, that may lead to death, persistent or significant disability that requires hospitalization or prolongation of a hospitalization period, or provoke invalidity, significant or permanent incapacity, or translate to congenital anomaly or malformation.

Participants were asked to immediately communicate any reactions to Evalulab. Tolerance to the test products was evaluated based on the observed reactions and their degree of severity, as well as on the reproducibility from one participant to another.

No "Severe Adverse Event" occurred during the entire length of the study.

9. Data Analysis

The tolerance to the product was evaluated by a dermatologist – considering the scores, observed reactions, their level of intensity and the reproducibility from one participant to another.

10. Amendment to Protocol

There were no amendments to the protocol.

11. HRIPT

- **Type of Study**

Monocentric and open-label, meaning the evaluator, participants, and sponsors alike, were aware of the nature of the test material.

- **Participants**

Recruitment of participants

A total of 53 participants were recruited based on the inclusion and exclusion criteria. The profile of each participant is presented in Table I in the Appendices.

Participant Demographics

Sex	Number	Age	Average Age
Male	15	24 to 70	50.87
Female	38	24 to 81	54.53
Total	53	24 to 81	51.28

Informed Consent Form

All participants had to sign and date the Informed Consent Forms explaining the conditions of the test, the risks involved and briefly describing the product to be tested. Each participant was informed verbally and in writing about the nature of the test and of the potential risks involved.

Confidentiality

Participation of the subjects in this study is confidential. The information gathered in the course of the study was recorded in individual Case Report Forms, that are numerically coded and do not contain the names of the participants.

Only the employees of Evalulab, auditors of the sponsor, and regulatory bodies (FDA, Health Canada and the Ethics Committee) may have access to the confidential information.

Inclusion Criteria

1. Participants of the feminine or masculine sex, aged 18 years or older,
2. With phototype I to IV (very clear to mat) and with a skin type that does not interfere with the assessment of cutaneous reactions,
3. Healthy and without any dermal anomalies on the areas to be tested that may interfere with the results of the study,
4. With no excessive body hair, especially on the test area,
5. Who will cooperate and be present for a follow-up at every visit, informed and sensitized about the duration and the importance of controls allowing complete compliance with the study protocol,
6. Who have read, signed and dated the Informed Consent Forms upon full knowledge of the risks involved with the study,
7. Women who use a method of contraception (oral contraceptive, condoms, spermicidal creams, an intra-uterine device (IUD), abstinence...), or are in a menopausal status.

Exclusion Criteria

1. Participants with a history of skin irritation or allergies to the type of products to be tested or in general, with allergies to certain foods, to certain chemical products, to jewellery, ...,
2. With a serious illness, health problem or chronic or progressive disease (asthma, diabetes, cancer, immunological deficiency, ablated organ...),
3. With a history of eczema, dermatitis, psoriasis or significant dermal anomalies on the test area,
4. On medication or having taken medication in the last 7 days prior to the study that could affect skin characteristics or could bias the study (antibiotics, anti-inflammatory drugs, steroids, antihistamines...),
5. Who frequent tanning salons or foresee exposure to the sun during the study,
6. Who abuse alcohol, drugs and/or tobacco,
7. Women, who are pregnant, breast feeding or expecting to become pregnant during the study.

- **Design of the Study**

Induction phase (or repeated skin contact test):

Prior to applying the patches, the test area (upper back, between the two shoulder blades) was carefully examined and wiped with alcohol if necessary (oily skin only). A patch containing the test products and the control were applied to the test area, and left in contact with the skin for 48 hours. Care was taken when positioning the patches to minimize the possibility of displacement or rubbing.

The test products were applied on the selected zones every second day, 3 times per week, over 3 consecutive weeks.

This is referred to as the Induction Phase.

Any deviation from the protocol or missed appointment or "non-application" was recorded in the attendance schedule for each participant. "Non-application" should not exceed 144 hours (6 days) and the induction phase should incorporate at least 9 applications over a 4 weeks period.

The first patch was removed at the laboratory 48 hours after application. The observation area was rinsed with water, dried, and examined for any skin changes, such as redness, irritation, inflammation, etc.

Following the examination, a new patch with "fresh" test product was applied.

With the exception of the first application, subsequent patches were removed by the participants themselves 24 hours after each application. Care was taken to re-apply the patches on the same test area every time. In the event of a significant skin irritation, for example if a reaction ranking between 2 and 4 is observed, a new application site is selected. If the reaction reoccurs and is identical in magnitude in the new test area, the Induction Phase for the test product in question is stopped.

Rest Period (or Incubation Phase):

After the completion of the Induction Phase described above, a Rest Period of 10 to 14 days is scheduled.

Challenge Phase (or Revealing Phase):

All test products are to be included in the Challenge Phase, except when there are strong indications of sensitivity (generally reactions of grade 3 to 4) caused by the test product that are observed during the Induction Phase.

The application site used during the Challenge Phase must be different than the one used in the Induction Phase.

For this phase, the patch was removed at the laboratory, 48 hours after application. The test site was cleaned and examined for any signs of intolerance or irritation.

Important notes:

The participants were to avoid wetting the patches, exposing them to sunlight or other sources of tanning; they were to keep the patches covered with clothing.

The use of topical pharmaceutical products or other skin care products on the test site was not permitted during the study. Ingestion of medication or any treatment that could have altered the results of the study was also prohibited.

• **Observations and Data collection**

The patch zones and their surrounding area were observed for erythema, oedema, vesicles, blisters, ulcerations, dryness and acne (papules). These parameters were evaluated and graded as follows:

Reaction Scale:

0 = No visible reaction,

+ = Erythema barely noticeable,

1 = Mild / slight erythema in the patch zone.

2 = Moderate but well defined erythema and presence of slight or barely visible oedema,

3 = Marked erythema, presence of oedema and vesicles.

4 = Severe erythema, presence of vesicles, blisters, and ulcerations.

In addition to the observations made and recorded by Evalulab, the participants were encouraged to observe and report to Evalulab any immediate or delayed reactions such as redness, irritation, itching, or other sensations on the application sites for up to 72 hours after application.

All observations and comments provided by the participants were recorded in their respective Case Report Form. The obtained scores were then entered in a tabular form showing the number of reactions after treatment.

RESULTS

Fifty-three (53) participants, men & women aged 24 to 81 years (Average Age = 51.28), were included in this study.

Two (2) participants did not complete the study due to schedule inconvenience. For the remaining fifty-one (51) participants, the data obtained during the study are presented in Table II in the Appendices.

No reaction with the control (Pure Vaseline USP) was observed.

CONCLUSION

Under the conditions of the study described herein, the test product "Raw unpreserved Glacial Oceanic Clay" has produced no signs of cutaneous irritation nor of skin sensitization.

It is therefore considered non-irritant and hypo-allergenic.

Also given the control provided by a dermatologist, the test product may bear the claim "Tested under the control of a dermatologist".

I the undersigned, Rébecca Padey, – Laboratory Assistant, declare that the study was conducted in accordance with the principles of "Good Clinical Practice". The recorded results show exactly and completely the raw data of the study.



Signature
Rébecca Padey, M.A.
Laboratory Assistant

Date: Mont-Royal – May 4th, 2018

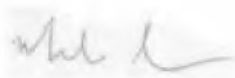
I the undersigned, Marie-Laure Oula, declare that the information provided in this report reflects in a complete and exact manner the results obtained during the study.



Signature
Marie-Laure Oula, M. Sc.
Investigator, Laboratory Director

Date: Mont-Royal – May 4th, 2018

I the undersigned, Marilou Nadeau, declare having validated the information provided in this report.



Signature
Marilou Nadeau, B. Sc.
Quality Assurance Director

Date: Mont-Royal – May 4th, 2018

APPENDICES

Table I. – Participants' Profile (Study #18J-0305-12)

Participant #			Initials	Age	Sex
01	-0305-	001	CR	73	F
01	-0305-	002	CB	52	F
01	-0305-	003	CB	24	F
01	-0305-	004	CD	69	F
02	-0305-	005	BB	58	M
01	-0305-	006	MY	31	F
02	-0305-	007	PC	70	M
02	-0305-	008	EC	38	M
01	-0305-	009	RV	59	F
01	-0305-	010	MM	37	F
01	-0305-	011	OL	69	F
01	-0305-	012	AD	70	F
01	-0305-	013	MA	75	F
01	-0305-	014	JL	62	F
01	-0305-	015	GH	70	F
02	-0305-	016	MR	70	M
01	-0305-	017	LM	47	F
01	-0305-	018	EM	31	F
02	-0305-	019	AM	65	M
01	-0305-	020	CD	56	F
01	-0305-	021	YL	37	F
02	-0305-	022	KL	57	M
01	-0305-	023	SY	60	F
02	-0305-	024	YO	68	M
01	-0305-	025	LL	65	F
01	-0305-	026	RO	59	F
02	-0305-	027	EL	61	M
01	-0305-	028	CL	58	F
01	-0305-	029	DM	63	F
01	-0305-	030	ZP	38	F
01	-0305-	031	AH	61	F
01	-0305-	032	AM	64	F
01	-0305-	033	MM	81	F
01	-0305-	034	PZ	45	F
01	-0305-	035	DV	61	F
01	-0305-	036	IS	66	F
01	-0305-	037	IS	24	F
01	-0305-	038	AN	35	F
02	-0305-	039	MB	54	M
01	-0305-	040	LP	43	F
01	-0305-	041	HC	53	F
01	-0305-	042	MG	35	F
02	-0305-	043	AM	30	M
02	-0305-	044	BC	24	M
01	-0305-	045	JL	38	F
01	-0305-	046	LS	35	F
02	-0305-	047	RT	38	M
01	-0305-	048	JF	37	F
02	-0305-	049	JB	38	M
01	-0305-	050	AV	42	F
02	-0305-	051	CH	43	M
01	-0305-	052	JM	30	F
02	-0305-	053	AP	49	M

Table II. – Individual results of HRIPT (Study #18J-0305-12)

Participant Identification			Induction Period										Challenge Period	
			Observations										Observations	
No.	Initials	Sex	#1	#2	#3	#4	#5	#6	#7	#8	#9	48h	72h	
01	-0305-001	CR	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-002	CB	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-003	CB	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-004	CD	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-005	BB	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-006	MY	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-007	PC	M	0	0	0	0	0	0	0	0	0	0	0
02	-0305-008	EC	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-009	RV	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-010	MM	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-011	OL	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-012	AD	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-013	MA	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-014	JL	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-015	GH	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-016	MR	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-017	LM	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-018	EM	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-019	AM	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-020	CD	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-021	YL	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-022	KL	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-023	SY	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-024	YO	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-025	LL	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-026	RO	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-027	EL	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-028	CL	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-029	DM	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-030	ZP	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-031	AH	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-032	AM	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-033	MM	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-034	PZ	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-035	DV	F	VS										
01	-0305-036	IS	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-037	IS	F	VS										
01	-0305-038	AN	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-039	MB	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-040	LP	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-041	HC	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-042	MG	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-043	AM	M	0	0	0	0	0	0	0	0	0	0	0
02	-0305-044	BC	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-045	JL	F	0	0	0	0	0	0	0	0	0	0	0
01	-0305-046	LS	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-047	RT	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-048	JP	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-049	JB	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-050	AV	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-051	CH	M	0	0	0	0	0	0	0	0	0	0	0
01	-0305-052	JM	F	0	0	0	0	0	0	0	0	0	0	0
02	-0305-053	AP	M	0	0	0	0	0	0	0	0	0	0	0

VS :Withdrawn from study

0 :No visible reaction

**In vitro evaluation of the eye irritation potential
on corneal epithelium
(according to OECD 492)**

<u>Study N°</u>	CAIC1529/21-01
<u>Study Protocol code</u>	REL/CA1582/2021/TOX
<u>Sponsor</u>	Ironwood Clay Company Inc. Unit 450-3771 Jacombs Road V6V 2 Richmond (BC) - Canada
<u>Analyzed substance</u>	SEA SILT Batch: WRCH210921-B

The results reported here in do exclusively refer to the tested sample

This report may not be reproduced, copied, downloaded or distributed in any way, in whole or in part, without the prior written authorization of the Study center

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ENCLOSURE:

- A) RAW DATA
- B) CoA

Note:

The results of the test in this report refer only to the tested product/s and to the particular experimental conditions here employed. This report cannot be partially duplicated without the preliminary written approval of the experimenters

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1 **PART ONE: GENERAL INFORMATION**

1.1 Sponsor

Ironwood Clay Company Inc.

1.2 Tested Sample

Sample	Internal code	Study Number	Description
SEA SILT Batch: WRCH210921-B	CA1204/21-01	CAIC1529/21-01	Clay paste

1.3 Controls

Controls	Supplier	Batch
Methyl acetate Positive control (PC)	MatTek	090821KMC
Phosphate Buffer(PBS) Negative control (NC)	MatTek	051221RAHA

1.4 Assay

Evaluation of eye irritation on 3D in vitro reconstituted human corneal epithelium (Mattek EpiOcular™) through the evaluation of:

- Cytotoxicity by MTT test

1.5 Entrusted laboratory

ABICH Inc
5160 Décarie Boulevard-suite # 330-340
Montréal (Québec) H3X 2H9- Canada

Study Dates

Start: 19/10/2021
End: 21/10/2021

Abich Inc

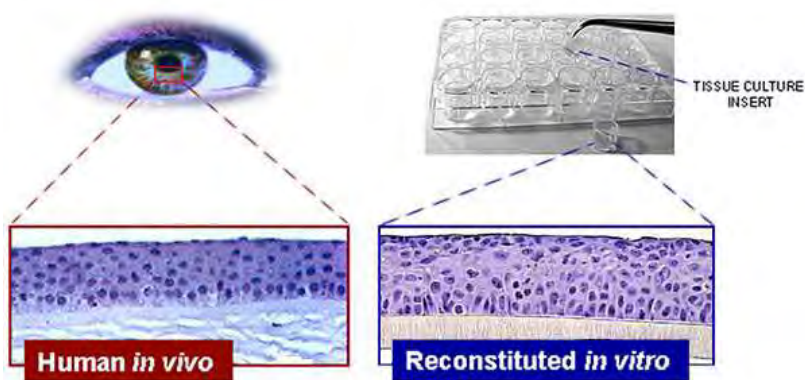
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2 **PART TWO: STUDY DESIGN**

2.1 **Purpose of the test:**

The purpose of this test is to evaluate the eye irritation potential of the tested sample, according to the method described in OECD 492. This method uses an artificial human corneal epithelial model (EpiOcular™ EIT) to assess the eye irritation of chemical substances and to properly label them according to the results.

The test is based on the evaluation of cell survival after the exposure to the substance through MTT assay and by comparison with epithelium treated with phosphate buffer only (negative control). The MTT method is a colorimetric assay that allows the determination of the percentage of cells alive within an in vitro cultured tissue. This assay is based on the ability of the mitochondrial succinate dehydrogenase enzyme to metabolise the nitro blue tetrazolium salt, giving a coloured compound that can be measured by spectrophotometer reading.



2.2 **Assay procedures**

The test has been carried out according to the method described in OECD 492 and in the MatTek validated protocol (2).

2.2.1 **Cell model**

The in vitro test systems employed consist of:

In vitro reconstituted human corneal epithelium.



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This system consists of normal, human-derived epidermal keratinocytes which have been cultured to form a stratified, squamous epithelium similar to that found in the cornea. Cultured on specially prepared cell culture inserts using serum-free culture medium, the cells differentiate to form a multi-layered structure which closely parallels the corneal epithelium. It was purchased from MatTek (Batch 32873).

2.2.2 Treatment and Exposure

The sample has been tested as follows: Pure

Phosphate buffer alone has been used as negative control while methyl acetate was used as positive control.

50µl of the product and controls have been applied on each epithelium unit in three replicas. The exposition has been carried out for 30 minutes at 37°C. At the end of the exposure period, the product was removed with multiple washes with PBS and the units were then incubated at 37°C for 2 hours. At the end of the exposure, the MTT assay was performed to evaluate the cell survival on the epithelium units.

2.2.3 MTT cell viability assay

Epithelium units are treated with 1mg/ml MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) for 3 h at 37°C. The solution is then removed and replaced with 1.5 ml/well of isopropanol, with further 2 h incubation at room temperature under medium speed shaking. 2 aliquots of every sample are transferred to a 96 well plate for the reading. The absorbance is read at the wavelength of 550-570 nm with a colorimeter (TECAN model Sunrise remote) equipped with a microplate reader. The absorbance values are corrected by subtracting the reading of the blanks (diluent only).

2.2.4 Expression of results

The results are expressed in terms of viability:

% of cell viability = [mean OD(550-570 nm) test product / mean OD(550-570 nm) negative control] x 100

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2.3 Acceptance criteria of method

Negative control (NC): The mean OD value of the 3 replica has to be >1.0 and <2.6 ,
The standard deviation has to be $\leq 18\%$

Positive control (PC): The viability mean (expressed as % of the NC) has to be $\leq 60\%$,
The standard deviation has to be $\leq 18\%$

Samples: The standard deviation has to be $\leq 18\%$

2.4 Results interpretation:

Mean cell viability is a predictive method of the ocular irritant potential of the sample.
In the following table, criteria for in vitro interpretation are reported:

Criteria for in vitro interpretation	Classification
Mean tissue viability $\leq 60\%$	No prediction can be made*
Mean tissue viability $> 60\%$	Not irritant

*According to OECD 492: Guideline for the testing of chemicals. Edition 2017 (Section 4.4. Interpretation of results and Prediction model)

3 **PART THREE:** RESULTS AND CONCLUSIONS

3.1 Assay validity requirements

	Value	Limits	Result
NC: mean OD from 3 replica	1.7237	$>1.0 \text{ e } < 2.6$	Complies
NC: % stand. Dev.	9.04%	$\leq 18\%$	Complies
PC: % mean viability	6.42%	$<60\%$	Complies
PC: % stand. Dev.	0.23%	$\leq 18\%$	Complies

Acceptance criteria of the assay (see § 2.3) comply, hence the assay is valid.

3.2 Results

	% mean cell viability (SD) 30 min treatment 2 hours incubation	Classification
SEA SILT Batch: WRCH210921-B Pure	87.93% (SD: 13.52%)	Not Irritant

*SD : Standard Deviation

3.3 Conclusion:

Based on the results here shown, the sample: **SEA SILT, Batch: WRCH210921-B** tested in normal use conditions

Is **NOT IRRITANT** for the human corneal epithelium.

Study Director

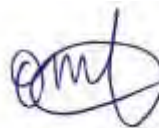
Michela Pollastri



Date: 09/11/2021

Laboratory Technician

Olivia Menoutis



Date: 27/10/2021

QA STATEMENT

The collected data derived from this study is managed according to internal procedures following the GLP directive and is verified by the QA manager who checks the different parts of this study (comparison between raw data and recorded data, laboratory books and files, protocol and report) according to the quality plan of ABICH Inc laboratory (internal audits, periodical calibration status of the instruments if they are involved in the test).

Quality Assurance Chemist



Nina Duru

Date 28/10/2021

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ANNEX 1: RAW DATA

Instrument: SUNRISE; Serial number: 1412005062; XFLUOR4 Version: V 4.51

Date of measurement: 21/10/2021

Time: 11:30 AM

Measurement mode: Absorbance

Measurement wavelength: 570 nm

Read mode: Accuracy

Raw data measurement

<>	1	2	3	4	5	6
Blank	0.003	0.002	0.002	0.002	0.003	0.001
Negative Control (NC)	1.783	1.859	1.866	1.605	1.625	1.617
Methyl acetate (PC)	0.117	0.119	0.111	0.103	0.114	0.113
Sample : SEA SILT, Batch: WRCH210921-B	1.349	1.370	1.340	1.707	1.647	1.694

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ANNEX 2: CoA

Certificate of Analysis



Product: EpiOcular™ Tissue

Lot Number: **32873**

Part#: OCL-200, OCL-212

Description: Reconstructed skin tissue containing normal human keratinocytes.
This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Cell source

All cells used to produce EpiOcular™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the cells or derivatives of the tissue for research purposes.

Keratinocyte Strain: **4F1B8**

II. Analysis for potential biological contaminants

The cells used to produce EpiOcular™ tissue are screened for potential biological contaminants. Tests performed for each of the potential biological contaminant listed in the analysis that follows, where performed according to the test method given. The product resulted in "no detection" for the following potential biological contaminants determined by the stated test method:

Keratinocytes:

HIV-1 virus - Oligonucleotide-directed amplification	Not detected
Hepatitis B virus - Oligonucleotide-directed amplification	Not detected
Hepatitis C virus - Oligonucleotide-directed amplification	Not detected
Bacteria, yeast, and other fungi - long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality

Test	Specification	Acceptance criteria	Result and QA Statement	
Tissue viability	MTT QC assay, 1 hour; n=3	OD (540-570 nm) { 1.1-3.0 }	LG ± 0.762	Pass
Barrier function	ET-50 assay, 100 µl 0.3% Triton X-100, 3 time-points, n=2 MTT assay	ET-50: {12.3-37.5 min}	20.64 min	Pass
Sterility	Long term antibiotic and antimycotic free culture	No contamination	Sterile	Pass

Tissue viability and the barrier function tests are within the acceptable ranges and indicate appropriate formation of the mucosal barrier and a viable basal cell layer.

Initials: **MP**
Date: **10/20/21**


Nelson Rivas
Quality Assurance Associate

October 20, 2021

Date

CAUTION: Whereas all information above is believed to be accurate and correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyewear and appropriate disposal procedures is strongly recommended.

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QC-10-012-0011 Rev. A

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REFERENCES

1. OECD 492 GUIDELINE FOR THE TESTING OF CHEMICALS Reconstructed Human Cornea-Like Epithelium (RhCE) Test Method Fo Identfyng Chemicals Not Requiring Classification And Labelling For Eye Irritation Or Serious Eye Damage. 28 July 2015.
2. Mattek Corporation. EpiOcular Eye irritation test (OCL-200-EIT) for the prediction of acute ocular irritation chemicals. For use with MatTek Corporation's Reconstructed Human EpiOcula Model. 7 July 2014

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Amended Safety Assessment of Naturally-Sourced Clays as Used in Cosmetics

Status: Final Amended Report
Release Date: March 31, 2023
Panel Meeting Date: March 6-7, 2023

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.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel members involved in this assessment: Ronald C. Shank, Ph.D. and Daniel C. Liebler, PhD. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

ABBREVIATIONS

16HBE	human bronchial epithelial cell line 16HBE14o-
AICIS	Australian Industrial Chemicals Introduction Scheme
BAL	bronchoalveolar lavage
CCK-8	cell counting kit-8
cfu	colony forming units
cGMPs	current good manufacturing practices
CHO	Chinese hamster ovary
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
ECVAM	European Centre for the Validation of Alternative Methods
EU	European Union
FDA	Food and Drug Administration
β-GLUC	β-glucuronidase
GRAS	generally recognized as safe
HRIPT	human repeated insult patch test
IARC	International Agency for Research on Cancer
ICP-OES	inductively coupled plasma-optical emission spectrometry
IL-1	interleukin-1
LDH	lactate dehydrogenase
MDGF	macrophages-derived growth factor
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
mU	milli units
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
OTC	over-the-counter
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PEL	permissible exposure limit
PM _{2.5}	particulate matter with aerodynamic equivalent diameter of 2.5 µm or less
PMN	polymorphonuclear
REL	recommended exposure limit
t ₅₀	50% decrease of tissue viability
TG	test guideline
UDS	unscheduled DNA synthesis
UV	ultraviolet light
VCRP	Voluntary Cosmetic Registration Program
WINCI Dictionary	web-based International Cosmetic Ingredient Dictionary and Handbook

ABSTRACT

The Expert Panel for Cosmetic Safety (Panel) assessed the safety of 8 naturally-sourced clay ingredients, of which 6 were previously reviewed, as used in cosmetic formulations. All of these ingredients are reported to function in cosmetics as absorbents and bulking agents; other cosmetic functions are also reported. The Panel reviewed all relevant data and concluded that Kaolin is safe in cosmetics in the present practices of use and concentration described in this safety assessment. The remaining 7 naturally-sourced clay ingredients are safe in cosmetics in the present practices of use and concentration, with the exception that the available data are insufficient to make a determination that these ingredients are safe in products that may be incidentally inhaled.

INTRODUCTION

The Expert Panel for Cosmetic Ingredient Safety (Panel) previously reviewed the safety of 6 naturally-sourced clay ingredients in a report that was published in 2003.¹ At that time, the Panel concluded that these ingredients are safe as used in cosmetic products. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment has been issued. This report has been reopened to reassess the safety of the 6 clays included in that original report, and also includes 2 additional ingredients, i.e., Clay and Illite. In total, this report assesses the safety of 8 naturally-sourced clay ingredients (listed below) as used in cosmetics. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*; see Table 1), all of these ingredients are reported to function in cosmetics as absorbents and bulking agents; other cosmetic functions are also reported.²

Attapulgite*

Bentonite*

Clay

Fuller's Earth*

Hectorite*

Illite

Kaolin*

Montmorillonite*

**Previously reviewed by the Panel.*

The Panel has also 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.³ In 2021, the Panel concluded that silicate ingredients, including aluminum silicate and magnesium aluminum silicate, are safe in cosmetics in the present practices of use and concentration when formulated to be non-irritating, with the exception that the data are insufficient to make a determination that naturally-sourced (i.e., mined) silicate ingredients are safe for use in products that may be incidentally inhaled.⁴ (The reports on these ingredients are available on the Cosmetic Ingredient Review (CIR) website (<https://www.cir-safety.org/ingredients>). Although the clay ingredients comprise silica and/or silicates, silicates, synthetically-manufactured amorphous silica, and hydrated silica are neither part of this safety assessment, nor are data from those reports included in this assessment.

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.

Excerpts from the summaries of the previous report on clay ingredients are disseminated throughout the text of this review document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or the summary section.)

CHEMISTRY

Definition and Structure

The definitions of Clay (CAS No. 53801-44-8) and the other clay ingredients included in this review are provided in Table 1.² These inorganic oxide ingredients, comprising in part silicon dioxide, are solids derived from naturally occurring minerals.

Clays in general have atomic lattices consisting of two structural units.¹ One unit consists of two sheets of closely packed oxygens or hydroxyls. Aluminum, iron, or magnesium atoms are embedded within these sheets in octahedral coordination, so that they are equidistant from the oxygen or hydroxyl groups. The second unit is composed of silica tetrahedrons. Assuming there are no distortions in each tetrahedron, a silicon atom is equidistant from four oxygens or hydroxyls, if needed to balance the structure, arranged in the form of a tetrahedron with a silicon atom in the center. The silica tetrahedral groups are arranged in a hexagonal network, which is repeated infinitely to form a sheet of composition $\text{Si}_4\text{O}_6(\text{OH})_4$. The tips of the tetrahedrons all point in the same direction and the bases are all in the same plane. Substantial distortion of these units occurs in order to fit into determined unit-cell dimensions of minerals.

Figure 1 depicts the general structure of clay ingredients. Clays are composed of magnesium, lithium, aluminum, and/or iron silicate sheets with various exchangeable cations. These sheet-like structures are in sharp contrast to the hexagonal crystalline structure of crystalline silica (e.g., quartz).

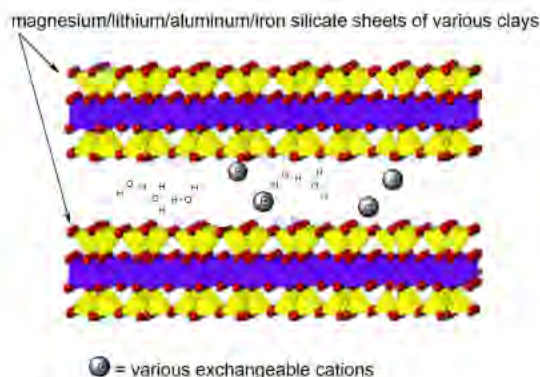


Figure 1. General structure of clay ingredients. CIR Staff

Attapulgite

The structurally important element is the amphibole double silica chain oriented with its long direction parallel to the *c*-axis.¹ Attapulgite consists of double silica chains situated parallel to the *c*-axis with the chains linked together through oxygens at their longitudinal edges. Tetrahedral apexes in successive chains point in the opposite direction. The linked chains form a kind of double-ribbed sheet with two rows of tetrahedral apexes at alternate intervals in the top and bottom of the sheets. The ribbed sheets are arranged so that the apex oxygens of successive sheets point together and are held together by aluminum and/or magnesium in octahedral coordination between the apex oxygens of successive sheets. Chains of water molecules run parallel to the *c*-axis and fill the interstices between the amphibole chains. Aluminum substitutions for silicon are considered probable.

Bentonite, Hectorite, and Montmorillonite

Bentonite, Hectorite, and Montmorillonite (also known as smectites) units comprise of two silica tetrahedral sheets with a central alumina octahedral sheet.¹ All tetrahedral tips point in the same direction and toward the center of the unit. The tips of the tetrahedrons of each silica sheet and one of the hydroxyl layers of the octahedral sheet form a common layer. As in Kaolin, the atoms common to both the tetrahedral and octahedral layer are O instead of OH. These layers are continuous in the *a* and *b* directions and are stacked one above the other in the *c* direction. As a consequence, O layers in the units become adjacent and a very weak bond is created with the possibility of cleavage. The preeminent feature of these clay ingredients is the ability of water and organic molecules to enter between unit layers and expand in the *c* direction. Expansion properties are reversible; however, the structure is completely collapsed by removal of interlayer polar molecules. Most of these clay ingredients have substitutions within their lattices: aluminum or phosphorous for silicon in the tetrahedral coordination and/or magnesium, iron, zinc, nickel, lithium, etc. for aluminum in the octahedral sheet.

Illite

Illite is a non-expanding, clay-sized, dioctahedral mineral that is considered part of the mica group.^{5,6} It is a layered alumino-silicate, known as a phyllosilicate. Its basic unit is a layer composed of two inward-pointing silica tetragonal sheets with a central alumina octahedral sheet. Poorly hydrated potassium cations occupy the space between the sequence of layers, which prevents swelling or the expansion of the layers. Illite is very similar structurally to common mica (muscovite), with slightly more silicon, magnesium, iron, and water, and slightly less tetrahedral aluminum and interlayer potassium.

Kaolin

Kaolin's structure is composed of a single silica tetrahedral sheet and a single alumina octahedral sheet combined in a unit so that the tips of the silica tetrahedrons and one of the layers of the octahedral sheet form a common layer.¹ All the tips of the silica tetrahedrons point in the same direction and toward the center of the unit made by the silica and octahedral sheets. Composite octahedral-tetrahedral layers are formed due to the similarity between the sheets *a* and *b* dimensions. The common layer between the octahedral and tetrahedral groups consists of two-thirds of shared atoms between silicon and aluminum that become O instead of OH. Analyses of Kaolin have shown there is little substitution within the lattice. In a small percentage of cases, iron and/or titanium has replaced aluminum. This has only been seen in the relatively poor crystalline varieties of Kaolin.

Chemical Properties

Chemical properties for clay ingredients are described in Table 2.^{1,7-9} Clay ingredients are generally described as insoluble in water.

A supplier has reported the particle size distribution at D₅₀ for Bentonite and Kaolin to be 61.1 and 3.1 µm, respectively.¹⁰ The mean particle sizes for 5 different Hectorite products were 19.9 - 25.4 µm, and the particle ranges for these products were 2.9 - 131.7 µm.¹¹ Another supplier provided specifications for a Clay raw material containing 75% Illite, 19% Kaolin, and 6% Montmorillonite (see Clay in Table 2); however, there is no information therein to indicate the particle size of resultant final cosmetic formulations.⁹

Method of Manufacture

Attapulgite

Attapulgite is produced through an opencast mining technique, stripping layers with heavy machinery.¹ The clay is then transported to a processing plant where crushing, drying, classification, and pulverizing take place. High-heat drying to remove water may occur to enhance absorbent qualities. Attapulgite is mined in 10 countries: Australia, China, France, India, Russia, Senegal, South Africa, Spain, Turkey, and the US.

Bentonite

Large deposits of Bentonite have been discovered in Canada, China, France, Germany, Great Britain, Greece, Hungary, Italy, Japan, Mexico, New Zealand, North Africa, Poland, South Africa, the post-Soviet states, and the US.¹ The mine ore of Bentonite is processed to remove grit and non-swelling materials.

A supplier has reported that Bentonite is mined mineral.¹² The material is then washed, filtered, dried, treated, and tested for quality.

Clay

A supplier has reported that Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) is naturally sourced, mechanically refined, and not chemically processed.⁹ A dehydration process is used to eliminate bacteria prior to sorting through induction.

Illite

Illite is formed by the weathering of silicates (feldspar), by the alteration of other clay minerals, or during the degradation of muscovite.^{5,6} The formation of Illite is generally favored by alkaline conditions and by high concentrations of aluminum and potassium. Deposits of Illite are widely distributed globally: it is commonly found in soil and argillaceous sedimentary rocks, as well as in some low-grade metamorphic rocks.^{5,6,8}

Kaolin

Deposits of Kaolin have been found in England, the US, France, the Czech Republic and Slovakia, Germany, and Japan.¹ Kaolin is extracted from kaolinized granite by washing it out with powerful water hoses. The clay stream is then pumped to the separation plant where sand and mica are removed. The purified clay is filtered when wet and then dried. The very fine powder is formed by milling.

Composition/Impurities

Attapulgite

Attapulgite commonly is found with smectites, amorphous silica, chert, and other minerals.¹ A typical mineral composition of Attapulgite is approximately 55% silicon dioxide, 10% aluminum oxide, 3.5% iron (III) oxide, 10.5% magnesium oxide, 0.5% potassium oxide, and 20% water.

Bentonite

The principal constituent of Bentonite is Montmorillonite.¹ However, other minerals such as Illite, kaolinite, and nonargillaceous detrital minerals can be present. Most bentonites appear relatively pure and other mineral contributions rarely exceed 10%. Cristobalite is often present. Montmorillonite compositions frequently vary either in its lattice structure or in the exchangeable ions present. A typical mineral composition of Bentonite is approximately 60% silicon dioxide, 20% aluminum oxide, 3% iron (II) oxide, 1.5% magnesium oxide, 0.6% calcium oxide, 0.6% potassium oxide, and 21% sodium oxide.

According to the *Food Chemicals Codex*, Bentonite is composed of natural smectite clays consisting primarily of colloidal hydrated aluminum silicates of the Montmorillonite or Hectorite type of minerals with varying quantities of alkalis, alkaline earths, and iron.⁷ Food-grade Bentonite should contain no more than 5 mg/kg arsenic, no more than 15 mg/kg lead, and no more than 1000 colony-forming units (cfu)/g aerobic microbes. Bentonite should be negative for *Escherichia coli* in 25 g.

In particle size analysis of Bentonite, 90% of the particles were smaller than 68 μm , 50% were smaller than 5 μm , and 10% were smaller than 2 μm .¹³ No particles were smaller than 0.9 μm . A second analysis of Bentonite showed that 90% of the particles were smaller than 25 μm , 50% were smaller than 6.5 μm , and 10% were smaller than 1.6 μm .¹⁴ No particles were smaller than 0.5 μm .

Clay

A supplier has reported that Clay contains 75% Illite, 19% Kaolin, and 6% Montmorillonite and does not contain quartz.⁹ Trace heavy metals may be present: < 0.5 ppm antimony, < 17 ppm arsenic, < 0.5 ppm cadmium, < 7 ppm cobalt, < 0.5 ppm tin, < 0.05 ppm mercury, < 20 ppm nickel, and < 20 ppm lead were detected using inductively coupled plasma-optical emission spectrometry (ICP-OES). Bacterial, yeast, and mold content were below the threshold of detection. Dioxin and polychlorinated biphenyl "results are near zero."

Fuller's Earth

Principal deposits of Fuller's Earth include Montmorillonite, Bentonite, Attapulgite, and sepiolite.¹

Hectorite

Principal impurities of Hectorite include calcite, dolomite, silica crystals, and grit.¹ A typical mineral composition of Hectorite is approximately 56% silicon dioxide, 0.1% aluminum oxide, 0.03% iron (III) oxide, 25% magnesium oxide, 0.1% potassium oxide, 3% sodium oxide, 1% lithium dioxide, 6% fluorine, and 12% water.

According to some suppliers of Hectorite products (97 - 100% pure), crystalline silica (also described as quartz and/or CAS No. 14808-6-7) may be an impurity.^{15,16} Quantities of crystalline silica were reported to be 1 - 3%.

Illite

The sheets of Illite are composed of silicon, magnesium, iron, aluminum, potassium, and water.⁶ In analysis of 0.5 kg samples of 3 clay products containing Illite, the major element composition (as mean concentration) comprises silicon (23.02%), aluminum (8.80%), calcium (4.55%), iron (4.22%), potassium (3.19%), titanium (0.48%), sulfur (0.21%), phosphorus (0.10%), and manganese (0.041%).¹⁷ Trace element impurities (as mean concentration) were identified as barium (426.70 mg/kg), rubidium (253.7 mg/kg), strontium (227.07 mg/kg), zinc (100.97 mg/kg), cesium (65.93 mg/kg), nickel (35.37 mg/kg), neodymium (30.23 mg/kg), lanthanum (28.00 mg/kg), lead (26.77 mg/kg), copper (25.07 mg/kg), arsenic (16.33 mg/kg), thorium (8.83 mg/kg), uranium (3.78 mg/kg), and bromine (< 1 mg/kg). Bulk composition analysis indicated the samples contained calcite and quartz.

Kaolin

Quartz, mica, and feldspar are often found associated with Kaolin as the crude mineral and are often removed through screening and elutriation.¹ Potentially pathogenic organisms were absent. The bacteria present were mostly gram-positive aerobic spore-formers. A typical mineral composition of Kaolin (reported as kaolinite) is approximately 45% silicon dioxide, 39% aluminum oxide, 0.8% iron (III) oxide, 0.08% magnesium oxide, 0.08% calcium oxide, 0.1% potassium oxide, 0.7% sodium oxide, 0.2% titanium (IV) oxide, and 14% water.

According to the *Food Chemicals Codex*, Kaolin is a purified clay consisting mainly of alumina, silica, and water.⁷ Food-grade Kaolin should contain no more than 3 mg/kg arsenic and no more than 10 mg/kg lead.

According to some suppliers of Kaolin products (up to 100% pure), crystalline silica (described as quartz, free respirable silica, and/or CAS No. 14808-6-7) may be an impurity.^{18,19} Quantities of crystalline silica were reported to be $\leq$ 2%.

Montmorillonite

A typical mineral composition of Montmorillonite is approximately 51% silicon dioxide, 20% aluminum oxide, 0.8% iron (III) oxide, 3% magnesium oxide, 2% calcium oxide, 0.1% potassium oxide, 0.04% sodium oxide, 0.1% zinc oxide, and 23% water.¹

USE

Cosmetic

The safety of the cosmetic ingredients 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 these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of

cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, Kaolin has the most reported uses in cosmetic products, with a total of 787; the majority of uses are in leave-on formulations (Table 3).²⁰ Bentonite has the second most reported uses in cosmetic products, with a total of 221; half are reported in leave-on formulations. The frequencies of use for both of these ingredients have greatly changed since the original safety assessment was finalized; in 1998, Kaolin was reported to have 509 uses, and Bentonite was reported to have 94.¹ The results of concentration of use surveys conducted by the Council in 2022 indicate Kaolin has the highest maximum concentration of use in leave-on formulations; it is used at up to 53.2% in manicuring preparations.²¹ For leave-on dermal preparations, specifically, Kaolin also has the highest reported maximum concentration of use, at 16% in face and neck products, and Bentonite has the next highest, at 8% in face and neck preparations. According to the original safety assessment, the maximum leave-on use concentration in 1999 for Kaolin was 100% in skin care preparations; the maximum leave-on use concentration for Bentonite was 8% in makeup foundations.¹

Clay ingredients may be used in products that can be incidentally ingested; for example, Kaolin is used in lipstick (at up to 14.5%).²¹ Additionally, clay ingredients have been reported to be used in products that may come into contact with the eyes and mucous membranes; for example, Kaolin is used at up to 8.5% in eye shadows and at up to 5% in bath soaps and detergents.

Moreover, clay ingredients are used in cosmetic formulations that could possibly be inhaled; for example, Bentonite is reported to be used at 0.9% in spray suntan products and Kaolin is reported to be used at up to 15% in face powders.²¹ In practice, as stated in the Panel's respiratory exposure resource document (<https://www.cir-safety.org/cir-findings>), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

While no data have been submitted from the cosmetics industry indicating that these clay ingredients are used in nanoform in cosmetic formulation, a report from a nanotechnology research company provides statistical data showing that nanoclays can be used as cosmetic additives for lipsticks, eyeliners, and toothpaste, functioning for rheology modification, viscosity control, thixotropic effect, as well as increased stability and pigment dispersibility.²²

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients, and without consumer habits and practices data or particle size data related to this use technology, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In regulations regarding cosmetic products in the European Union (EU), no restrictions were listed for Attapulgit, Clay, Fuller's Earth, Hectorite, Illite, or Montmorillonite.²³ Bentonite and Kaolin are listed in Annex IV-Allowed Colorants under CI 77004 with the chemical name of "natural hydrated aluminum silicate...containing calcium, magnesium or iron carbonate, ferric hydroxide, quartz-sand, mica, etc. as impurities;" the remaining clay ingredients named in this report are not restricted from cosmetic use in any way. Note, these ingredients are not approved as colorants in the US.² Bentonite, Hectorite, and Kaolin were included in the scientific opinion by the EU Scientific Committee on Consumer Safety (SCCS) on the safety of aluminum in cosmetic products.²⁴ The SCCS concluded that the use of aluminum compounds is safe at the following equivalent aluminum concentrations up to 6.25% in non-spray deodorants/antiperspirants, 10.60% in spray deodorants/antiperspirants, 2.65% in toothpaste, and 14% in lipstick.

According to the Australian Industrial Chemicals Introduction Scheme (AICIS), the following ingredients are Tier I chemicals (not considered to pose an unreasonable risk to the health of workers and public health): Bentonite, Fuller's Earth, Kaolin, and Montmorillonite.²⁵ Attapulgit is a Tier II chemical (requires risk management measures to be instituted for safe use for human health). Hectorite is listed as a chemical unlikely to require further regulation to manage risks to human health.

Non-Cosmetic

Based on the properties of broad surface area, rich porosity, diverse morphology, good adsorption performance, and high ion exchange capacity, nanoclays have been widely applied in many fields, such as drug delivery systems,²⁶ food and beverage packaging,²⁷ paper manufacturing,²⁸ and constructional material.²⁹

Attapulgit

Attapulgit is reported to be used in absorbents, pesticides, oil and petroleum treatment, and as a filler in many products.¹

Bentonite

Bentonite is reported to be used in foundry sand bonding, bleaching clay in oil refining and decolorizers, filtering agents, water impedance, animal feed, pharmaceuticals, paint, plasticity increasers, and iron-ore pelletizing.¹

Bentonite is generally recognized as safe (GRAS) as a direct food additive for humans (21 CFR§184.1155) and animals (21 CFR§582.1155). Bentonite is also GRAS as an indirect food additive in adhesives and components of coatings (21 CFR§175.105), in paper and paperboard components (as a colorant only, 21 CFR§176.170), in adjuvants as a colorant for polymers (21 CFR§178.3297),

Clay

Clay (natural) is GRAS as an indirect food additive in polymers (cellophane; 21 CFR§177.1200).

Fuller's Earth

Fuller's Earth is reported to be used as a military decontaminant for removal of hazardous materials from the skin.³⁰

Hectorite

Hectorite has been approved for use in internally and externally applied products, as well as dentifrices and externally approved pharmaceuticals.¹

Hectorite is reported to be used as drug-delivery system in anticancer therapy because of its biocompatibility, mechanical strength, and natural availability.³¹

Illite

Illite has been studied for use in environmental remediation of contaminated soils and water as an adsorbent.³²⁻³⁵ It also has been studied for use in veterinary applications, such as dietary supplements for swine³⁶ and topical therapeutic treatment in equine injuries.³⁷

Kaolin

Kaolin is reported to be used in the paper industry to fill and coat the surface of paper, as a filler in rubber and plastics, paint extender, ceramics manufacture, ink, adhesives, insecticides, medicines, food additives, bleaching, adsorbents, cement, fertilizers, crayons, pencils, detergents, porcelain enamels, paste, foundries, linoleum, floor tiles, and textiles.¹ It has been classified by the National Formulary as a tablet and/or capsule diluent.

Kaolin clay is GRAS as an indirect food additive with no limitation other than current good manufacturing practice (21 CFR§186.1256). It is used in the manufacture of paper and paperboard that contact food. Kaolin (colloidal) is an approved over-the-counter (OTC) drug as an antidiarrheal active ingredient (21 CFR§335.10), an anorectal active ingredient (21 CFR§346.14), and a skin protectant active ingredient (from 4% to 20%; 21 CFR§347.10). Kaolin is used as a digestive aid (21 CFR§310.545); however, the data are currently inadequate to establish general recognition of the safety and effectiveness of this ingredient for this specified use. Kaolin is exempted from the requirement of a tolerance for pesticide residues when used on or in food commodities to aid in the control of insects, fungi, and bacteria (food/feed use; 40 CFR§180.1180).

Kaolin minerals (specifically kaolinite) have been studied for use in environmental remediation of contaminated soils and water.^{34,35} This clay material has also been studied for use in veterinary applications, such as dietary supplements for swine³⁶ and topical therapeutic treatment in equine injuries.³⁷

Montmorillonite

Montmorillonite is reported to be used for food packaging and in paper manufacturing.^{27,28} It also has been studied for use in environmental remediation of contaminated soils and water^{34,35} and in veterinary applications, such as use in dietary supplements for swine.³⁶

TOXICOKINETIC STUDIES

Absorption, Distribution, Metabolism, and Excretion (ADME)

Clay

In ex vitro bioavailability studies using human skin models, the ability of transcutaneous passage of heavy metals (vanadium, lead, arsenic, barium, nickel, chromium, and aluminum) in 3 clay pastes was analyzed.³⁸ The clay pastes were white Montmorillonite, Kaolin, and Clay (composed of 75% Illite, 19% Kaolin, and 6% Montmorillonite). Approximately 150 g of each product were tested with human skin samples in Franz cells and incubated for 24 h. The tested products, the diffusion liquids, and the storage liquids were then analyzed for metal content (details not provided). No detectable quantities of heavy metals were found in the diffusion or storage liquids. It was determined that the traces of heavy metal in the clay pastes did not penetrate cutaneous tissue.

Kaolin

In a dietary study, a group of 10 male Sprague-Dawley rats were fed a control diet plus 0.5 ml 20% Kaolin – 1% pectin for 48 h.¹ Stool samples were collected 72 h later and analyzed for volume, sodium, potassium, and fat content. The results were a 103% increase in sodium, a 184% increase in potassium, and fat excretion remained at baseline.

Montmorillonite

Polydisperse and monodisperse [¹³⁴Cs]-fused Montmorillonite suspensions were administered to groups of 40 rats and mice and to 120 beagle dogs by a multiport nose-only inhalation exposure system.¹ Aerosol concentrations ranged from 0.1 to 0.001 mg of fused Montmorillonite/l of air. Equal numbers of male and female rats and mice and 74 male and 46 female dogs were utilized. Exposure times for rats and mice ranged from 25 to 45 min and for dogs 15 to 50 min. All animals were whole-body counted for the labeled particles. Five rats and 5 mice from each group were killed 4 h after exposure. The remaining rats and mice were killed at various times after exposure. Tissues from rats and mice were collected on post-exposure days 2, 4, 8, 16, 32, 64, 128, 256, 365, 512, 730, and 850. Tissues and excreta from the dogs were also collected on the same schedule, but also at 4, 5, 7, and 9 yr after inhalation exposure. Two dogs were scheduled for termination at times ranging from 4 h to 9 yr. All animals were necropsied and tissues from lungs, lung-associated lymph nodes, gastrointestinal tract, spleen, kidneys, abdominal lymph nodes, blood, skeleton, muscle, and skin were prepared for analysis of [¹³⁴Cs]-exposure. The mass of material deposited into the lungs of rats and mice was ~0.01 to 0.1 mg and for dogs was ~1 to 10 mg. The mass of Montmorillonite for all three species was < 0.1 mg/g of lung. Clearance of the initial ¹³⁴Cs occurred by dissolution and mechanical clearance. Mechanical clearance from the nasopharynx was rapid, and the clearance rate was decreased to a negligible value for all three species within a few days. Most initial deposits cleared via the gastrointestinal tract. Long-term mechanical clearance from the pulmonary region occurred at a constant rate for all species. Solubilization was the primary factor in long-term lung clearance for most particles inhaled by dogs and mechanical clearance was dominant in rats and mice. Most of the long-term clearance of deposited particles went to lung-associated lymph nodes in dogs and occurred at a slower rate as compared to rats and mice. Rats and mice had a rapid clearance from the pulmonary region, where most of the mechanical clearance occurred via the gastrointestinal tract. Long-term clearance of the particles in dogs occurred at 3500-d half-time in the lymph nodes and 6900-d half-time clearance in the gastrointestinal tract. The transport rate of the particles in the dog was 0.0002/d of the lung burden. The long-term biological clearance half-term day was 690 d for rats and 490 d for mice. The lymph node accumulation process was modeled by a short-term process that became negligible after a few days.

Radiolabeled, [¹³⁴Cs]-fused Montmorillonite particles were instilled into specific lung lobes or injected intraperitoneally into 32 beagle dogs.¹ Necropsy was performed 34, 182, and 365 d later. Specific sites of instillation included right apical lobe, right cardiac lobe, right diaphragmatic lobe, right intermediate lobe, left apical lobe, left diaphragmatic lobe, and intraperitoneal. Initial burdens in the peritoneal cavity or the lungs ranged from 0.50 to 14 μ Ci for 29 dogs and from 42 to 64 μ Ci lung burdens for the other 3 dogs. Effective translocation half-time of lung instillations was 390 d. The accumulation rate of [¹³⁴Cs]-fused particles in the lymph nodes was 0.03% per day. Individual lung lobes cleared particles to 1 - 2 lymph nodes, and specific lymph nodes accumulated particles from 1 - 3 lung lobes. Lymph nodes that collected particles from the lung included the left mediastinal node and the left, left-middle, right, and right-middle tracheobronchial lymph nodes. The destination for translocated particles was primarily the nodes proximate to the tracheal bifurcation. Particles injected into the peritoneal cavity were translocated mainly to mesenteric lymph nodes and left and right sternal lymph nodes. A small percentage of particles went to the left tracheobronchial lymph node.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Clay

In an acute dermal toxicity study performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 402, rats received 2000 mg/kg bw of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) on clipped skin.³⁸ The test material was moistened with 0.2 ml distilled water. A control group received only distilled water. The rats were observed for 14 d. No mortality or clinical signs of toxicity were observed. The LD₅₀ was greater than 2000 mg/kg. No further details were provided.

Oral

Clay

In an acute oral study performed in accordance with OECD TG 423, rats were exposed to a single dose of 2000 mg/kg bw of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) in distilled water.³⁸ No mortality or clinical signs of toxicity were observed. Visceral examination did not reveal any lesions of pathological significance except uterine distension in one rat, which was not considered to be treatment-related. The LD₅₀ was greater than 2000 mg/kg bw. No further details were provided.

Hectorite

Five male and 5 female Sprague-Dawley rats were administered a single dose of 5 g/kg of Hectorite by gavage.¹ None of the animals died; the acute oral LD₅₀ was > 5.0 g/kg bw.

Kaolin

In an acute oral study, 120 rats were fed doses of Kaolin ranging from 100 to 210 g/kg.¹ Fourteen rats were controls. Kaolin was inert and non-static except for the danger of bowel obstruction resulting in perforation. The clinical signs were listlessness, anorexia, oliguria, hypothermia, and dyspnea. These were pathological reactions from overdistention of the alimentary canal by an inert solid. The number of fatalities and the incidence and advance of bowel obstruction along the small intestine were dose-related. The dose that killed 50% of the rats by bowel obstruction was 149 g/kg.

Inhalation

Clay

In an acute inhalation study performed in accordance with OECD TG 403, rats were exposed to 3.856 mg/l air of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite).³⁸ A control group was exposed to air passed through inert bed material (iron grit). Both groups of rats were exposed for 4 h and then observed for 14 d. No mortality or clinical signs of toxicity were observed. The LC₅₀ was greater than 3.856 mg/l. No further details were provided.

Illite, Montmorillonite, Kaolin

The inhalation toxicity of an environmental dust sample containing Illite, Montmorillonite, Kaolin and α -quartz was determined in a group of 6 male CD(SD)BR rats exposed to an aerosol of the dust (12 mg/m³, ~ 10 μ m mean diameter) for 3 h in open cages.³⁹ The dust was composed of approximately 75% of the 3 clay aluminum-silicates and approximately 20% α -quartz. A control group of 6 male rats were exposed to room air only. Animals were killed at recovery periods of 0 h, 24 h, 8 d, and 30 d. Lung tissues underwent microscopic and histopathological examination. Rats that were exposed to the dust exhibited preferential particle deposition at the first alveolar duct bifurcations after the terminal bronchiole immediately after the 3 h exposure. No extracellular particles were observed in the recovery periods after this point, in either treated or control animals. The average number of particles observed at the first bifurcation after the 3 h exposure (recovery time 0 h) was 4.6 ± 1.0 particles per bifurcation. Histological sections showed prominent first bifurcation characterized by accumulation of mononuclear cells 24 h after exposure. The presence of macrophages with ingested aluminum silicate particles were observed. Macrophage migration to the bifurcations was observed to a lesser degree immediately after exposure. At 24 h, $87 \pm 12\%$ of the first bifurcation contained a significantly increased number of macrophages. After 8 and 30 d, particles and alveolar macrophages were not significantly elevated and histology was back to normal.

Parenteral

Illite, Montmorillonite, Kaolin

In a study of the environmental dust sample described above, 6 male albino Wistar rats were instilled with 50 mg of the dust (3.2 μ m mean diameter) in 300 μ l of sterile phosphate-buffered saline (PBS).³⁹ The dust particles were injected into the trachea. Control animals (groups of 6) received either 300 μ l of PBS, 50 mg of carbonyl iron in 300 μ l PBS, or 50 mg of α -quartz in 300 μ l PBS. The animals were killed 30 d later. Tissues, particularly the lungs and trachea, underwent microscopic and histopathological examination. Multifocal interstitial lung disease was observed using light microscopy. Mononuclear cell infiltrates, composed of macrophages and lymphocytes located mainly around the small airways and alveolar walls, were identified during examination of all lung sections. No nodules or granulomata were observed. Collagen fibers were observed in the interstitial lesions. The α -quartz instillation resulted in multiple silicotic nodules, iron instillation did not produce any interstitial lesions (some alveolar macrophages with intracellular iron spheres were identified), and no alterations were observed in the animals that received PBS alone.

Short-Term Toxicity Studies

Parenteral

Kaolin

Nano-sized Kaolin (primary particle size 4.8 μ m) was instilled intratracheally in groups of 4 male *gpt* delta transgenic mice (a strain that allows for analysis of deletion mutations) as either a single dose of 0.2 mg/animal or multiple doses of 0.2 mg/animal/wk for 4 consecutive instillations.⁴⁰ Control mice received solvent alone intratracheally. The mice were killed at 12 wk after instillation (for a single dose) or 8 wk after the last instillation (for multiple doses). Tissues, particularly the lungs and kidneys, underwent histopathological examination. Kaolin-phagocytized alveolar macrophages were found, diffusely distributed in the lungs. Focal granulomatous formation, with or without phagocytized alveolar macrophages, were also frequently observed in the lungs of mice that received multiple instillations. Similar observations were made in the mice that received a single instillation, but with a slight degree of particle accumulation and granuloma formation in the lungs. No abnormalities were observed in the kidneys.

Subchronic Toxicity Studies

Oral

Montmorillonite

In a 90-d feed study, 10 male Wistar rats received a Montmorillonite clay (40 mg/kg/d; modified with hexadecyltrimethylammonium bromide) in the diet.⁴¹ Another 10 male rats received only standard diet as control. During the treatment period, clinical signs, body weight, and feed and water consumption were recorded weekly. At the end of the treatment period, the rats were fasted for 18 h before being killed. Histopathological examinations were performed, and liver, kidneys, lungs, spleen, brain, testes, gastrointestinal tract, and heart were weighed. Blood samples were obtained for analysis. No rats died during the treatment period and no remarkable clinical signs were observed. Body weight gains and feed and water consumption were comparable to controls. No significant changes were noted in clinical biochemistry, organ weights, or in the histopathological examinations when compared to controls.

Parenteral

Kaolin

Toxicity of some of the minerals present in coal-mine dust was examined in groups of 10 SPF Sprague-Dawley rats.¹ The rats were exposed over a period of 3 mo to 50 mg/animal intratracheal instillations of Kaolin. The following assessments were made: weight of the fresh lungs; macroscopic and microscopic lesions in the lungs; amount of collagen and dust present in the lungs; and calculation of the toxicity index from the amount of collagen formed per mg of dust. The weight of fresh lungs subjected to Kaolin was 1.76 g. Collagen formed per lung was 23.9 mg. The dust per lung was 30.2 mg and the collagen/dust ratio was 0.79. Microscopic examinations of the lungs showed no alveolar proteinosis but Kaolin was detected in the bronchovascular lymphoid sheaths. No information regarding nonexposed lungs was presented. The opinion of the investigators was that exposure to Kaolin results in "pulmonary toxicity" and possesses "fibrogenic capacity."

Chronic Toxicity Studies

Oral

Montmorillonite

The potential toxicity of a naturally-occurring calcium Montmorillonite clay, an anti-caking agent in animal feed, was studied using groups of 10 male and 10 female Sprague-Dawley rats for 28 wk.⁴² The rats received the test material at 0, 0.25, 0.5, 1.0, or 2.0% w/w in their feed. Rats were observed daily for clinical signs and deaths. Feed consumption was recorded daily for the first month and then every fourth day. Body weights were measured weekly. At the treatment end, final body weights were recorded and blood was drawn for analysis. After the rats were killed, histopathological examinations were performed, and select organs were weighed. Total feed consumption, cumulative feed consumption, body weight, total body weight gain, and relative organ weights were not affected in either sex at any dose tested. No differences in relative organ weights or gross or histopathological changes compared to controls were observed. Non-dose-dependent significant changes were observed in mean corpuscular hemoglobin, serum calcium, serum vitamin A, and serum iron.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Kaolin

Groups of 12 Sprague-Dawley female rats were fed a control diet, 20% Kaolin diet, or iron-supplemented 20% Kaolin diet.¹ The diets were fed for 37 to 86 d, 69 to 85 d, and 96 to 117 d prior to fertilization. These same diets were fed for the duration of the gestation period. The animals fed the 20% Kaolin diet had significant reductions in hemoglobin, hematocrit, and red blood cell numbers, indicating maternal anemia. Significant reduction in the birth weight of the pups was observed. Animals fed the iron-supplemented diet maintained their hematocrit, hemoglobin, and red blood cell levels.

In a study of 12 fetal lambs and 6 fetal rhesus monkeys, sterile suspensions of 2% Kaolin in saline were injected into the cisterna magna.¹ Fetal lambs received 1 to 3 ml of Kaolin and fetal monkeys received 0.5 to 1.0 ml of Kaolin. After injection, the fetuses were replaced into the uterus. Prenatal ultrasound monitoring was used to document the progression of fetal ventriculomegaly. Cesarean sections were scheduled for gestation days 140 to 145 for the sheep and gestation days 160 to 165 for the monkeys. Newborn animals with gross head enlargement were killed 2 h after birth and necropsy was performed. Brains were sectioned for gross and microscopic examination. Five lambs and 1 monkey underwent ventriculoamniotic shunting at 120 d after gestation. Ventricular dilatation was apparent at 1 wk following Kaolin injections. The cerebral mantle was markedly thinned, with relative preservation of the cortex and severe attenuation of the white matter. The average cortical thickness of the cingulate gyrus in the Kaolin-injected sheep was 716 μ m compared to 1225 μ m in control animals. The corpus callosum was an average of 125 μ m in thickness in the sheep compared to 475 μ m in control animals. Microscopic examination found the cortical neurons well preserved and contained the complexity and density of neural processes. A mild-to-moderate fibrotic reaction and inflammatory cell response along the basal meninges was apparent. A large number of macrophages containing Kaolin infiltrated the subarachnoid space. In 5 fetuses, Kaolin was injected mistakenly into either the epidural tissues superficial to the cisterna magna or into the cervical musculature. None of these fetuses had hydrocephalus at birth.

GENOTOXICITY STUDIES

In vitro and in vivo genotoxicity studies summarized here are described in Table 4.

Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; 5000 µg) was not mutagenic in an Ames test, with or without metabolic activation.³⁸ Unmodified Montmorillonite clay (at up to 125 µg/ml) and one type of cation-exchanged (hexadecyltrimethyl-ammonium) montmorillonite clay (at up to 250 µg/ml) also were not mutagenic in an Ames test with or without metabolic activation, but significant increases in revertant colonies were observed in one strain with metabolic activation in 2 other cation-exchanged montmorillonite clays.⁴³ No mutagenic activity was observed in a *Salmonella*/microsome assay with and without metabolic activation when tested in Montmorillonite and cation-exchanged montmorillonite in both nano- and non-nano-sized material at up to 141 µg/ml and up to 14.1 µg/plate, respectively.⁴⁴ However, the cation-exchanged montmorillonite material (both nano- and non-nano-sized, at up to 226 µg/ml and 170 µg/ml, respectively) in this study was genotoxic in a concentration-related manner in a Comet assay with Caco-2 cells. The natural clay was not considered genotoxic. Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cell cultures when tested at up to 5000 µg/ml, with or without metabolic activation.³⁸ Micronucleus induction was observed in a dose-dependent manner to micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, with fine particles having a higher genotoxic potency than coarse particles.⁴⁵ A 4-fold increased frequency of micronucleated cells was observed in human lung cancer A549 cells following exposure to nano-sized Kaolin.⁴⁰ Statistically significant increases in the frequency of micronuclei were induced by Montmorillonite clay at 62.5 µg/ml in a cytokinesis block micronucleus cytome assay in human hepatoma cell lines, but this effect was not observed at a concentration of 31.25 µg/ml or lower.⁴⁶ No effects in nucleoplasmic bridges or nuclear buds were observed at any concentration in this study. In an in vitro micronucleus assay and kinetochore analysis using human lung fibroblasts, the genotoxic potential of Bentonite at up to 15 µg/cm² was determined to be generally low, but could be altered by the content of quartz and available transition metals.⁴⁷ In an in vitro Comet assay with micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, the test materials promoted DNA damage in a dose-dependent manner, and the particles that were 200 nm had a higher DNA-damaging potency than those that were 4.8 µm.⁴⁵

In an in vivo Comet assay with nano-sized Kaolin intratracheally instilled in mice, DNA damage was induced with 0.2 mg/mouse, but not with 0.05 mg/mouse, after a 3-h exposure. No difference in induction was observed after the 24 h exposure compared to the 3 h exposure.⁴⁰ Increased *gpt* and *Spi*⁻ mutant frequencies were observed in the lungs of the mice following intratracheal instillation with either single or multiple doses of 0.2 mg nano-sized Kaolin. A mutation spectra analysis showed > 60% of G:C to C:G transversion occurred in the *gpt* genes. In another Comet assay, rats were given 2 oral doses of up to 1000 mg/kg bw cation-exchanged montmorillonite clay.⁴⁸ There was no statistically significant difference in % tail DNA between the negative controls and the different treatment groups for any of the cells (liver, kidneys, colon) tested.

Attapulgit

In 2 studies looking at unscheduled DNA synthesis (UDS) in rat hepatocytes, Attapulgit did not cause a significant increase in DNA-specific activity at up to 10 µg/ml with no cytotoxicity.¹ However, in another UDS study using rat pleural mesothelial cells, Attapulgit tested at 2, 4, or 10 µg/cm² produced a significant increase in UDS and inhibited cell growth at 10 µg/cm². Attapulgit did not induce point mutations in a third DNA study.

Hectorite

In the Salmonella typhimurium LT2 spot test (TA98, TA100, TA1535, TA1537, and TA1538) with or without metabolic activation, Hectorite was found to be non-mutagenic.¹ In primary hepatocyte cultures, the addition of Attapulgit at 10 µg/cm² caused significant increases in UDS in rat pleural mesothelial cells.

CARCINOGENICITY STUDIES

The International Agency for Research on Cancer (IARC) has determined there is inadequate evidence in humans for the carcinogenicity of Attapulgit (IARC uses the mineralogical term “palygorskite” for Attapulgit).⁴⁹ Further, IARC has determined there is insufficient evidence in experimental animals for the carcinogenicity of short Attapulgit fibers (< 5 µm); however, there is sufficient evidence in experimental animals for the carcinogenicity of long Attapulgit fibers (> 5 µm). Overall, long Attapulgit fibers (> 5 µm) are possibly carcinogenic to humans (Group 2B) and short Attapulgit fibers (< 5 µm) cannot be classified as to their carcinogenicity to humans (Group 3).

Attapulgit (palygorskite fibers > 5 µm in length) is listed by California Proposition 65 as a carcinogen.⁵⁰

Inhalation

Attapulgit

In a rat inhalation study, groups of 40 (20 male and 20 female) SPF Fischer rats were exposed to samples of Attapulgit dust mined in Lebrija or Leicester in inhalation chambers at a concentration of 10 mg/m³ for 6 h/d for 5 d/wk until they were killed.¹ Negative and positive control groups received Kaolin and crocidolite, respectively, at 10 mg/m³. Four

animals were killed at 3, 6, and 12 mo, and the remaining rats were allowed to live their life span. All animals were subject to necropsy; the lungs, liver, spleen, kidneys, and other relevant organs were examined microscopically. At microscopic examination, 1 peritoneal mesothelioma, 1 adenocarcinoma, and 3 bronchoalveolar hyperplasia were found in rats treated with Lebrija Attapulgit. Thirty-five rats had no proliferative changes. In rats treated with Leicester Attapulgit, proliferative lesions observed included 2 mesothelioma, 1 peritoneal mesothelioma, 1 malignant alveolar neoplasm, 2 benign alveolar neoplasms, and 8 bronchoalveolar hyperplasias. Twenty-seven rats had no proliferative lesions. Rats exposed to the negative-control Kaolin had 2 bronchoalveolar hyperplasias. Rats in the positive-control crocidolite group had 1 adenocarcinoma and 3 bronchoalveolar hyperplasias.

Kaolin

Kaolin was reported to be the negative control in the above rat inhalation study.¹ The rats received 10 mg/m³ Kaolin for 6 h/d for 5 d/wk. Two bronchoalveolar hyperplasias were reported.

Parenteral

Attapulgit

In an intratracheal study, groups of 5 rats received a single instillation of Attapulgit at 1, 5, and 10 mg.¹ One month after treatment, bronchoalveolar lavage and microscopic examination of the lungs were performed. The average length of the fibers was 0.8 μ m, and 100% of the fibers were less than 3 μ m. Every test animal had type A lesions, which are characterized by an accumulation of inflammatory cells, mostly macrophages, and epithelioid cells around fiber deposits. These inflammatory cells form a compact cellular infiltrate at the periphery of the deposits and some are focally dispersed throughout the alveolar region. The bronchoalveolar lavage (BAL) had mostly macrophages and a small number of neutrophils at 5- and 10-mg doses. At the 5-mg dose, 3.6% of the cells were lymphocytes.

Two groups of 30 to 50 female Osbourne-Mendel rats received a single direct application to the left pleural surface by open thoracotomy of 40 mg of 1 of 2 Attapulgit samples.¹ The samples were 90% pure with quartz being the other component. One dose consisted of fibers > 4 μ m and the other contained no fibers > 4 μ m. The rats were killed at the end of 2 yr. Pleural sarcomas were seen in 2/29 rats. The incidences of pleural sarcomas in the untreated groups were 3/491 and 17/615 of the rats receiving the pleural implants of Attapulgit. Of rats receiving the positive control, crocidolite, 14/29 developed pleural mesotheliomas.

Attapulgit (20 mg/ml of 0.9% sodium chloride) was injected into the pleural cavities of 36 Sprague-Dawley rats.¹ The median fiber length was 0.77 μ m. Two control groups, untreated and saline-injected, were utilized. Necropsy was performed after the rats died or killed when moribund. No mesothelial neoplasms were found in either controls or in rats treated with Attapulgit. Survival times between the Attapulgit-treated group and the controls were not statistically different.

Attapulgit was injected intrapleurally as a single dose of 0.5, 2, 4, 8, 16, or 32 mg into 6 groups of 25 Fischer 344 rats.¹ Nearly all the fibers were < 1 μ m in length. Mesotheliomas were present in 2/140 treated rats compared to 1/79 incidences in control groups. The median life span was 839 days for Attapulgit-treated animals and 729 days for nontreated animals.

In another intrapleural study, injections of 20 mg of different Attapulgit fiber samples in 1 ml of saline were given to 2-mo-old Sprague-Dawley rats.¹ The control group received only a saline injection. All rats were allowed to live full life span. The mean length of Attapulgit fibers in this experiment was 0.77 μ m. The number of groups were not reported; however, 36 rats were reported to comprise each group. Pulmonary and thoracic neoplasms were fixed and processed for histopathological examination. The survival time of the treated groups (788 $\pm$ 155 days) was very similar to that of the control groups (809 $\pm$ 110 days). The incidence of mesothelioma was 0% for control groups and treated groups. The researchers concluded Attapulgit was not carcinogenic in this study.

Samples of Attapulgit from Lebrija, Torrejon, and Leicester were injected intrapleurally as a single injection in groups of 20 male and 20 female SPF Fischer rats.¹ Concentrations were not reported; however, fiber length was reported as < 2 μ m, for Lebrija Attapulgit, at most 0.54% > 6 μ m for Torrejon Attapulgit, and 19% > 6 μ m for Leicester Attapulgit. Kaolin and saline were used as negative controls, and crocidolite was used as a positive control. The animals were allowed to live their life span but were killed if they appeared distressed. Upon death, necropsy and microscopic examination of tissue were performed. Dust extraction was obtained from granulomas removed from the diaphragm or mediastinal tissue. Mesotheliomas were reported in 2, 14, 30, and 34 rats for Lebrija Attapulgit, Torrejon Attapulgit, Leicester Attapulgit, and crocidolite, respectively. In the negative controls, no mesotheliomas were reported for the Kaolin and 1 mesothelioma was reported for the saline group. Lebrija Attapulgit dust extracted from the lung had fibers < 2 μ m. Material examined from Torrejon Attapulgit was fibrous and had fiber length up to 8 μ m. Leicester Attapulgit fibers from extracted lungs were up to 25 μ m. The investigators considered these fibers to be tumorigenic.

Three samples of 25 mg of Attapulgit dust were injected intraperitoneally into 40 Wistar rats.¹ Electron microscopy of the sample revealed 37.5% of fibers < 2 μ m long and 70.0% < 5 μ m. All animals were observed until they died either

spontaneously or were killed. Saline was injected into 80 control animals. The time required to produce the first tumor in the rats was 257 days and the tumor incidence rate was 65%.

In a carcinogenicity study conducted with 3 samples of Attapulgit labeled Georgia, Lebrija, and Morimoiron, female Wistar rats (112, 115, and 114 per sample type, respectively) were injected intraperitoneally.¹ Each sample was injected 1/wk for 9 wk at 60 mg per injection. Fiber dimensions for each of the samples Morimoiron, Georgia, and Lebrija were as follows: <50% fiber length was 0.7, 0.5, and 0.8 μm , respectively and <50% fiber diameter of 0.07, 0.07, and 0.04 μm , respectively. Some rats died spontaneously or others in poor health were killed. Surviving animals were killed 2.5 yr after treatment for necropsy. At necropsy, neoplasms or organs with suspected neoplasm tissue were fixed for microscopic examination. The percentage of rats with tumors were 3.5%, 3.5%, and 3.6% for the Morimoiron, Lebrija, and Georgia samples, respectively. These 3 samples were determined to be noncarcinogenic.

In another experiment by the same investigators, a fourth sample of Attapulgit from Caceres was tested in 30 rats.¹ Intraperitoneal injections of 2, 4, and 4 mg were administered consecutively for 3 wk. The fiber length and diameter of this sample were <50% 1.3 and 0.07 μm , respectively. Animals in poor health were killed. Surviving animals were killed 2.5 years after treatment for necropsy. At postmortem examination, parts of neoplasms or organs with suspected neoplasm tissue were fixed for microscopic examination. Forty percent of the rats had tumors. The results were considered moderate in relation to the dose.

Montmorillonite

Heat-treated Montmorillonite in doses of 5, 15, and 45 mg was given to groups of 4 Sprague-Dawley rats by intratracheal instillation.¹ Following a 3-mo postexposure period, the animals were killed and tissues were subjected to microscopic examination. The Montmorillonite particles were mainly restricted to alveoli within and adjacent to alveolar ducts regardless of dose. Most particles were contained within small to moderate numbers of pulmonary alveolar macrophages. However, some particles were free in alveoli. Adjacent alveoli septae were mildly thickened. Interstitial fibrosis was present in all groups. At the 5- and 15-mg doses, fibrosis was mild to moderate, multifocal, and loose, meaning less collagen. The 45-mg dose produced dense fibrosis. Macrophages contained clay particles and lymphocytes were present in the lesions. Occasionally giant multinucleate cells were seen.

OTHER RELEVANT STUDIES

Adsorption

Clay ingredients are reported to adsorb various chemicals, molecules, and microorganisms.¹ These compounds include, but are not limited to, strychnine, quinine, atropine, ampicillin, amoxycillin, *Agrobacterium radiobacter*, *Escherichia coli*, *Serratia marcescens*, *Bacillus species*, bacterial endotoxins and enterotoxins, and aflatoxins.

Cytotoxicity

Numerous studies with various cell lines on the cytotoxic and hemolytic effects of clay ingredients have been reported.¹ Results varied and may have been dependent on different factors, including mineral composition of the test materials.

Illite and Montmorillonite

The protective effect of Illite and Montmorillonite (up to 1 mg/ml each) on alterations in cell viability and epithelial barrier function induced by mycotoxins was evaluated using Caco-2 cells in a colorimetric 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and a lactate dehydrogenase (LDH) assay.⁵¹ Both clays provided protection against mycotoxin effects. Aflatoxin B1- and fumonisin B1-induced cytotoxicity were completely abolished by Illite. Decreases in the gene expression of specific claudin isoforms and the reduction of trans-epithelial electrical resistance of cell monolayers (an indicator of the epithelial barrier integrity) induced by mycotoxins were reversed by Illite. Montmorillonite also provided protection against mycotoxin effects, but at a lesser degree.

Illite, Montmorillonite, Kaolin

The cytotoxicity of an environmental dust sample (dust samples collected from a Mexican city) comprising approximately 75% Illite, Montmorillonite, and Kaolin, and approximately 20% α -quartz was studied in alveolar macrophages obtained from male albino Wistar rats.³⁹ LDH release was used as an indicator of cell membrane disruption. The alveolar macrophages were incubated with and without the dust (3.2 μm mean diameter) for up to 2 h. LDH was measured in the supernatant at 0, 1, and 2 h, with controls run in parallel. Rat alveolar macrophages incubated with the dust released increasing amount of LDH into the medium as a function of time. Significant levels above control values (2.2 ± 2.6 LDH U/l) were observed by 1 h (19.5 ± 2.6 LDH U/l) and 2 h (29.5 ± 4.0 LDH U/li) of incubation.

The hemolytic activity of the dust was also investigated by incubating different particle concentrations (0.1, 0.5, 1.0, 1.5, and 2.0 mg/ml) with a 0.6% suspension of human red blood cells obtained from normal donors. Cell-particle suspensions were incubated at 37°C in PBS for 1 h under moderate agitation. The results indicated the dust was highly hemolytic. An amount of 2 mg/ml produced $95 \pm 3\%$ hemolysis. The effect was observed starting at 1 mg/ml in a dose-related manner.

Kaolin

In a study examining the toxic mechanisms of typical fine particulate air pollution (PM_{2.5}), human bronchial epithelial (16HBE) cells were treated with nano-scale Kaolin at concentrations of 40 to 240 µg/ml.⁵² The particle size information was not available; however, the authors stated the nano-scale Kaolin utilized in the study was to imitate Kaolin in atmospheric fine particles (PM_{2.5}). Cytotoxicity results of the cell counting kit-8 (CCK-8) assay showed the 16HBE cells had high viability after exposure to 40 µg/ml nano-sized Kaolin, but cell viability decreased significantly at doses greater than 80 µg/ml. A lactate dehydrogenase assay indicated that nano-sized Kaolin caused membrane disruption in a dose-dependent manner.

Hemostatic Response

Bentonite

The ability for Bentonite (2/3 weight) and a zeolite (type not specified; 1/3 weight) to act as a hemostatic agent was studied in 12 male Sprague-Dawley rats.⁵³ Another 12 rats served as controls. Approximately 8 g of the material was applied on wounded skin. Wounds were circular, full-thickness and 2 cm in diameter; skin samples were excised and evaluated stereologically after scarification. On days 12 and 21, 6 rats from the test group and 6 rats from the control group were killed. At day 12 termination, a reduction in the length density of the blood vessels (31%) and diameter of the large and small vessels (38% and 16%, respectively) was observed in the rats that received the test material. At day 21 termination, volume density of both the dermis and collagen bundles was reduced by 25% in the treated rats when compared to the controls. The researchers concluded the hemostatic agent containing Bentonite may cause vasoconstriction and inhibition of neoangiogenesis.

Other Parenteral Studies

Attapulgit

In an intratracheal study, groups of 5 rats received a single instillation of Attapulgit at 1, 5, and 10 mg.¹ One month after treatment, BAL and microscopic examination of the lungs were performed. The average length of the fibers was 0.8 µm, and 100% of the fibers were less than 3 µm. Every test animal had type A lesions. Type A lesions are characterized by an accumulation of inflammatory cells mostly macrophages, and epithelioid cells around fiber deposits. These inflammatory cells form a compact cellular infiltrate at the periphery of the deposits and some are focally dispersed throughout the alveolar region. The BAL had mostly macrophages and a small number of neutrophils at 5 and 10 mg doses. At the 5 mg dose, 3.6% of the cells were lymphocytes.

Groups of 5 male Wistar rats received 1, 5, or 10 mg of Attapulgit by transtracheal injection to examine alveolar macrophage production of interleukin-1 (IL-1) and macrophages-derived growth factor (MDGF) from fibroblasts.¹ Saline and chrysotile B asbestos were used as controls. At 1 mo, Attapulgit produced granulomas and the chrysotile B produced fibrosis. At 8 mo, the granulomatous reactions had either resolved or were greatly diminished, whereas the fibrosis persisted. Cells obtained by BAL included multinucleated giant macrophages in animals treated with Attapulgit, but not in those treated with chrysotile B. Enhanced production of IL-1 was seen in all treated groups. MDGF production was only seen in animals with lung fibrosis.

Attapulgit with a mean fiber length of 0.8 µm and diameter of 0.02 µm was delivered to the lungs of sheep by bronchoscopic cannulation.¹ The tracheal lobe of 16 sheep was subjected to a single exposure of 100 mg of Attapulgit in 100 ml of saline. A BAL was conducted at 2, 12, 24, 40, and 60 d, and necropsy was conducted on day 60. Total BAL cells, macrophages, and neutrophils, fibronectin content, and LDH and β-glucuronidase (β-GLUC) activity were examined. Nine samples of the tracheal lobe of the lung were obtained each time for microscopic examination. The controls were saline-exposed sheep and had no changes in BAL or pulmonary morphology. The total BAL cells/ml and subpopulations increased significantly above control numbers at days 12, 24, and 40 but returned to control levels by day 60. Albumin and procollagen III did not differ from controls, whereas fibronectin, LDH, and β-GLUC activities were significantly above the controls. Microscopic examination revealed infiltrates that were predominantly alveolar and peribronchial lesions. Macrophagic alveolitis with minimal airway distortion was seen. Three sheep had lesions of peribronchiolar alveolitis.

Bentonite

The ability of Bentonite to increase susceptibility to bacterial pneumonia was studied in mice.¹ The animals were injected intratracheally with 1, 10, or 100 µg Bentonite. In vivo bacterial-infectivity screening assays were conducted by exposing the animals to aerosolized Group C Streptococcus species. The severity of infection was calculated by recording the deaths of the mice over a 15-d period. Control animals were exposed to titanium dioxide. At the 100 µg dose, Bentonite increased the infectivity of the bacteria. Mortality was 85%. Even at 10 µg, Bentonite caused increased animal mortality (43.3%). Control dusts at 100 µg produced only a 5% mortality.

The effects of Bentonite dust in rats was analyzed in a 2-part intratracheal study.¹ A 0.5 mg dose of Bentonite with a mean size of 0.3 µm was instilled. Control animals were injected with sterile saline and titanium dioxide. Animals were killed at 1, 2, 6, 24, and 48 h and 4 and 7 d after instillation. Bronchopulmonary lavage was carried out and alveolar

macrophages and polymorphonuclear leukocytes were recovered. The activity of LDH and protein content of the lavage fluid were also determined. In the first experiment, a rapid influx of polymorphonuclear (PMN) leukocytes was detected at 6 h. PMN leukocyte response peaked at approximately 19×10^6 cells after instillation and started declining more slowly up to 4 d. At 7 d, the polymorphonuclear leukocyte numbers were 2.5×10^6 . The greatest increase in the numbers of alveolar macrophages recovered occurred at 4 and 7 d. The mean diameter of macrophages increased from 11.0 to 12.5 μm over the first 48 h after instillation. The mean diameter decreased at 4 and 7 d. LDH activity at 24 h was maintained at 40 milli units (mU)/ml and then increased (73 mU/ml) with the influx of polymorphonuclear leukocytes into the lungs after 48 h. Protein concentration was calculated at 500 $\mu\text{g}/\text{cm}^3$ for the first 24 h and was maintained for 48 h.

In the second experiment, after instillation of 5 mg of Bentonite, the animals were killed at 1, 7, 49, and 100 d.¹ In addition to the above measured parameters, peroxidase and lysozyme activity were also measured. A large number of polymorphonuclear leukocytes were recovered at day 1. However, the severity of the response did not differ significantly from the 0.5 mg dose. By 7 d, the numbers had decreased and were similar to control values. A significant decrease in the number of alveolar macrophages compared to controls was observed at 24 h after instillation. This decrease was followed by a sharp increase that exceeded control values by 7 d. Total number estimates were similar to those of the first experiment. LDH activity and protein concentration from Bentonite and titanium dioxide were very similar. The initial rise at day 1 following administration was short-lived. Peroxidase activity was minimal. Lysozyme activity rose sharply between 1 and 7 d, but returned to control values at 49 and 100 d.

In an intratracheal study, a single dose of 40 mg of Bentonite suspended in 1 ml of physiological saline containing 40,000 IU of crystalline penicillin was administered to male CFY rats.¹ The Bentonite composition consisted of 73% Montmorillonite, 18% cristobalite, 3% quartz, 3% feldspar, and 3% other minerals. Particle sizes were $< 2 \mu\text{m}$. The control group received 1 ml of physiological saline containing 40,000 IU of crystalline penicillin. Animals were killed 12, 24, 48, or 72 h or 90 d after exposure. Body and lung weights of the rats were measured. The right lung was fixed and sectioned for microscopic examination. The lipids and phospholipids were analyzed in the left lung. The body weights of the rats were moderately decreased and the lung weight increased 72 h after Bentonite exposure. After 90 d, the lung weight was only slightly greater than that of the control animals. Upon microscopic examination at 12 h, Bentonite exposure had resulted in a nonspecific inflammation of mostly neutrophils with perivascular edema, alveolitis, and incipient bronchopneumonia. A small number of macrophages and lymphocytes were detected. Dust particles were observed in the leukocytes and macrophages or extracellularly in the alveoli. After 24 h, bronchopneumonia was present after coalescence of the inflammatory foci; the pneumonia then became necrotizing and desquamative. Necrotic neutrophilic leukocytes and eosinophil leukocytes were observed. The reticular network collapsed between 48 and 72 h. After 90 d of exposure, Bentonite caused storage focal tissue reaction (large foamy cells with pale cytoplasm). Closely-packed cells with dark cytoplasm and nuclei were located at the periphery. After 12 and 24 h, the amount of lipids and phospholipids in the lungs was not altered. However, between 48 and 72 h, the lipid and phospholipid content increased but distribution remained the same. After 90 d, the value was the same as seen at 72 h.

In another study by the same research group, male CFY rats were given a single intratracheal dose of 60 mg of Bentonite in 1 ml of physiological saline containing 40,000 IU crystalline penicillin.¹ Bentonite particle size was less than 5 μm . Control groups received 1 ml physiological saline containing 40,000 IU penicillin. Animals were killed at the end of 72 h, weeks 2 and 4, and months 3, 6, and 12. The acid phosphatase activity and the progression of fibrosis were determined. The lungs were processed for microscopic examination and fibrosis determined by Belt and King's classification. Acid phosphatase activity was increased at 72 h and had returned to normal by the first month. Loose reticulin fibrils, but no collagen, were observed after months 1 - 12.

Bentonite dust was administered intratracheally as a single 60-mg dose to Sprague-Dawley rats.¹ The animals were killed 3, 6, and 12 mo after exposure. The right lung was studied microscopically and the lipids, phospholipids, and hydroxyproline values were determined. Significantly greater phospholipid values compared to controls were observed. Among the phospholipid fractions, the greatest quantitative increase was seen in phosphatidylcholine (more than twice the control) and the smallest increase was seen in phosphatidylethanolamine (less than 1.6 times). After 6 and 12 mo, the values were similar. Lung lipids had a greater range of values than did the phospholipids (no details given). The wet weight of the lung in grams increased in 5% to 10% Bentonite-treated rats compared to controls at month 3. No difference was detected at 6 and 12 mo. Hydroxyproline content of treated rats (mg/g lung wet weight) was very similar to controls at 3, 6, and 12 mo.

Subplantar injections of 0.05 ml of a 5% solution of Bentonite were given to male Wistar rats.¹ The rats either received Bentonite injections in both hind paws at an interval of 24 h, or their left paw was injected with Bentonite and their right paw injected with 0.05 ml of a 10% solution of Kaolin (control). Subcutaneous Bentonite granulomas were produced on the left side, both dorsally and ventrally. Simultaneously, Kaolin granulomas were produced on the right side analogous to the Bentonite injection. Sodium salicylate and prednisone suppressed the Bentonite edema during the first 24 h. The presence of mononuclear cells was confirmed.

Kaolin

The ability of Kaolin to increase susceptibility to bacterial pneumonia was studied in mice.¹ The animals were injected intratracheally with 100 µg Kaolin. In vivo bacterial infectivity screening assays were conducted by exposing the animals to aerosolized Group C Streptococcus species. The severity of infection was calculated by recording the deaths of the mice over a 15-d period. Control animals were exposed to titanium dioxide. A 100-µg dose of Kaolin caused statistically significant but modest (< 50%) increased death due to infection by a large dose. Mortality was calculated at 38.9%. Control dusts at 100 µg produced only a 5% increase in mortality.

DERMAL IRRITATION AND SENSITIZATION

In vitro, animal, and human dermal irritation, sensitization, and photoallergy studies are summarized in Table 5.

A formulation containing 38% Montmorillonite was predicted to be non-irritating in an EpiDerm™ skin model when tested neat.⁵⁴ Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) was not irritating to rabbit skin when tested at 500 mg in distilled water.³⁸ A formulation containing 1.75% Bentonite was not irritating to 25 human subjects in a 14-d cumulative irritation assay, nor was a mud mask containing 8% Bentonite irritating in a single-insult occlusive patch test in 19 subjects.^{55,56} No visible irritation was observed in a 4-wk clinical use test (50 subjects) of a facial cleanser containing 2% Bentonite and 2% Kaolin; however, some subjects reported perceived discomfort and/or irritation.⁵⁷

A formulation containing 38% Montmorillonite was predicted to be non-sensitizing in a KeratinoSens™ assay.⁵⁴ No sensitization was observed in guinea pig studies of 50% Hectorite (Buehler test, details not provided) or of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; intradermal induction at 5%; no further information on dosing for topical induction or challenge provided).^{11,38} Dermal sensitization was not reported in human repeated insult patch tests (HRIPTs) with a foot mask containing 3.5% Bentonite (102 subjects), a clay mask containing 3.8% Bentonite (108 subjects), or in a face cream containing 7.5% Bentonite (52 subjects).⁵⁸⁻⁶⁰ No sensitization was observed in HRIPTs with a lip product containing 14.5% Kaolin (54 subjects) or a clay mask containing 40% Kaolin (51 subjects); however, one subject in a study of a clay mask containing 14.5% Kaolin (103 subjects) had moderate erythema progressing to erythema and edema with papules through the induction and challenge phase.⁶¹⁻⁶³ A sunscreen with 1.75% Bentonite was not a photosensitizer in 23 human subjects.⁶⁴

Hectorite

A primary irritation study patterned after the Draize method was conducted using 6 white rabbits.¹ Either a 0.5 ml or a 0.5 g sample of Hectorite was applied to two sites, one on abraded skin, and the other on intact skin of the backs of the rabbits. The test sites were occluded for 24 h. At the end of the 24 h, the binders were removed and the sites were gently wiped clean. One-half hour later, the sites were examined and scored for erythema and edema. The sites were examined again at 72 h. The average score was 0.0. Hectorite was nonirritating to the skin of rabbits.

OCULAR IRRITATION STUDIES

Animal

Clay

In an ocular irritation study performed in accordance with OECD TG 405, 100 mg of Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite) was instilled into one eye of rabbits.³⁸ The other eye served as a control and was instilled with 0.1 ml normal saline. No adverse effects were noted following treatment up to 72 h after instillation. The test material was considered to be non-irritating to rabbit eyes. No further details were provided.

Hectorite

In a primary eye irritation study using 9 New Zealand white rabbits, a 0.1 ml liquid or semisolid (100 mg of the solid) sample was instilled into the one eye of each rabbit.¹ The eyes of 6 rabbits were not rinsed, and the eyes of 3 rabbits were rinsed approximately 4 s. All untreated eyes served as controls. The eyes were then examined with sodium fluorescein and an ultraviolet lamp at 24, 48, and 72 h and at 7 d. The mean score at 24 h was 2.0. All subsequent scores were 0.0. The test sample was considered moderately irritating to rabbit eyes without rinsing and practically nonirritating to the eyes with rinsing 4 s after instillation.

Kaolin

The potential for ocular irritation from a clay mask with 14.5% Kaolin was investigated in a tissue equivalent assay with EpiOcular™ cultures.⁶⁵ The EpiOcular™ human cell constructs were exposed to 100 µl test material under standard culture conditions for 4, 8, 16, and 24 h. Tissue viabilities were then examined by MTT assay. The duration of exposure resulting in a 50% decrease of tissue viability (t_{50}) was calculated to be 5.2 h (at 4 h, tissue viability was 63.4%). The positive control yielded expected results. As residual test article may bind to the tissue and result in a false MTT reduction signal, a freeze-killed tissue control was used, and calculations were performed to correct for the amount of MTT reduced directly by the test article residues in the tissues. The clay mask with 14.5% Kaolin was predicted not to be an ocular irritant in this assay.

CLINICAL STUDIES

In a study of total pulmonary non-asbestos mineral content in lung tissue from 20 individuals with no occupational dust exposure, Attapulgite and Kaolin were identified in 12 individuals.¹ No correlations were made between numbers or types of fibers and age, sex, or smoking. Approximately 8400 out of 106,000 fibers (7.9%) were identified as Attapulgite and approximately 3500 out of 106,000 fibers (3.3%) were identified as Kaolin. Mineralogical analysis found that 100% of the Attapulgite fibers and 94% of the Kaolin fibers were 1 - 4.9 μ m in length.

Oral

Montmorillonite

The effect of oral ingestion of Montmorillonite on protection against the adverse effects of the ingestion of aflatoxins were studied in 23 male and 27 female human subjects.⁶⁶ The subjects received 1.5 g/d or 3.0 g/d in capsules. A total of 9 capsules were ingested over a 2-wk period. The study was randomized and double-blinded. Blood and urine samples were collected before and after the study. Mild gastrointestinal effects were reported with no statistical significance found between the treatment groups. No significant differences in hematology, liver and kidney function, or electrolytes were reported in either group.

Case Reports

Bentonite

Several case studies involving Bentonite workers have been reported.¹ Some milling plants had dangerous concentrations of silica that ranged from 2 to 10 times the safe maximal concentration according to the US Bureau of Mines. Silicotuberculosis developed in four patients studied.

Fuller's Earth

A patient that reported working no more than 15 yr in a Fuller's Earth plant as a young man was diagnosed with terminal aspiration pneumonia, pneumoconiosis due to Fuller's Earth exposure, bilateral emphysema, and fibrous pleural adhesions.¹ The lesions differed from typical silicotic lesions of the lungs; no formations of the whorled, acellular collagen typical of silicotic nodules were observed. Isolated cavities in the apices were filled with black sludge and surrounded by vascular and cellular collagen. The dust in the lymph nodes had only stimulated the formation of reticulin fibers. No subpleural nodules were present. At mineralogical analysis, the Fuller's Earth deposits were constituted mainly of Montmorillonite (85.2% to 90%).

Two additional cases of pneumoconiosis in employees that worked in processing or milling Fuller's Earth for at least 28 yr were reported.¹

Kaolin

A patient was reported with multiple pulmonary Kaolin granulomas.¹ The man had a history of bilateral recurrent pneumothorax. Both pleural spaces were destroyed with a suspension of liquid Kaolin. Recurrent right-sided pneumothorax devolved and reobliteration was again performed. In a follow-up chest radiograph, multiple well-defined peripheral nodules were in both lungs and pathological analysis revealed a bland acellular material surrounded by chronic inflammatory cells. By light microscopy, the particles were consistent with Kaolin. It was presumed that Kaolin entered the lungs through pleuroalveolar or pleurobronchial openings.

In another investigation, the death of a 62-yr-old man who worked in a cotton textile mill for 43 yr was reported.¹ The patient complained of progressive dyspnea and a productive cough. After being admitted to the hospital, a bronchoscopy was performed and no endobronchial lesions were found. A lung biopsy had lesions of severe interstitial fibrosis with bronchioalveolar structures extensively involved in the fibrotic process. Pathological alterations such as bronchiectasis, interstitial fibrosis with thickening of alveolar septa, mobilization of macrophages, and multinucleated giant cells were identified. Neither ferruginous bodies nor pleural hyaline plaque was identified. Kaolin particles were present with a mean size of 0.88 μ m. Chrysotile asbestos was also detected, but the majority of particles were Kaolin. The man died as a consequence of respiratory failure despite an aggressive therapy of antibiotics and tuberculosis therapy.

The lungs and chest x-ray films were evaluated in a pair of case studies of men who worked in a Kaolin-processing plant for many years.¹ The first case was a 36-yr-old man who worked on the plant for 17 yr. Chest films were taken at the end of his career and detected lesions of extensive confluent consolidation and nodule formation of advanced pneumoconiosis with infection. Autopsy and microscopic findings included alveolar spaces uniformly expanded, three areas of whorled fibrous tissue, scattered areas of cystic spaces, hilar nodes heavily pigmented, deposits of brownish black particulate matter, a large vessel with recent thrombus, hemorrhage, and necrosis, marked fibrous thickening of the pleura, and dense fibrous scarring of the lymph nodes. The final diagnosis was pneumoconiosis (kaolinosi) with pulmonary thrombosis and infarction of the lungs. The second case study was a 35-yr-old man who worked in the Kaolin-processing plant for 21 yr. Within his last 3 yr, he had dyspnea and a slight cough with small amounts of dark colored sputum. The sputum was negative for bacteria. Chest films revealed advanced pneumoconiosis with infection, confluent consolidation, nodular infiltration, cavitation, and emphysema. Autopsy and microscopic findings included nodules in the right and middle

lobes, pleural spaces were thickened and shaggy, large bulbous emphysematous blebs, a pulmonary artery with organizing thrombus, heavily pigmented hilar lymph nodes, whorled fibrous collagenous tissue, and spaces and walls with macrophages. The final diagnosis was pneumoconiosis (kaolinosi).

A 35-yr-old man who worked at a Kaolin-processing plant for 17 yr presented with chest pain and was hospitalized.¹ For the previous 2 yr before admittance, the man had packaged dried, processed Kaolin. Chest films revealed diffuse reticulonodular pulmonary infiltrates and a well-defined, noncalcified mass in the upper right lobe. A thoracotomy was performed and an 8 cm x 12 cm x 10 cm conglomerate pneumoconiotic lesion containing large amounts of Kaolin was found. X-ray diffraction material from the lesion had peaks corresponding to Kaolinite. The presence of silica was not confirmed by x-ray diffraction.

Pulmonary tissue was obtained from 5 Kaolin workers with advanced pneumoconiosis.¹ Chest radiographs detected small irregular shadows and large opacities typical of Kaolin pneumoconiosis. At autopsy, firm, grey-brown nodules and masses were in the parenchyma and in the hilar lymph nodes. Microscopic lesions were extensive pulmonary Kaolinite deposition associated with the formation of peribronchiolar nodules. The nodules were composed of Kaolinite aggregates transversed by bands of fibrous tissue rather than dense whorled collagen. Kaolin was detected in the lungs. Silica was not detected by either analytical scanning electron microscopy or x-ray diffractometry.

Six additional cases of pneumoconiosis in employees of 12 yr in Kaolin processing or milling facilities were reported.¹

Montmorillonite

A 73-yr-old Montmorillonite worker developed signs of pneumoconiosis, but subsequently died of acute gastrointestinal hemorrhage from a benign gastric ulcer.¹ A chest radiograph taken 2 yr before his death showed a bilateral fine reticulonodular shadowing, while another radiograph taken a few weeks before his death indicated a slight increase in the reticulonodular opacities and a mass at the left hilum and apex. At autopsy, numerous soft stellate grey-black dust lesions 4 - 5 mm in diameter were observed occupying most of the lungs. No lesions of progressive massive fibrosis were identified. Also present were lesions of severe emphysema and a 4 cm diameter neoplasm arising from the bronchus of the left upper lobe. At microscopic examination, numerous interstitial collections of dust-laden macrophages were situated around the respiratory bronchioles and along the adjacent alveolar septa. A slight degree of fibrosis associated with the dust lesions was observed, and the neoplasm was a poorly differentiated adenocarcinoma containing giant cell areas. Mineralogical analysis showed a large amount of calcium Montmorillonite.

OCCUPATIONAL EXPOSURE

Attapulgit

A cohort of 2302 men employed for at least 1 mo between January 1, 1940 and December 31, 1975 at an Attapulgit mining and milling facility was studied.¹ A significant deficit of mortality from nonmalignant respiratory disease was observed based on age, calendar year, and rates. A marked deficit of nonmalignant respiratory disease was seen regardless of presumed dust exposure level, induction-latency period, or duration of employment. A statistically significant excess of mortality from lung cancer was observed among whites, but a deficit occurred among nonwhites. Lung-cancer risk in either race was not altered substantially with presumed dust exposure level, induction-latency period, or duration employed, with one exception—those employed for at least 5 yr in high-exposure level jobs. An increased mortality was observed for gastric cancer (6 observed) and a deficit due to nonmalignant respiratory disease was observed (9 observed).

Kaolin

A study was performed on the prevalence of ventilatory impairment, chest symptoms, and radiographic abnormalities in over 2000 Kaolin workers representing over 95% of the employees in the industry at the time.¹ Of the participants, 19% admitted having a cough. Of those participants with a cough, 17% had an abnormal forced expiratory volume and 14% had an abnormal vital capacity. Of those without a cough, 5.5% had an abnormal forced expiratory volume and 7% had an abnormal vital capacity. Also, 18% of the participants admitted to chronic sputum production. Of those with sputum production, 16% had abnormal forced expiratory volume, and 12.5% had abnormal vital capacity. Of those without sputum production, 6% had an abnormal forced expiratory volume, and 7.5% had an abnormal vital capacity. About 30% of the participants complained of shortness of breath, 3.1% of the cases were classified as severe. Wheezing was reported by 29% of the subjects. Satisfactory chest films for 2069 of the subjects were available for examination. Radiographic findings of 90 subjects revealed simple pneumoconiosis. Eighteen subjects (0.89%) had complicated pneumoconiosis. Of men with either case of pneumoconiosis, 51.1% were dry processors, compared to 6.3% of the men who worked in wet processing. Of the nonsmoking participants (549), 542 and 537 men had a satisfactory forced expiratory volume and forced vital capacity, respectively, in addition to an acceptable chest radiograph. Of these nonsmoking workers, 516 were studied for dust exposure and pulmonary function. Among the nonsmokers with no pneumoconiosis, those persons working in calcined clay had a greater prevalence of lung function abnormalities. This group had a significant increase in the risk of having an abnormal forced expiratory volume but tended to have less incidences of pneumoconiosis. In short, ventilatory impairment was related to the presence of complicated pneumoconiosis, employment in clay calcining, and cigarette smoking. Also work in dry processing was associated with a greater risk of developing pneumoconiosis.

The lungs of 62 recently deceased men between the years of 1968 to 1981 were taken for an assessment of the severity of lung disease.¹ Fifty-four of the 62 men worked with Kaolin or related kaolinized mineral stone. All the test subjects were employed in the mining industry. Chest radiographs were available for 39 of the 62 cases. Sections of lung tissue were examined microscopically for nodular and interstitial fibrosis and an overall grade ranging from 0 (none) to 3 (severe). Samples from 42 cases were analyzed for mineral content by x-ray diffraction and lung-dust concentrations. Radiographic lesions included 13 cases of progressive massive fibrosis and 22 cases of simple pneumoconiosis. Only four cases had no evidence of any disease. Nodular opacities tended to reflect a high quartz content, whereas high-Kaolin lung content had interstitial changes and irregular radiological changes. An increasing quartz concentration appears to be related to nodular fibrosis. The degree of interstitial fibrosis appeared to be more related to dust lung concentrations, although these results failed to reach statistical significance.

The Occupational Safety and Health Administration (OSHA) lists the following permissible exposure limit (PEL) for 8 h work shifts for Kaolin: total dust - 15 mg/m³ and respirable fraction - 5 mg/m³.^{67,68} The National Institute for Occupational Safety and Health (NIOSH) lists the following recommended exposure limit (REL) for up to 10 h time weighted average for Kaolin: total dust - 10 mg/m³ and respirable fraction - 5 mg/m³.

Bentonite

In a toxicological and occupational epidemiological review of Bentonite, the authors concluded Bentonite is probably not more toxic than any other inert insoluble dusts.⁶⁹ However, because some forms may contain variable amounts of respirable crystalline silica, prudent management and adherence to occupational exposure limits is appropriate.

SUMMARY

This report assesses the safety of 8 clay ingredients as used in cosmetics. All of these ingredients are reported to function as absorbents and bulking agents; other cosmetic functions are also reported. The Panel previously reviewed the safety of Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, and Montmorillonite in a report that was published in 2003. In that report, the Panel concluded that these ingredients were safe as used in cosmetic ingredients. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment has been issued.

According to 2023 VCRP survey data, Kaolin has the most reported uses in cosmetic products, with a total of 787; the majority of uses are in leave-on formulations. Bentonite has the second most reported uses in cosmetic products, with a total of 221; a little more than half are reported in leave-on formulations. The frequencies of use for both of these ingredients have greatly changed since the original safety assessment was finalized; in 1998, Kaolin was reported to have 509 uses and Bentonite was reported to have 94. The results of concentration of use surveys conducted by the Council in 2022 indicate Kaolin also has the highest maximum concentration of use in leave-on formulations; it is used at up to 53.2% in manicuring preparations. For leave-on dermal preparations, specifically, Kaolin also has the highest reported maximum concentration of use, at 16% in face and neck products, and Bentonite has the next highest, at 8% in face and neck preparations. According to the original safety assessment, the maximum leave-on use concentration in 1999 for Kaolin was 100% in skin care preparations; the maximum leave-on use concentration for Bentonite was 8% in makeup foundations.

Several of these clay ingredients are GRAS as direct and/or indirect food additives. Kaolin is also an approved OTC drug.

Ex vitro bioavailability studies were performed using human skin models. Trace heavy metals in 3 clay pastes (white Montmorillonite, Kaolin, and Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite)) did not penetrate cutaneous tissue.

In acute dermal and oral toxicity studies in rats, Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) had an LD₅₀ greater than 2000 mg/kg. The same product was tested in an acute inhalation study in rats and had an LC₅₀ greater than 3.856 mg/l. Inhalation studies in rats with an environmental dust sample composed of approximately 75% of Illite, Montmorillonite, and Kaolin and approximately 20% α -quartz demonstrated that the majority of particles were deposited at the first alveolar duct bifurcations, and at 24-h later, numerous particles had been ingested by alveolar macrophages. Rats instilled intratracheally with the same dust developed a multifocal interstitial lung disease.

Nano-sized Kaolin (primary particle size 4.8 μ m) instilled intratracheally in mice (single and multiple (4) instillations) produced diffuse alveolar macrophages containing Kaolin in the lungs. Focal granulomatous formation, with or without alveolar macrophages containing Kaolin, were also frequently observed in the lungs of mice that received multiple instillations. Similar observations were made in mice that received a single instillation, but with a slight degree of particle accumulation and granuloma formation in the lungs. No abnormalities were observed in the kidneys.

In a 90-d oral study in male rats, a modified Montmorillonite clay at 40 mg/kg/d did not cause any deaths during treatment, and no significant changes were noted in clinical biochemistry, organ weights, or in histopathological examinations when compared to controls. As an anti-caking agent in animal feed, calcium Montmorillonite clay produced non-dose-dependent significant changes in mean corpuscular hemoglobin, serum calcium, serum vitamin A, and serum iron when tested at up to 2.0% w/w in rats in a dietary study; however, no adverse effects were noted in feed consumption, body weight, organ weights, or in gross or histopathological exams.

Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; 5000 µg) was not mutagenic in an Ames test, with or without metabolic activation. Unmodified Montmorillonite clay (at up to 125 µg/ml) and one type of cation-exchanged Montmorillonite clay (at up to 250 µg/ml) also were not mutagenic in an Ames test with or without metabolic activation, but significant increases in revertant colonies were observed in one strain with metabolic activation in 2 other cation-exchanged Montmorillonite clays. No mutagenic activity was observed in a *Salmonella*/microsome assay with and without metabolic activation when tested in Montmorillonite and cation-exchanged montmorillonite in both nano- and non-nano-sized material at up to 141 µg/ml and up to 14.1 µg/plate, respectively. However, the cation-exchanged Montmorillonite material (both nano- and non-nano-sized, at up to 226 µg/ml and 170 µg/ml, respectively) in this study was genotoxic in a concentration-related manner in a Comet assay with Caco-2 cells. Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cell cultures when tested at up to 5000 µg/ml, with or without metabolic activation. Micronucleus induction was observed in a dose-dependent manner to micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, with fine particles having a higher genotoxic potency than coarse particles. A 4-fold increased frequency of micronucleated cells was observed in human lung cancer A549 cells following exposure to nano-sized Kaolin. Statistically significant increases in the frequency of micronuclei were induced by Montmorillonite clay at 62.5 µg/ml in a cytokinesis block micronucleus cytome assay in human hepatoma cell lines, but this effect was not observed at a concentration of 31.25 µg/ml or lower. No effects in nucleoplasmic bridges or nuclear buds were observed at any concentration in this study. In an in vitro micronucleus assay and kinetochore analysis using human lung fibroblasts, the genotoxic potential of Bentonite at up to 15 µg/cm² was determined to be generally low, but could be altered by the content of quartz and available transition metals. In an in vitro Comet assay with micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, the test materials promoted DNA damage in a dose-dependent manner, with greater DNA-damaging potency in the nano-sized Kaolin than in the micro-sized Kaolin.

In an in vivo Comet assay with nano-sized Kaolin intratracheally instilled in mice, DNA damage was induced at 0.2 mg/mouse but not at 0.05 mg/mouse after 3 h exposure. No difference in induction was observed after 24 h exposure compared to the 3 h exposure. Increased *gpt* and Spi- mutant frequencies were observed in the lungs of the mice following intratracheal instillation with either single or multiple doses of 0.2 mg nano-sized Kaolin. A mutation spectra analysis showed > 60% of G:C to C:G transversion occurred in the *gpt* genes. In another Comet assay, rats were given 2 oral doses of up to 1000 mg/kg bw cation-exchanged montmorillonite clay. There was no statistically significant difference in % tail DNA between the negative controls and the different treatment groups for any of the cells (liver, kidneys, colon) tested.

IARC has determined there is inadequate evidence in humans for the carcinogenicity of Attapulgite. Further, IARC has determined there is insufficient evidence in experimental animals for the carcinogenicity of short Attapulgite fibers (< 5 µm); however, there is sufficient evidence in experimental animals for the carcinogenicity of long Attapulgite fibers (> 5 µm). Overall, long Attapulgite fibers (> 5 µm) are possibly carcinogenic to humans (Group 2B) and short Attapulgite fibers (< 5 µm) cannot be classified as to its carcinogenicity to humans (Group 3). Attapulgite (palygorskite fibers > 5 µm in length) is listed by California Proposition 65 as a carcinogen.

The ability for Bentonite (2/3 weight) and a zeolite (type not specified; 1/3 weight) to act as a hemostatic agent was studied in 12 male Sprague-Dawley rats. This hemostatic agent may cause vasoconstriction and inhibition of neoangiogenesis. Illite and Montmorillonite were observed to have protective effects on cytotoxicity induced by mycotoxins in MTT and LDH assays. An environmental dust sample composed of approximately 75% of Illite, Montmorillonite, and Kaolin and approximately 20% α-quartz induced LDH release from alveolar macrophages of rats, and showed hemolytic effects on human red blood cells. Cytotoxicity results of the CCK-8 assay showed the 16HBE cells had high viability after exposure to 40 µg/ml nano-sized Kaolin, but cell viability decreased significantly at doses greater than 80 µg/ml. A LDH assay indicated that nano-sized Kaolin caused membrane disruption in a dose-dependent manner.

A formulation containing 38% Montmorillonite was predicted to be non-irritating in an EpiDerm™ skin model when tested neat. Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) was not irritating to rabbit skin when tested at 500 mg in distilled water. A formulation containing 1.75% Bentonite was not irritating to 25 human subjects in a 14-d cumulative irritation assay, nor was a mud mask containing 8% Bentonite irritating in a single-insult patch test in 19 subjects. No visible irritation was observed in a 4-wk clinical use test (50 subjects) of a facial cleanser containing 2% Bentonite and 2% Kaolin; however, some subjects reported perceived discomfort and/or irritation.

A formulation containing 38% Montmorillonite was predicted to be non-sensitizing in a KeratinoSens™ assay. No sensitization was observed in guinea pig studies of 50% Hectorite (further dosing information not provided) or Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; intradermal induction at 5%, no further information on dosing for topical induction or challenge provided). Dermal sensitization was not reported in HRIPTs with a foot mask containing 3.5% Bentonite (102 subjects), a clay mask containing 3.8% Bentonite (108 subjects), or in a face cream containing 7.5% Bentonite (52 subjects). No sensitization was observed in HRIPTs with a lip product containing 14.5% Kaolin (54 subjects) or a clay mask containing 40% Kaolin (51 subjects); however, one subject in a study of a clay mask containing 14.5% Kaolin (103 subjects) had moderate erythema progressing to erythema and edema with papules through the induction and challenge phase. A sunscreen with 1.75% Bentonite was not a photosensitizer in 23 human subjects.

A clay mask with 14.5% Kaolin was predicted to not be an ocular irritant in a tissue equivalent assay with EpiOcular™. In an ocular irritation study in rabbits, Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) produced no adverse effects and was considered to be non-irritating.

Nine capsules containing Montmorillonite (up to 3.0 g/d) were administered to 50 human subjects over a 2-wk period. Only mild gastrointestinal effects were reported.

OSHA lists the following PEL for 8-h work shifts for Kaolin: total dust - 15 mg/m³ and respirable fraction - 5 mg/m³. NIOSH lists the following REL for up to 10-h time weighted average for Kaolin: total dust - 10 mg/m³ and respirable fraction - 5 mg/m³. In a toxicological and epidemiological review of Bentonite, the authors concluded Bentonite is probably not more toxic than any other particulate. However, because some forms may contain variable amounts of respirable crystalline Silica, prudent management and adherence to occupational exposure limits is appropriate.

DISCUSSION

In 2003, the Panel published a final report that included Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, and Montmorillonite, and concluded that the ingredients named in that report were safe as used in cosmetic products. In accordance with its Procedures, the Panel re-evaluates the conclusions of previously-issued reports approximately every 15 years, and when appropriate, additional ingredients are included in the resulting re-review. Accordingly, the Panel re-reviewed the safety of these 6 clays, with the addition of 2 related ingredients (Clay and Illite), and concluded that the available data are sufficient to determine that Kaolin is safe in cosmetics in the present practices of use and concentration as described in this safety assessment. Furthermore, the Panel concluded that the remaining 7 naturally-sourced clay ingredients are safe in cosmetics in the present practices of use and concentration, except for those products that may be incidentally inhaled, for which the available data are insufficient.

The Panel expressed concern regarding heavy metals that may be present in these ingredients. Heavy metals associated with clay ingredients did not penetrate the skin. The Panel stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit these impurities in cosmetic formulations.

The Panel was made aware that nanoforms of clay ingredients could potentially be used in cosmetic formulations, including those that could result in incidental ingestion (e.g., lipstick and toothpaste; categories of sprayable products were not reported based on current available data). However, use of nanoform ingredients is unlikely to translate into nanoparticle form within final formulations under in-use conditions (or under in-use exposure scenarios). In these formulations, low concentrations of use (e.g., maximum reported use concentration of Kaolin in lipstick is 14.5%) would limit exposure, and in addition, processing would be expected to result in much larger particle sizes (by, for example, agglomeration) in the consumer product.

Additionally, some naturally-sourced clay ingredients were reported to be used in spray and powder products that could possibly be inhaled. For example, Bentonite is reported to be used at 0.9% in spray suntan products and Kaolin is reported to be used at up to 15% in face powders. For Kaolin, the data available from inhalation studies, including acute, chronic, and carcinogenicity data, suggest little potential for adverse respiratory effects at relevant doses for this naturally-sourced clay ingredient. These data have mitigated the concern of the use of Kaolin in cosmetic products which may be incidentally inhaled.

Conversely, the data are insufficient to determine the safety of the remaining 7 naturally-sourced clays for use in formulations which may be incidentally inhaled. The Panel noted that Bentonite and Hectorite may contain crystalline silica (cristobalite), which is a human carcinogen, as an impurity. Furthermore, the Panel noted that Attapulgite with long fibers (> 5 µm) is possibly carcinogenic to humans and animals. The additional data needed to determine safety of these 7 ingredients for such use are composition and impurities data, especially quantification of crystalline silica, and negative repeated-dose inhalation data on naturally-sourced clay ingredients.

The Panel's respiratory exposure resource document (<https://www.cir-safety.org/cir-findings>) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Kaolin is safe in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel also concluded that the following 7 ingredients are safe in cosmetics in the present practice of use and concentration described in this safety assessment, with the exception that the available data are insufficient to make a determination of safety for these ingredients in products that may be incidentally inhaled.

Attapulgite
Bentonite
Clay
Fuller's Earth

Hectorite
Illite
Montmorillonite

TABLES

Table 1. Definitions and reported cosmetic functions of the ingredients in this safety assessment.²

Ingredient, CAS No.	Definition	Reported Functions
Attapulgit 12174-11-7 1337-76-4	Attapulgit is a variety of Fuller's Earth found typically near Attapulgas, Georgia. It is characterized by having a chain structure rather than the usual sheet structure of other clay minerals.	Abrasives; Absorbents; Bulking Agents; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Bentonite 1302-78-9	Bentonite is a native hydrated colloidal aluminum silicate clay.	Absorbents; Bulking Agents; Dispersing Agents - Nonsurfactant; Emulsion Stabilizers; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Clay 53801-44-8	Clay is a group of phyllosilicate minerals produced by the chemical and physical weathering of rock. It consists chiefly of varying amounts of hydrated silica and alumina, and is characterized by a particle size of less than 2 micrometers.	Absorbents; Binders; Bulking Agents; Skin-Conditioning Agents - Misc.; Viscosity Increasing Agents - Aqueous
Fuller's Earth 8031-18-3	Fuller's Earth is a non-plastic variety of kaolin containing an aluminum magnesium silicate.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents
Hectorite 12173-47-6 68084-71-9	Hectorite is one of the montmorillonite minerals that are the principal constituents of bentonite clay.	Absorbents; Bulking Agents; Dispersing Agents - Nonsurfactant; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Illite 12173-60-3	Illite refers to a group of clay sized micas that have a higher lattice water content and lower potassium content than mica.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents
Kaolin 1332-58-7	Kaolin is a native hydrated aluminum silicate with an approximate composition of $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Skin Protectants; Slip Modifiers
Montmorillonite 1318-93-0	Montmorillonite is a complex aluminum/magnesium silicate clay.	Abrasives; Absorbents; Bulking Agents; Emulsion Stabilizers; Opacifying Agents; Viscosity Increasing Agents - Aqueous

Table 2. Chemical properties

Property	Value	Reference
<i>Attapulgite</i>		
Physical Form	White, gray, or transparent, dull, elongated, lath-shaped crystals in bundles that comprise thin sheets of minute interlaced fibers; surface is protonated and hydrated	1
Chemical Formula	$\text{Mg}(\text{Al}_{0.5-1}\text{Fe}_{0.5})\text{Si}_4\text{O}_{10}(\text{OH}) \cdot 4\text{H}_2\text{O}$	1
Density (g/ml)	2.2	1
Solubility	Insoluble in water	1
Particle Size/Length (μm)	< 5	1
<i>Bentonite</i>		
Physical Form	Crystalline, claylike material, available as an odorless, pale buff or cream to grayish-colored fine powder, which is free from grit; dioctahedral	1
Chemical Formula	$\text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot 2\text{H}_2\text{O}$	1
Formula Weight (Da)	359.16	1
Solubility	Insoluble in water, alcohol, fixed oils, glycerin, dilute acid, and alkali solutions	1,7
Particle Size/Length (μm)	Mainly 50-150 with some 1-2	1
<i>Clay</i>		
Physical Form	Beige green powder	9
pH	Between 8 and 9	9
Grain size	Variation 1: 80% < 5 μm ; 100% < 10 μm Variation 2: 90% < 20 μm ; 100% < 40 μm Variation 3: 90% < 77 μm ; 100% < 100 μm Variation 4: 90% < 750 μm ; 100% < 1100 μm	9
<i>Fuller's Earth</i>		
Physical Form	Non-plastic variety of Kaolin; sheet structure	1
<i>Hectorite</i>		
Physical Form	Translucent colorless mineral when mined and turns white when dried; tridecahedral	1
Chemical Formula	$\text{Na}_{0.33}(\text{Mg}_{2.67}\text{Li}_{0.33})\text{Si}_4\text{O}_{10}(\text{OH})_2$	1
Specific Gravity (g/ml)	2.65	1
<i>Illite</i>		
Physical Form	Gray-white to silvery-white, greenish-gray claylike material; waxy, greasy, earthy, or dull luster; dioctahedral	8
Chemical Formula	$\text{K}_{0.65}\text{Al}_{2.0}[\text{Al}_{0.65}\text{Si}_{3.35}\text{O}_{10}](\text{OH})_2$	8
Specific Gravity (g/ml)	2.79 - 2.80	8
<i>Kaolin</i>		
Physical Form	White or yellowish white, earthy mass or white powder; unctuous when moist	1
Chemical Formula	$\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$	1
Formula Weight (Da)	258.2	1
Solubility	Insoluble in water, alcohol, dilute acids, and alkali solutions	1,7
<i>Montmorillonite</i>		
Chemical Formula	$\text{R}^{+}_{0.33}(\text{Al}, \text{Mg})_2\text{Si}_4\text{O}_{10}(\text{OH})_2$, where $\text{R}^{+} = \text{Na}^{+}, \text{K}^{+}, \text{Mg}^{2+}$ or Ca^{2+}	1

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
	Attapulgit				Bentonite			
Totals	1	10	NR	8	221	94	0.00025-17.3	0.5-80
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	1	5	NR	8	110	37	0.00025-8	0.8-8
Rinse-Off	NR	5	NR	8	98	57	0.22-17.3	0.5-80
Diluted for (Bath) Use	NR	NR	NR	NR	13	NR	NR	5
Exposure Type**								
Eye Area	1	NR	NR	NR	20	8	0.00025-4.5	0.8-5
Incidental Ingestion	NR	NR	NR	NR	8	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	8 ^b	14 ^a ; 40 ^b	5 ^a ; 7 ^b	0.9	1-3 ^a ; 2-5 ^b
Incidental Inhalation-Powder	NR	5	NR	8 ^b	2; 1 ^c ; 40 ^b	7 ^b	8 ^c	2-5 ^b
Dermal Contact	1	10	NR	8	185	88	0.00025-10	0.5-80
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	6	4	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	17.3	NR
Nail	NR	NR	NR	NR	12	1	2.8	1
Mucous Membrane	NR	NR	NR	NR	29	3	0.22-4.7	0.5-5
Baby Products	NR	NR	NR	NR	3	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams					1	NR	NR	NR
Other Baby Products					2	NR	NR	NR
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts					11	NR	NR	5
Bath Capsules								
Other Bath Preparations					2	NR	NR	NR
Eye Makeup Preparations								
Eyebrow Pencil								
Eyeliners					4	6	0.00025	5
Eye Shadow					2	NR	4.5	NR
Eye Lotion					2	NR	NR	NR
Eye Makeup Remover					1	NR	NR	NR
Mascara					10	1	1.5	0.8
Other Eye Makeup Preparations	1	NR	NR	NR	1	1	NR	NR
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)	NR	5	NR	NR				
Hair Preparations (non-coloring)								
Hair Conditioner					NR	1	NR	NR
Hair Sprays (aerosol fixatives)								
Hair Straighteners					NR	3	NR	NR
Rinses (non-coloring)								
Shampoos (non-coloring)								
Tonics, Dressings, and Other Hair Grooming Aids					4	NR	NR	NR
Wave Sets								
Other Hair Preparations					2	NR	NR	NR
Hair Coloring Preparations								
Hair Tints								
Hair Shampoos (coloring)								
Hair Lighteners with Color								
Hair Bleaches					NR	NR	17.3	NR
Makeup Preparations								
Blushers (all types)					2	NR	4	NR
Face Powders					2	NR	NR	NR
Foundations						5	NR	2-8
Leg and Body Paints								
Lipstick					1			
Makeup Bases					1	3	NR	1
Rouges								
Makeup Fixatives								
Other Makeup Preparations					1	NR	0.6	NR
Manicuring Preparations (Nail)								
Basecoats and Undercoats								
Cuticle Softeners					NR	1	NR	1
Nail Polish and Enamel					11	NR	NR	NR
Nail Polish and Enamel Removers								
Other Manicuring Preparations					1	NR	2.8	NR

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Oral Hygiene Products								
Dentifrices					4	NR	NR	NR
Other Oral Hygiene Products					3	NR	NR	NR
Personal Cleanliness Products								
Bath Soaps and Detergents					6	1	0.22 - 4.7	0.5
Deodorants (underarm)								
Other Personal Cleanliness Products					2	2	NR	NR
Shaving Preparations								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)					1	NR	NR	NR
Shaving Cream								
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing					15	6	1.3 - 7	NR
Depilatories								
Face and Neck (exc shave)					38	1	8	2 - 5
Body and Hand (exc shave)	NR	NR	NR	8	2	6	NR	2 - 5
Moisturizing					9	2	NR	3
Night					NR	1	NR	NR
Paste Masks (mud packs)	NR	5	NR	8	66	44	1.5 - 10	12 - 80
Skin Fresheners					1	1	NR	NR
Other Skin Care Preparations					13	8	NR	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids					NR	1	2.5 (not spray) 0.9 (spray)	NR
Other Suntan Preparations					NR	NR	NR	1
	Clay				Fuller's Earth			
Totals	53	NA	4.5	NA	8	3	NR	NR
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	37	NA	NR	NA	3	1	NR	NR
Rinse-Off	14	NA	4.5	NA	5	2	NR	NR
Diluted for (Bath) Use	2	NA	NR	NA	NR	NR	NR	NR
Exposure Type**								
Eye Area	12	NA	NR	NA	NR	NR	NR	NR
Incidental Ingestion	8	NA	NR	NA	NR	NR	NR	NR
Incidental Inhalation-Spray	1 ^a ; 11 ^b	NA	NR	NA	1 ^a ; 1 ^b	NR	NR	NR
Incidental Inhalation-Powder	1; 11 ^b	NA	NR	NA	1 ^b	NR	NR	NR
Dermal Contact	38	NA	4.5	NA	7	3	NR	NR
Deodorant (underarm)	NR	NA	NR	NA	NR	NR	NR	NR
Hair - Non-Coloring	4	NA	NR	NA	1	NR	NR	NR
Hair-Coloring	NR	NA	NR	NA	NR	NR	NR	NR
Nail	NR	NA	NR	NA	NR	NR	NR	NR
Mucous Membrane	14	NA	NR	NA	1	NR	NR	NR
Baby Products	NR	NA	NR	NA	NR	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams								
Other Baby Products								
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts	2	NA	NR	NA				
Bath Capsules								
Other Bath Preparations								
Eye Makeup Preparations								
Eyebrow Pencil	1	NA	NR	NA				
Eyeliner								
Eye Shadow	8	NA	NR	NA				
Eye Lotion								
Eye Makeup Remover								
Mascara	3	NA	NR	NA				
Other Eye Makeup Preparations								
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)								
Hair Preparations (non-coloring)								
Hair Conditioner	1	NA	NR	NA				
Hair Sprays (aerosol fixatives)								

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Hair Straighteners								
Rinses (non-coloring)								
Shampoos (non-coloring)	2	NA	NR	NA				
Tonics, Dressings, and Other Hair Grooming Aids					1	NR	NR	NR
Wave Sets								
Other Hair Preparations	1	NA	NR	NA				
Hair Coloring Preparations								
Hair Tints								
Hair Shampoos (coloring)								
Hair Lighteners with Color								
Hair Bleaches								
Makeup Preparations								
Blushers (all types)	3	NA	NR	NA				
Face Powders	1	NA	NR	NA				
Foundations	1	NA	NR	NA				
Leg and Body Paints								
Lipstick	6	NA	NR	NA				
Makeup Bases	1	NA	NR	NA				
Rouges								
Makeup Fixatives								
Other Makeup Preparations								
Manicuring Preparations (Nail)								
Basecoats and Undercoats								
Cuticle Softeners								
Nail Polish and Enamel								
Nail Polish and Enamel Removers								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Other Oral Hygiene Products	2	NA	NR	NA				
Personal Cleanliness Products								
Bath Soaps and Detergents	4	NA	NR	NA	1	NR	NR	NR
Deodorants (underarm)								
Other Personal Cleanliness Products								
Shaving Preparations								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream								
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	2	NA	4.5	NA				
Depilatories								
Face and Neck (exc shave)	10	NA	NR	NA	1	NR	NR	NR
Body and Hand (exc shave)	1	NA	NR	NA				
Moisturizing	1	NA	NR	NA				
Night								
Paste Masks (mud packs)	3	NA	NR	NA	4	2	NR	NR
Skin Fresheners								
Other Skin Care Preparations					1	1	NR	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids								
Other Suntan Preparations								

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
	Hectorite				Illite			
Totals	45	18	0.057-1.5	0.4-100	57	NA	0.0015-3.8	NA
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	31	10	0.057-1.5	0.7-15	29	NA	0.0015-0.025	NA
Rinse-Off	14	8	0.1-0.13	0.4-100	26	NA	0.34-3.8	NA
Diluted for (Bath) Use	NR	NR	NR	NR	2	NA	NR	NA
Exposure Type**								
Eye Area	15	4	0.057	0.7	NR	NA	NR	NA
Incidental Ingestion	NR	NR	NR	NR	1	NA	NR	NA
Incidental Inhalation-Spray	1 ^a ; 8 ^b	1 ^a	NR	8 ^b	3 ^a ; 18 ^b	NA	0.25 ^a	NA
Incidental Inhalation-Powder	1; 8 ^b ; 1 ^c	NR	0.1 ^c	8 ^b	3; 18 ^b	NA	NR	NA
Dermal Contact	26	10	0.057-1.5	0.4-100	53	NA	0.0015-3.8	NA
Deodorant (underarm)	NR	1 ^a	NR	0.7 ^a	NR	NA	NR	NA
Hair - Non-Coloring	6	NR	0.1-0.13	1	3	NA	0.25	NA
Hair-Coloring	1	5	0.1	NR	NR	NA	NR	NA
Nail	1	2	NR	NR	NR	NA	NR	NA
Mucous Membrane	NR	1	NR	NR	3	NA	0.34	NA
Baby Products	1	NR	NR	NR	NR	NA	0.0015	NA
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams	1	NR	NR	NR				
Other Baby Products					NR	NA	0.0015	NA
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts					1	NA	NR	NA
Bath Capsules								
Other Bath Preparations					1	NA	NR	NA
Eye Makeup Preparations								
Eyebrow Pencil								
Eyeliner	3	3	0.057	NR				
Eye Shadow	1	NR	NR	NR				
Eye Lotion								
Eye Makeup Remover								
Mascara	11	1	NR	0.7				
Other Eye Makeup Preparations								
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)								
Hair Preparations (non-coloring)								
Hair Conditioner	3	NR	0.13	NR				
Hair Sprays (aerosol fixatives)								
Hair Straighteners								
Rinses (non-coloring)	1	NR	NR	NR				
Shampoos (non-coloring)	1	NR	0.1	1	1	NA	NR	NA
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	0.13 (not spray)	NR	2	NA	0.025	NA
Wave Sets								
Other Hair Preparations	1	NR	NR	NR				
Hair Coloring Preparations								
Hair Tints								
Hair Shampoos (coloring)	1	NR	0.1	NR				
Hair Lighteners with Color								
Hair Bleaches	NR	5	NR	NR				
Makeup Preparations								
Blushers (all types)								
Face Powders	1	NR	NR	NR	2	NA	NR	NA
Foundations	NR	NR	1.5	15	1	NA	NR	NA
Leg and Body Paints								
Lipstick								
Makeup Bases	2	NR	NR	NR				
Rouges								
Makeup Fixatives								
Other Makeup Preparations	NR	1	NR	NR				
Manicuring Preparations (Nail)								
Basecoats and Undercoats	NR	1	NR	NR				
Cuticle Softeners								
Nail Polish and Enamel	NR	1	NR	NR				
Nail Polish and Enamel Removers								
Other Manicuring Preparations	1	NR	NR	NR				

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Oral Hygiene Products								
Dentifrices	NR	NR	NR	NR	1	NA	NR	NA
Other Oral Hygiene Products								
Personal Cleanliness Products								
Bath Soaps and Detergents					1	NA	0.34	NA
Deodorants (underarm)	NR	1	NR	0.7				
Other Personal Cleanliness Products	NR	1	NR	NR				
Shaving Preparations								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream								
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	5	NR	NR	100	6	NA	3.8	NA
Depilatories								
Face and Neck (exc shave)	8	NR	0.1 (not spray)	NR	18	NA	NR	NA
Body and Hand (exc shave)	NR	NR	NR	8				
Moisturizing					2	NA	NR	NA
Night	1	NR	NR	NR				
Paste Masks (mud packs)	3	2	NR	0.4 - 8	17	NA	1 - 2	NA
Skin Fresheners								
Other Skin Care Preparations	1	1	NR	NR	6	NA	NR	NA
Suntan Preparations								
Suntan Gels, Creams, and Liquids	NR	1	NR	NR				
Other Suntan Preparations								
	Kaolin				Montmorillonite			
Totals	787[†]	509	0.0005-53.2	0.01-100	54	NR	0.1-3	NR
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	502	501	0.0015-53.2	2-100	20	NR	1.2-2	NR
Rinse-Off	262	7	0.0005-33	0.01-84	32	NR	0.1-3	NR
Diluted for (Bath) Use	23	1	NR	NR	2	NR	NR	NR
Exposure Type**								
Eye Area	89	231	0.001-8.5	3-48	2	NR	NR	NR
Incidental Ingestion	135	6	0.0053-14.5	12-30	1	NR	NR	NR
Incidental Inhalation-Spray	1; 35 ^a ; 90 ^b	NR	0.0015-25 ^a	2-25 ^a ; 3 ^b	2 ^a ; 10 ^b	NR	NR	NR
Incidental Inhalation-Powder	38; 90 ^b ; 1 ^c	98	0.01-15; 0.11-16 ^c	5-30; 3 ^b	1 ^c ; 10 ^b	NR	1.2-2 ^c	NR
Dermal Contact	562	466	0.001-33	0.01-100	48	NR	0.1-3	NR
Deodorant (underarm)	6 ^a	NR	2.6	NR	NR	NR	NR	NR
Hair - Non-Coloring	46	5	0.0005-26	4-15	5	NR	NR	NR
Hair-Coloring	8	1	5-19.5	NR	NR	NR	NR	NR
Nail	11	NR	0.023-53.2	53-54	NR	NR	NR	NR
Mucous Membrane	182	8	0.0053-14.5	3-30	8	NR	NR	NR
Baby Products	1	NR	0.0085	NR	1	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams	1	NR	NR	NR	1			
Other Baby Products	NR	NR	0.0085	NR				
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts	15	NR	NR	NR	1	NR	NR	NR
Bath Capsules								
Other Bath Preparations	8	1	NR	NR	1	NR	NR	NR
Eye Makeup Preparations								
Eyebrow Pencil	3	5	4.3	15 - 17	1	NR	NR	NR
Eyeliner	6	9	0.82 - 2	25 - 48				
Eye Shadow	42	171	0.35 - 8.5	3 - 29				
Eye Lotion	1	NR	NR	NR	NR	NR	NR	NR
Eye Makeup Remover	NR	NR	0.001	NR	1	NR	NR	NR
Mascara	25	31	0.5 - 4.7	8 - 18				
Other Eye Makeup Preparations	12	15	NR	20				
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)	NR	40	NR	5				
Hair Preparations (non-coloring)								
Hair Conditioner	6	5	0.0005 - 26	4	1	NR	NR	NR
Hair Spray (aerosol fixatives)	1	NR	NR	NR				
Hair Straighteners								

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Rinses (non-coloring)								
Shampoos (non-coloring)	14	NR	0.058 - 0.14	NR	1	NR	NR	NR
Tonics, Dressings, and Other Hair Grooming Aids	14	NR	0.0015 - 25	15				
Wave Sets	1	NR	NR	NR				
Other Hair Preparations	10	1	0.1	5	3	NR	NR	NR
Hair Coloring Preparations								
Hair Tints	NR	NR	5	NR				
Hair Shampoos (coloring)								
Hair Lighteners with Color	2	NR	19.3	NR				
Hair Bleaches	6	NR	19.5	NR				
Makeup Preparations								
Blushers (all types)	10	72	0.05 - 15	14 - 20				
Face Powders	38	58	0.01 - 15	30				
Foundations	29†	45	0.5 - 6	6 - 36				
Leg and Body Paints	1	NR	NR	NR				
Lipstick	118	6	0.0053 - 14.5	12 - 30				
Makeup Bases	2	24	1	7 - 25				
Rouges	3	2	NR	NR				
Makeup Fixatives	NR	3	NR	NR				
Other Makeup Preparations	11	20	2 - 4	10 - 24	1	NR	NR	NR
Manicuring Preparations (Nail)								
Basecoats and Undercoats	1	NR	NR	NR				
Cuticle Softeners								
Nail Polish and Enamel	9	NR	0.023	NR				
Nail Polish and Enamel Removers								
Other Manicuring Preparations	1	NR	35.5 - 53.2	53 - 54				
Oral Hygiene Products								
Dentifrices	16	NR	NR	NR	1	NR	NR	NR
Other Oral Hygiene Products	1							
Personal Cleanliness Products								
Bath Soaps and Detergents	15†	1	1 - 5	3	2	NR	NR	NR
Deodorants (underarm)	6	NR	2.6 (not spray)	NR				
Other Personal Cleanliness Products	9	NR	NR	NR	3	NR	NR	NR
Shaving Preparations								
Beard Softeners	1	NR	NR	NR				
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream	NR	NR	0.25	NR				
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	72	NR	3.4 - 20	0.01	8	NR	0.3	NR
Depilatories	NR	NR	4	NR				
Face and Neck (exc shave)	76	NR	0.11 - 16 (not spray)	3	7	NR	1.2 - 2 (not spray)	NR
Body and Hand (exc shave)	14	NR	3.3 - 12 (not spray)	NR	3	NR	NR	NR
Moisturizing	18	NR	0.2 - 1 (not spray)	25	2	NR	NR	NR
Night	2	NR	NR	NR				
Paste Masks (mud packs)	120†	NR	5 - 33	12 - 84	15	NR	0.1-3	NR
Skin Fresheners	1	NR	0.25	2				
Other Skin Care Preparations	46†	NR	10 - 20.4	3 - 100	2	NR	NR	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids	NR	NR	NR	25				
Other Suntan Preparations								

NR – not reported

NA – not applicable

*likely duration and exposure is derived based on product category (see Use Categorization <https://www.cir-safety.org/cir-findings>)

**Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

*It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

† 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

‡ It is possible these products are powders, but it is not specified whether the reported uses are powders.

† Includes entries for Kaolinite from the VCRP database.

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VITRO						
Bentonite particles with α -quartz content (up to 6%) and with different chemical modifications (acid, alkaline, organic, untreated); gypsum and quartz were negative and positive controls	1 to 15 $\mu\text{g}/\text{cm}^2$	isotonic NaCl solution	Human lung fibroblasts (IMR90)	Micronucleus assay and kinetochore analysis; treated cells incubated for 36, 48, or 72 h	In acidic sample, formation of micronuclei was only slightly increased after exposure to samples with a quartz content of 4% - 5% for 36 h (15 $\mu\text{g}/\text{cm}^2$), 48 h (5 $\mu\text{g}/\text{cm}^2$), and 72 h (1 $\mu\text{g}/\text{cm}^2$); and with a quartz content of 1% for 72 h (1 $\mu\text{g}/\text{cm}^2$). In the alkaline sample, the formation of micronuclei was only slightly increased after exposure to sample with a quartz content of 5% for 48 h and 72 h (15 $\mu\text{g}/\text{cm}^2$). Untreated and organic activated Bentonite particles did not show genotoxic effects; Bentonite particles with a quartz content of < 1% were in negative in the micronucleus assay. Statistically significant reductions in kinetochore-positive micronuclei were not observed with the Bentonite samples	47
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	5000 μg	Not reported	<i>S. typhimurium</i> strains TA98, TA100, TA102, TA1535, TA1537	Ames test in accordance with OECD TG 471; with and without metabolic activation	Not mutagenic with or without metabolic activation	38
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	156.25, 312.5, 625, 1250, 2500, or 5000 $\mu\text{g}/\text{ml}$	culture medium	CHO - K1 cell cultures	Mammalian chromosome aberration test in accordance with OECD TG 473; with and without metabolic activation	Test material did not induce chromosomal aberrations, with or without metabolic activation	38
Kaolin; particle size 4.8 μm and 200 nm	0.2 - 200 $\mu\text{g}/\text{ml}$	particles suspended in saline containing 0.05% Tween 80; suspensions then mixed with culture medium supplemented with 10% fetal bovine serum prior to treating cells	CHO AA8 cells; primary normal human diploid epidermal keratinocytes and fibroblasts	Micronucleus assay; cells were incubated with test materials for 6 h; mitomycin C was positive control	Micronucleus induction in a dose-dependent manner, with frequencies of micronucleated cells increased 3- to 4-fold at 200 $\mu\text{g}/\text{ml}$ in all cell types; fine particles of Kaolin had higher genotoxic potency than coarse particles, with no significant differences detected among the 3 cell types	45
Kaolin; primary particle size 4.8 μm	0.02 - 200 $\mu\text{g}/\text{ml}$	particles suspended in saline containing 0.05% Tween 80; suspensions then mixed with culture medium supplemented with 10% fetal bovine serum prior to treating cells	Human lung cancer A549 cells	Micronucleus assay; cells were incubated with test materials for 6 h	A 4-fold increased frequency of micronucleated cells was observed	40

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
Kaolin; particle size 4.8 µm and 200 nm	0.2 - 200 µg/ml	particles suspended in saline containing 0.05% Tween 80; suspensions then mixed with culture medium supplemented with 10% fetal bovine serum prior to treating cells	CHO AA8 cells; primary normal human diploid epidermal keratinocytes and fibroblasts	Comet assay; 1-h treatment	Kaolin particles promoted DNA damage in a dose-dependent manner; %tail DNA was increased 8- to 20-fold by exposure to the particles at 200 µg/ml for all cells tested; 200 nm particles had a higher DNA-damaging potency than the 4.8 µm particles, while no significant difference was detected among three cell types	45
Montmorillonite-based clay minerals (1 unmodified clay and 3 cation-exchanged clays)	unmodified clay tested at up to 125 µg/ml; modified clays tested at up to 250 µg/ml	MilliQ water	<i>S. typhimurium</i> strains TA97A, TA98, TA100, TA102, TA104	Ames test; with and without metabolic activation	No significant increases in revertant colonies observed in the unmodified clay (tested at up to 125 µg/ml) or in one of the modified clays (tested at up to 250 µg/ml); however, significant increases in revertant colonies observed in S9 in strain TA98 in the other two modified clays (tested at up to 8 µg/ml and 125 µg/ml, respectively); no changes observed for the same strain without S9 or in the rest of the strains for these 2 modified clays	43
Montmorillonite, natural and organo-modified	0-14.1 µg/plate for unfiltered material; 0-141 µg/ml for filtered material (particles larger than nano-range removed)	MilliQ water	<i>S. typhimurium</i> TA98 and TA100	<i>Salmonella</i> /microsome assay; with and without metabolic activation; suspensions were tested both filtered (removing particle larger than nano-range) and unfiltered material	No mutagenic activity observed with or without metabolic activation	44
Montmorillonite, natural and cation-exchanged	56.5, 85, 113, or 170 µg/ml for unfiltered material; up to 226 µg/ml for filtered material (particles larger than nano-range removed)	culture medium	Caco-2 cells	Comet assay; suspensions were tested both filtered (removing particle larger than nano-range) and unfiltered material	Unfiltered and filtered modified Montmorillonite was genotoxic in a concentration-related manner, with statistical significance at the 2 highest concentrations tested for each, when compared to negative controls; no genotoxic effects were observed in the unfiltered and unfiltered natural Montmorillonite	44
Unmodified Montmorillonite clay	15.65, 31.25, or 62.5 µg/ml	serum-free medium supplemented with B27 (no further details on supplement)	Human hepatoma cell lines (HepG2)	Cytokinesis block micronucleus cytome assay; cells were incubated with test material for 4 or 24 h; positive controls were benzo(a)pyrene and etoposide	Test material induced statistically significant increases ($p < 0.0001$) only in the frequency of micronuclei in 1000 binucleated cells at the highest concentration tested; no effects observed in nucleoplasmic bridges or nuclear buds; positive controls yielded expected results	46

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VIVO						
Kaolin; primary particle size 4.8 μm (major peak average 357.6 $\pm$ 199.4 nm)	Single dose of 0.05 or 0.2 mg/animal	particles suspended in saline containing 0.05% Tween 80; particles agglomeration in suspensions observed (low-density tabular structures with rectangular or hexagonal shape were observed)	Groups of 5 male C57BL/6J mice	Comet assay; mice were intratracheally instilled with particles; negative control received solvent; 3 h after instillation, mice were killed and lungs were removed for analysis; additional exposure time also examined (24 h)	DNA damage was induced at 0.2 mg/mouse (up to 2 - 3-fold), but not at 0.05 mg/mouse, after 3 h exposure; DNA damage induced at 24 h did not differ from 3 h exposure	40
Kaolin; primary particle size 4.8 μm (major peak average 357.6 $\pm$ 199.4 nm)	Single dose or multiple doses (4x) of 0.2 mg/animal	particles suspended in saline containing 0.05% Tween 80; particles agglomeration in suspensions observed (low-density tabular structures with rectangular or hexagonal shape were observed)	Groups of 10 male <i>gpt</i> delta transgenic mice	<i>gpt</i> mutagenesis assay; mice were intratracheally instilled with particles; negative control received solvent; mice killed at 12 (single dose) or 8 (multiple doses) wk; lungs and kidneys removed for analysis	Increased <i>gpt</i> and <i>Spi</i> ⁺ mutant frequencies observed in the lungs of the mice; mutation spectra analysis showed > 60% of the base substitution occurred in the <i>gpt</i> genes	40
cation-exchanged Montmorillonite	0, 250, 500, or 1000 mg/kg bw; 1000 mg/kg bw in range finding study	Water or cell-culture medium	Groups of 3 male and 3 female Wistar Hannover Galas rats; 2 rats of each sex in a range finding study	Comet assay; rats received 2 single doses of test material by gavage 24 h apart; 3 h after dosing, rats were killed and examined macroscopically, and liver, kidneys, and colon were dissected and analyzed for DNA damage; water or cell-culture medium was negative control and ethylmethanesulfonate was positive control	No statistically significant difference in % tail DNA between the negative controls and the different treatment groups for any of the organs tested for DNA damage; positive control yielded expected results; all rats survived during treatment period with no clinical signs of abnormalities	48

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
IN VITRO						
Formulation containing 38% Montmorillonite	25 µl calcium and magnesium free Dulbecco's phosphate buffered saline	Tested neat; 25 mg	EpiDerm™ tissues	EpiDerm™ skin model in accordance with OECD TG 439	Not an irritant; mean viability = 105.7%	54
ANIMAL						
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	0.5 ml distilled water	500 mg	rabbits (no further details)	Acute dermal irritation study in accordance with OECD TG 404; patches were applied to intact skin with gauze patch for 4 h; after exposure period, residual test substance was removed with cotton soaked in distilled water; skin reactions scored at 1, 24, 48, and 72 h post-patch removal	Not irritating; mean dermal irritation scores for erythema and edema were 0.0	38
HUMAN						
Formulation containing 1.75% Bentonite	none	0.05 ml	25 subjects	14-d cumulative irritation assay under occlusive patches; test sites on the back or upper arm and patches were 15 mm diameter disks; positive control site received 0.05 ml of 0.25% sodium lauryl sulfate and negative control site was a plain patch	Irritation potential was negligible; no adverse events reported	55
Mud mask containing 8% Bentonite	none	As supplied	19 subjects	Single-insult occlusive patch test	No irritation; primary irritation index = 0.0	56
Facial cleanser containing 2% Bentonite and 2% Kaolin	none	As supplied	50 subjects	4-wk clinical use test; single-blind baseline controlled monadic design; subjects instructed to use test material daily in the morning as a facial cleanser, twice weekly in the evening as an exfoliating scrub, twice weekly in the evening as a purifying mask, and 3 times weekly in the evening as a facial cleanser	No visible irritation; however, 8 subjects reported perceived discomfort and/or irritation, including burning/stinging and/or redness/dryness while using the test material; 6/8 subject responses were not considered related to the use of the test material and 2/8 subject responses were not sufficient intensity to warrant discontinuation of product	57
SENSITIZATION						
IN VITRO						
Formulation containing 38% Montmorillonite	Cell culture medium	Not reported	KeratinSens™ cells	KeratinSens™ assay (validated by the European Centre for the Validation of Alternative Methods (ECVAM)); 12 concentrations in 3 repetitions, in 3 replicates (details not reported); luciferase induction and cellular viability determined after 48 h incubation time	Not a sensitizer; test material weakly toxic to cells	54
ANIMAL						
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	Distilled water	5% (w/v) for intradermal injection; concentration details not provided for topical induction or challenge; topical dose was 100 mg test material in 0.2 ml vehicle	10 guinea pigs/sex	Skin sensitization study in accordance with OECD TG 406; no further details provided	Negative for sensitization; no positive skin responses at 24 or 48 h post-patch removal at challenge; no clinical signs during treatment	38
50% Hectorite	Not reported	Not reported	Guinea pigs; number/sex not provided	Buehler test; no further details provided	Non-sensitizing	11

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
HUMAN						
Foot mask containing 3.5% Bentonite	As supplied	0.2 g	102 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; 10-15 d non-treatment period followed by challenge patch applied to naïve site and scored at 48 h and 72 h post-application; patch was ~ 2 cm ²	No dermal sensitization; no adverse events reported	⁶⁰
Clay mask containing 3.8% Bentonite	As supplied	0.1 - 0.15 g	108 subjects; 25% with self-perceived sensitive skin	HRIPT under semi-occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and scored at 24 h and 72 h post-application; patch was ~ 2 cm ² and contained ~ 25 - 38 mg/cm ² test material	No dermal irritation or sensitization	⁵⁸
Face cream containing 7.5% Bentonite	As supplied	0.1 - 0.15 g; volatilized for 30-90 min prior to application	52 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and scored at 24 h and 72 h post-application; no further details on patches provided	No dermal irritation or sensitization	⁵⁹
Lip product containing 14.5% Kaolin	As supplied	0.1 - 0.15 g	54 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and score at 24 h and 72 h post-application; patch contained ~ 25 - 38 mg/cm ² test material	No dermal irritation or sensitization	⁶¹
Clay mask containing 14.5% Kaolin	Neat	0.1 ml	103 subjects	HRIPT under occlusive patches; induction patch applied on the back or upper arm ;after a 10-15 d non-treatment period, challenge patch applied to naïve site and scored at 48 and 72 h post-application; material was applied to a 2 x 2 cm ² Webril pad	Not sensitizing; moderate erythema at the first induction in one subject mildly progressed through the induction period to erythema and edema with papules; no other adverse events reported during study	⁶³
Clay mask containing 40% Kaolin	As supplied	0.1 - 0.15 g	51 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and scored at 24 h and 72 h post-application; no further details on patches provided	No dermal irritation or sensitization	⁶²
PHOTOSENSITIZATION						
HUMAN						
Sunscreen containing 1.75% Bentonite	As supplied	0.2 ml	23 subjects	Photoallergy test under occlusive patch; on day 1, subjects received test material on 2 sites on the back for 24 h, after which, subjects received a total of 6 applications (twice weekly) on the back; 24 h after application, one site was irradiated with UVB + UVA with full Xenon lamp spectrum, other site was non-irradiated control; after a non-treatment period of 10-17 d, challenge patches on naïve sites were placed and one site was irradiated with 6 J/cm ² UVA and ½ the minimal erythral dose of UVB; sites were evaluated at 24, 48, and 72 h after irradiation	Not a photosensitizer	⁶⁴

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SAFETY DATA SHEET

SECTION 1 – IDENTIFICATION

Product name: Glacial Oceanic Clay Powder

Product code: ICC-POWCLAY10K

Recommended use: Personal care

Restrictions on use: None

Manufacturer's name: Ironwood Clay Company Inc.

Address: Unit 450 – 3771 Jacombs Road
Richmond, B.C.
V6V 2L9 Canada

Telephone: (604) 279-9498

Emergency telephone: Call 911 for emergency OR
(604) 279-9498 for SDS information

SECTION 2 – HAZARD(S) IDENTIFICATION

Hazard classification:

Classification according to WHMIS 2015 and GHS:

The substance does not meet the criteria for a hazardous substance.

Classification according to hazard communication standard (HCS) (29 CFR 1910.1200):

The substance does not meet the criteria for a hazardous substance.

Label elements (Pictograms): The substance does not require a hazard warning label.

Signal word(s): Not applicable.

Hazard statement(s): Not applicable.

Precautionary statement(s): Not applicable.

Other hazards which do not result in classification: Dust explosion hazard.

SECTION 3 - COMPOSITION / INFORMATION ON INGREDIENTS

Composition:

INCI Name	CAS#	%
Sea Silt	-- / 999999-99-4*	100.0%

*Naturally occurring substances

SECTION 4 - FIRST-AID MEASURES

First-aid measures by route of exposure:

Inhalation: The substance is not volatile in the form supplied. Remove person into fresh air.

Skin contact: Wash off with plenty of water.

Eye contact: Flush immediately with large amounts of water for at least 15 minutes. Eyelids should be held away from the eyeball to ensure thorough rinsing. Consult a physician if irritation persists.

Ingestion: If swallowed, clean mouth with water and afterwards drink plenty of water. Get immediate medical attention if necessary.

Most important symptoms and effects (acute or delayed):

No significant symptoms and hazards are expected under normal intended use and appropriate handling.

Immediate medical attention and special treatment, if necessary:

No immediate medical attention and treatment normally needed.

SECTION 5 – FIRE-FIGHTING MEASURES

Suitable extinguishing media: Carbon dioxide or dry chemical. Water may be ineffective but should be used to cool fire-exposed containers, structures and to protect personnel. Use water to dilute spills and to flush them away from sources of ignition.

Unsuitable extinguishing media: Not known.

Specific hazards arising from the substance or mixture:

Fire hazard: Not known.

Explosion hazard: Dust.



Hazardous combustion products: Irritating or toxic substances may be emitted upon thermal decomposition. Thermal decomposition products may include oxides of carbon, smoke & hydrocarbons.

Special protective equipment and precautions for fire-fighters:

Exercise caution when fighting fire. Firefighters should wear full protective gear. Do not enter fire area without proper protective equipment, including respiratory protection.

SECTION 6 – ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures:

General measures: Wear glove, respirator, goggles. Handle in accordance with good industrial hygiene and safety practice.

Environmental precautions: Non-hazardous but prevent further leakage or spillage.

Methods and materials for containment and cleaning up:

Clear up spills immediately and rinse with as much water as possible. Use caution walking around when handling broken product package. May cause slip.

SECTION 7 – HANDLING AND STORAGE

Precautions for safe handling:

Keep at ambient temperature. Wear a respirator when handling. Do not get in eyes or on clothing. Keep container closed. Use good personal and industrial hygiene practices.

Wash hand before eating, drinking, smoking and again when leaving work. Remove contaminated clothing and clean before re-use.

Conditions for safe storage (including incompatible materials):

Store in a cool, dry, well-ventilated place. Keep container closed when not in use. Store at ambient temperature and avoid direct sunlight. Do not freeze.

SECTION 8 - EXPOSURE CONTROLS / PERSONAL PROTECTION

Control parameters, including occupational exposure guidelines or biological exposure limits and the source of those values:

The substance, as supplied, does not contain any hazardous substance with occupational exposure limits.

Appropriate engineering controls:

Practice proper hygienic and work-place safety procedures.

Individual protection measures (PPEs):

Eye protection: Wear goggles or safety glasses. Have eye-wash stations available where eye contact can occur.

Skin and body protection: Wear gloves, lab coat or coverall.

Respiratory protection: Wear NIOSH/MSHA approved respirator.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Information on basic physical and chemical properties:

Appearance	:	Fine powder.
Colour	:	Light grey.
Odour	:	Standard dry clay odour.
Odour threshold	:	No data available.
pH	:	Not applicable.
Melting point	:	No data available.
Freezing point	:	No data available.
Initial boiling point/boiling range	:	No data available.
Flash point	:	No data available.
Evaporation rate	:	No data available.
Flammability (solid, gas)	:	No data available.
Lower flammable/explosive limit	:	No data available.
Upper flammable/explosive limit	:	No data available.
Vapour pressure	:	No data available.
Vapour density	:	No data available.
Relative density	:	No data available.
Solubility	:	No data available.
Partition coefficient – n-octanol/water	:	No data available.
Auto-ignition temperature	:	No data available.
Decomposition temperature	:	No data available.
Viscosity	:	Not applicable.
Specific gravity	:	Not applicable.

SECTION 10 - STABILITY AND REACTIVITY

Reactivity: No data available.

Chemical Stability: Stable under recommended handling and storage conditions (see section 7).

Possibility of hazardous reactions: No data available.

Conditions to avoid: Extremely high or low temperatures and direct sunlight.



Incompatible materials: None

Hazardous decomposition products: Thermal decomposition products may include oxides of carbon, smoke & hydrocarbons.

SECTION 11 - TOXICOLOGICAL INFORMATION

Information on the likely routes of exposure:

Inhalation: No data available.

Ingestion: Ingestion of large amount may cause irritation to the gastrointestinal tract and blockage.

Skin contact: May cause mild skin irritation.

Eye contact: May cause mild eye irritation.

Symptoms related to the physical, chemical and toxicological characteristics:

No information available.

Delayed and immediate effects, and chronic effects from short-term and long-term exposure:

Skin corrosion / irritation: No data available.

Serious eye damage / irritation: No data available.

Respiratory or skin sensitization: No data available.

Germ cell mutagenicity: No data available.

Carcinogenicity: No data available.

Reproductive Toxicity: No data available.

Specific target organ toxicity: No data available.

Aspiration hazard: No data available.

Numerical measures of toxicity, including acute toxicity estimates (ATEs):

Acute toxicity: No data available.

SECTION 12 – ECOLOGICAL INFORMATION

Ecotoxicity: No data available.

Persistence and degradability: Biodegradable.

Bioaccumulation potential: No information available.

Mobility in soil: No information available.

Other adverse effects: No data available.

SECTION 13 – DISPOSAL CONSIDERATIONS

As sold, this product is not classified as a hazardous waste according to US Federal regulations (40 CFR 261) and Hazardous waste legislation guide, Ministry of Environment, and The province of B.C., Canada.

Waste disposal recommendations: Dispose in accordance with applicable federal, provincial and local regulations

SECTION 14 – TRANSPORT INFORMATION

Canada Transportation of Dangerous Goods (TDG), U.S. Department of Transportation (DOT), International Air Transport Association (IATA) and International Maritime

Dangerous Goods (IMDG):

TDG (Canada):

UN number:	Not applicable.
UN proper shipping name:	Not regulated.
Transport hazard class(es):	Not classified.
Packing group:	Not applicable.
Environmental hazards (Marine pollutant):	No information available.
Special precautions:	Ship in climate-controlled transport. Do not freeze.

DOT (US):

UN number:	Not applicable.
UN proper shipping name:	Not regulated.
Transport hazard class(es):	Not classified.
Packing group:	Not applicable.
Environmental hazards (Marine pollutant):	No information available.
Special precautions:	Ship in climate-controlled transport. Do not freeze.

IATA:

UN number:	Not applicable.
UN proper shipping name:	Not regulated.
Transport hazard class(es):	Not classified.
Packing group:	Not applicable.
Environmental hazards (Marine pollutant):	No information available.
Special precautions:	Ship in climate-controlled transport. Do not freeze.

IMDG:

UN number:	Not applicable.
UN proper shipping name:	Not regulated.
Transport hazard class(es):	Not classified.
Packing group:	Not applicable.
Environmental hazards (Marine pollutant):	No information available.
Special precautions:	Ship in climate-controlled transport. Do not freeze.

SECTION 15 – REGULATORY INFORMATION

Safety, health and environmental regulations specific to the substance or mixture:

Canadian Controlled Products Regulation, Hazardous Products Act: This substance is not classified as hazardous substance.

Canadian Environmental Protection Act: All components of this substance are either on the Domestic Substances List (DSL), the Non-Domestic Substance List (NDSL) or exempted.

U.S. Toxic Substances Control Act (TSCA) Section 8(b) Inventory:

All components of this substance are either on the TSCA Inventory List or exempted.

U.S. 40 CFR Appendices A and B to Part 355:

This substance is not subjected to reporting requirements of Title III of SARA Section 355 (extremely hazardous substances).

U.S. Emergency Planning and Community Right-To-Know Act (EPCRA) Section 313:

This substance is not subjected to reporting requirements of Title III of SARA Section 313 (specific toxic chemical listings).

U.S. 40 CFR Table 1, 2, 3 and 4 to Part 68.130 List of substances (Clean Air Act, Section 112(r)):

This substance is not subjected to reporting requirements of Clean Air Act section 112(r).

SECTION 16 – OTHER INFORMATION

Preparation date: December 2, 2021

Revision date: December 2, 2021

This document has been prepared in accordance with the SDS requirements of the WHMIS 2015, OSHA, A guide to the globally harmonized system of classification and labeling of chemicals (GHS) and Hazard communication standard 29 CFR 1910.1200.



Disclaimer: The information provided in this Safety Data Sheet is based on our current knowledge and information from manufacturers of components at the date obtained. It is designed only as a guidance to describe the product for the purposes of safe handling, use, storage, transportation and disposal only and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with other substances.

Determination of the Efficacy and Tolerance of a Facial Mask

Study N° 16AO-0627-01 Final Report

Study Sponsor:

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***This final report was prepared by a clinical testing laboratory with quality management
system registered to ISO 9001.***

This report is composed of 36 pages including appendices (21 pages).

August 19th, 2016

Revised on July 25th, 2018

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Notice: This report is an updated version of Final Report #16AO-0627 modified on July 25th, 2018 upon study sponsor request.

STUDY OBJECTIVE

The primary aim of this study was to assess the effectiveness of a facial mask developed by Ironwood Clay Company Inc. The test product was evaluated for its effects on skin sebum level as well as the appearance of pores on the face. Twenty (20) healthy female participants were included in this study and have applied the test product three times per week during a 28-day period.

The secondary objective of the study was to evaluate consumer acceptance and tolerance to the test product under normal conditions of use.

PROTOCOL

1. Ethics Committee

This standard procedure and associated documents were reviewed and approved by an Ethics Committee prior to the commencement of the study. The Ethics Committee is an independent organization whose members' responsibility is to ensure the protection of the rights, safety and well-being of the subjects participating in the study.

2. Duration

The study took place from June 27th to July 28th, 2016.

3. Investigation Site

Evalulab Inc. located at 5475 Paré, Suite 206, Mont-Royal, Québec, H4P 1P7, Canada.

4. Personnel

The study was conducted by Evalulab Inc., represented by Marie-Laure Oula, M. Sc. – Investigator, Laboratory Director.

5. Test Product

Upon reception, the test product was registered in the "Receptions Book" and assigned a code followed by its storage at ambient humidity and temperature in its original container (as received) in an area allocated for this purpose. The test product was provided in twenty-five (25) homogeneously labelled jars of 325g and detailed as follows:

Product name:	Glacial Oceanic Clay Mask
Category:	Mask
Client lot #:	BN 3274 (Lot# 10584B)
Evalulab lot #:	160427.EA.01

Safety Data:

The safety and/or innocuity of the test product was based on MSDS (Material Safety Data Sheet) and toxicology data available from ingredients suppliers. The sponsor has issued a certificate attesting its innocuous nature and thus suitability for use in this study, conforming to Canadian regulations.

6. Quality Assurance

The study was conducted in accordance with Good Clinical Practice (GCP). GCP is defined as the totality of the pronouncements put in place for ensuring the quality and authenticity of the trials and the obtained data on one hand and the respect for the ethics on the other.

The data obtained for each participant is recorded in individual Case Report Forms. The data entry is made in black ink. In case of errors or omissions, the initial entry is crossed out and initialled by the investigator.

All recorded data is validated by the investigator, who assumes responsibility for the quality of the work presented and verifies that all gathered data is in agreement with the protocol.

The records obtained during the study will be kept by Evalulab Inc. for a period of 2 years.

7. Study Design

• Type of Study

This study was a monocentric open-label trial, meaning the evaluator, participants and sponsor alike were aware of the nature of the test material.

• Participants

Recruitment of Participants:

Twenty (20) participants were recruited based on the inclusion and exclusion criteria. The profile of each participant is presented in Table I in the Appendices.

Inclusion Criteria:

1. Male or female participants in good health, over 45 years of age.
2. With mature and normal to oily skin on the face.
3. With fine lines and/or moderate wrinkles in the crow's feet area of the face.
4. With skin redness, skin irritation or signs of skin inflammation on the face.
5. Who agree to avoid sun exposure during the study.
6. Cooperating in the study, able to be monitored at each visit, aware of the demands and duration of the controls, thus allowing perfect adherence to the established protocol.
7. Who have signed and dated the Informed Consent Forms upon full knowledge of the risks involved with the study.
8. For women who use a method of contraception (e.g., oral contraceptive, condoms, spermicidal creams, an intra-uterine device (IUD), abstinence...) or who are in a menopausal status.

Exclusion Criteria:

1. Participants with a history of skin irritation or allergies to skin care products in general (e.g., facial masks, peeling products, clay...), with allergies to certain foods, to certain chemical products, to jewellery...
2. Who refuse to only use the test product in their weekly mask routine (with the exception of their regular skin care, cleansing and make-up products).
3. Who have a history of severe acne, eczema, topical dermatitis, psoriasis or significant skin anomalies on the areas to be tested.
4. Who suffer from a serious illness or health problem or a critical or progressive disease (e.g., asthma, diabetes, cancer, immunological deficiency, removed organ ...).
5. Who have taken prescription or over the counter medication (at a frequency equal to or more than 3 doses per week) that could affect skin characteristics or could bias the study (e.g., antibiotics, antihistamines, anti-inflammatories...) within 7 days of study start.
6. Who frequent tanning salons, foresee exposure to the sun during the study or use sunless/self-tanning products.
7. Who abuse alcohol, drugs and/or tobacco.
8. Women who are pregnant, breastfeeding or expecting to become pregnant during the study.

Informed Consent Forms:

Each participant was informed verbally and in writing of the nature of the test and of the potential risks involved. All participants were required to read, sign and date the Informed Consent Form explaining the conditions of the test, the risks involved and briefly describing the product to be tested.

A photography consent form was also given to the participants, since photographs of their face were taken and provided to the study sponsor.

Confidentiality:

Subject participation in this study is confidential. The information gathered during the course of the study was recorded in Case Report Forms that are numerically coded and that do not contain the names of the participants. Only the employees of Evalulab, the affiliated dermatologist, auditors of the sponsor and regulatory bodies (FDA, Health Canada and the Ethics Committee) may have access to the confidential information.

Moreover, it is forbidden to use the participants' photographs taken during the study (even if their identity is unrecognizable) for commercial purposes without the written consent of the participant in question.

8. Evaluation

• Schedule

The study was carried out according to the schedule summarized in Figure 1.

Figure 1. Study Schedule

DESCRIPTION	D0-T0	D0-T30	D28
Participant selection according to inclusion and exclusion criteria	X		
Signing of the Informed Consent Forms and Photograph Consent Forms	X		
Completion of the Medical Questionnaire	X		
Distribution of Samples + Forms (instructions + log of applications)	X		
Digital photographs using the Visia-CR Imaging System (frontal + 2 profiles)	X	X	X
Measurements of Cutaneous Sebum Level using the Sebumeter® SM810	X	X	X
First application of the test product at the laboratory	X		
Verification of product use (weigh sample container)	X		X
Return of completed follow-up form (log of applications)			X
Completion of the electronic self-evaluation questionnaire at the laboratory		X	X

• Procedure

On the first visit (Day 0), the participants were scheduled to arrive at the laboratory at least two hours after having washed their face, without make-up or skin care products on their face nor eye contour area.

After verification of the inclusion/exclusion criteria and signing of the appropriate documents, each participant received a sample of the test product and a follow-up sheet to be completed after every application.

Directions for use were then explained to the participants, according to the recommendations of the study sponsor as follows:

- Wash your hand and face with your regular cleansing products,
- Pat dry your skin,
- Apply a generous amount of clay mask (using your fingers, a sponge...) evenly over the entire face, avoiding the eye and mouth areas – but including wrinkles in the crow's feet area of the face,
- Allow to dry for 15 to 20 minutes,
- Rinse off with warm water,
- Repeat three times per week.

The use of all other mask or peeling products was prohibited during the entire length of the study. Changes regarding the brand of regular skin care products, facial cleansing products or make-up products were not permitted during the week prior to the beginning of the study nor during the study. Participants were also instructed to avoid sun exposure during the study.

The study was conducted in a laboratory room with controlled temperature and relative humidity. On visit Day 0, after 15 minutes of acclimatization in the controlled room, digital photographs of clean face with no product (standard lighting mode) were taken using the Visia-CR Imaging System. Additionally, measurements of skin sebum level using the Sebumeter® were taken.

First application of the tested mask was performed at the laboratory under laboratory personnel supervision. Thirty minutes (30 minutes) after mask removal photographs and measurements of skin sebum level were performed. Additionally, the participants evaluated the effects of the test product after first application by completing an electronic self-evaluation questionnaire using a computer at the laboratory (with the help of the laboratory personnel, if needed).

Photographs and sebum level measurements were repeated on the second visit (Day 28). On Day 28, participants had to return their completed daily log as well as the sample containers with the unused portion of product to the laboratory. The unused portion of product and the daily log were intended for verification of the participants' adherence to the protocol. Participants also evaluated the test product by completing an electronic self-evaluation questionnaire using a computer at the laboratory (with the help of the laboratory personnel, if needed).

All observations were recorded for each participant in their respective Case Report Forms. In addition to the observations made and recorded by Evalulab, the participants were encouraged to observe and report to Evalulab any immediate or delayed reactions such as redness, irritation, itching and/or other sensations on the application area.

- **Test Methods**

Product Acceptance:

Product acceptance is defined by the overall participation of the subjects in the study and the level of tolerance to the test product in question. Tolerance to the product was evaluated based on the observed reactions if any, their degree of severity and reproducibility from one participant to another. These parameters were also evaluated using a self-evaluation questionnaire, which included a section on tolerance.

Perception of Product Performance by Self-Evaluation Questionnaire:

Each participant completed a self-evaluation questionnaire at the end of the study (Day 28). A section of the self-evaluation questionnaire was designed to gauge the participants' perception of sensory attributes (cosmetic qualities) and overall performance of the test product. Participants were also encouraged to write down the comments that they may have had regarding the test product.

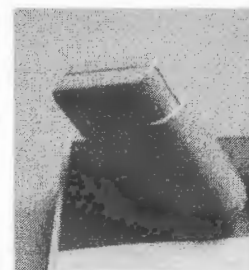
Skin Surface Sebum Level:

Sebum level was determined by using Sebumeter® SM810.

The measurement is based on grease spot photometry. The mat tape of the Sebumeter® SM 810 is brought into contact with skin (forehead). It becomes transparent in relation to the sebum on the surface of the measurement area. Then the tape is inserted into the aperture of the device and the transparency is measured by a photocell. The light transmission represents the sebum content. Values are expressed in μg of sebum / cm^2 of skin.

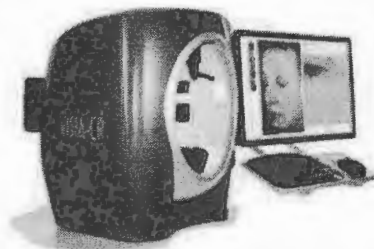
Below is an indication of the interpretation of the values measured on the forehead.

Skin type	Value (μg / cm^2)
Dry skin	< 100
Normal skin	100-220
Oily skin	> 220



Digital Photographs using Visia-CR Imaging System:

Digital photographs using the Visia-CR Imaging System (Canfield® Ltd, USA) were taken under standard conditions for illustration of overall skin appearance at D0-T0, D0-T30 and D28 for all participants. Photographs were taken under Standard lighting mode, resulting in 3 photos per participant (i.e., front full face, left profile and right profile) at each study time point. Photographs were intended for illustrative purposes and skin pores analysis.



Pores Analysis:

Pores analysis was performed using ImageJ Software (National Institute of Health, USA). Pores algorithm is able to detect pores within a Region of Interest (ROI) on Standard light images and to calculate their number (closed and open pores) and fractional area.

The analysis was performed automatically by the image analysis software. The program also has the ability to keep the same ROI and to replace it at the same place from a visit to another.



9. Statistical Method

Statistical analysis was carried out on all pertinent parameters (i.e., measurements of skin sebum level, pores results). Results obtained after treatment (D0-T30 and D28) were compared to the baseline (D0-T0) using the Student's paired t-Test.

Whenever appropriate, the results were expressed as the mean of all participants' measurements.

10. Adverse Events or Serious Adverse Events

The participants were instructed to immediately communicate any reactions to Evalulab.

An "Adverse Event" is defined as any noxious and unintended response observed in a participant testing a product that does not necessarily have a causal relation with the test product in question.

The risks for adverse events associated with this test may vary amongst the participants. Participants may be subject to rash (intense redness), stinging and burning sensations, cracking, exfoliation effect, dryness or even pain if the test product is strongly irritant or if the participant is particularly sensitive to the product. Participants may also develop an allergic sensitization to the test product or to its components.

The term "Serious Adverse Event" refers to any untoward medical occurrence, related or not to the test product that may lead to death, persistent or significant disability, that requires hospitalization or prolongation of a hospitalization period or that provokes invalidity, significant or permanent incapacity, or that translates to congenital anomaly or malformation.

11. Amendments to Protocol

No amendments were made to the protocol.

RESULTS

1. Product Acceptance

• Participation

Twenty (20) participants between 47 and 73 years of age (Average Age = 61.20) were recruited for this study. All participants completed the study without incident. The profile of the participants is described below:

Sex	Number	Age	Average Age
Male	3	65 to 70	67.67
Female	17	47 to 73	60.06
Total	20	47 to 73	61.20

• Adherence to Protocol

Adherence to the protocol was in part qualified by following product usage by the participants between Day 0 and Day 28. Product usage details are presented in the Appendices in Tables II.

During the entire length of the study (Day 0 to Day 28), the average use for the test product "Glacial Oceanic Clay Mask" was recorded at 9.89g per application.

Based on these results, it is considered that all participants were compliant with the test protocol.

• Tolerance after first application (D0-T30) and at D28

No "Serious Adverse Events" were noted by the investigator during the entire length of the study for all participants.

All data for tolerance collected at D0-T30 and D28 from the self-evaluation questionnaires completed by the participants are presented in the Appendices – Table III.A for D0-T30 and Table III.B for D28.

As part of the questionnaire, participants were to judge their tolerance to the test product by selecting "None", "Slight", "Moderate" or "High" for each of the intolerance criteria. The list of intolerance criteria and the responses expressed as the percentage of participants responding in each category after first application (D0-T30) and after 28 days of use are provided in Figures 2 & 3 below.

Figure 2. Overall Scores for Tolerance at D0-T30 (After first application)

Criteria	"None"	"Slight"	"Moderate"+"High"
Burning sensation	95%	5%	0%
Ocular discomfort	95%	5%	0%
Stinging	85%	10%	5%
Redness	80%	10%	10%
Tightness	70%	30%	0%

Thirty minutes (D0-T30) after first application of the test mask, thirty percent (30%) of the participants declared having experienced "Slight" tightness. Additionally, ten percent (10%) of the participants reported "Slight" redness and stinging, while there were five percent (5%) of them who experienced "Slight" burning sensation or ocular discomfort.

Finally, there were also few reports of "Moderate" redness (10%) and stinging (5%).

According to the participants' comments (see Table III.A in the Appendices – Details), the participants experienced those intolerance signs while the tested mask was drying on their skin.

Figure 3. Overall Scores for Tolerance at D28

Criteria	"None"	"Slight"	"Moderate" + "High"
Burning sensation	100%	0%	0%
Ocular discomfort	100%	0%	0%
Stinging	95%	5%	0%
Redness	90%	10%	0%
Tightness	45%	40%	15%

During the 28 days of the study, a total of fifty-five percent (55%) of the participants reported skin tightness (40% at a "Slight" level and 15% at "Moderate" + "High" levels). Ten percent (10%) of the participants experienced "Slight" skin redness and five percent (5%) of them experienced "Slight" stinging.

It is important to note that most of the reported intolerance signs were experienced by the participants during the drying time and have disappeared spontaneously after mask removal or after applying a cream (*See Table III.B in the Appendices – Details*)

Overall, the test product may be considered as well tolerated by the participants.

2. Product Efficacy

The purpose of the self-evaluation questionnaire was to assess the participants' opinions on sensory attributes and their perception of product efficacy after first application (D0-T30) and after 28 days of use.

• Sensory Attributes after first application (D0-T30)

Highlights of the sensory scores are expressed as the combined percentage of participants who indicated either "Highly appreciated" or "Appreciated" (Total Appreciated) for each sensory attribute.

All scores for sensory attributes collected at D0-T30 from the self-evaluation questionnaires completed by the participants are presented in Table IV in the Appendices.

The results obtained are summarized in Figure 4 and discussed below.

Figure 4. Overall Scores for Sensory Attributes after First Application (D0-T30)

Criteria	"Total Appreciated"
Feeling after application	90%
Hydrating effect after application	50%

The overall level of appreciation for each sensory attribute was good for this type of product.

The great majority of the participants (90%) appreciated the feeling after application of the tested mask. Fifty percent (50%) of participants considered that the test product provided a hydrating effect after application.

• Perception of Product Performance at D0-T0 and D28

To assess the perception of product performance, the participants were asked to answer questions regarding the effect of the tested mask on various parameters such as signs of aging, skin sebum level, skin texture, skin tone as well as its potential cooling and soothing effects.

Participants were asked to evaluate the test product effectiveness after first application (D0-T30) and after 28 days of use. All scores for performance collected from the self-evaluation questionnaires completed by the participants are presented in the Appendices – Table V.A for D0-T30 and Table V.B for D28.

The performance scores collected at D0-T30 and at D28 have been summarized as the total percentage of positive responses ("Somewhat agree" + "Strongly agree") for each criterion in Figure 5.

Figure 5. Overall Scores for Performance at D0-T30 and D28

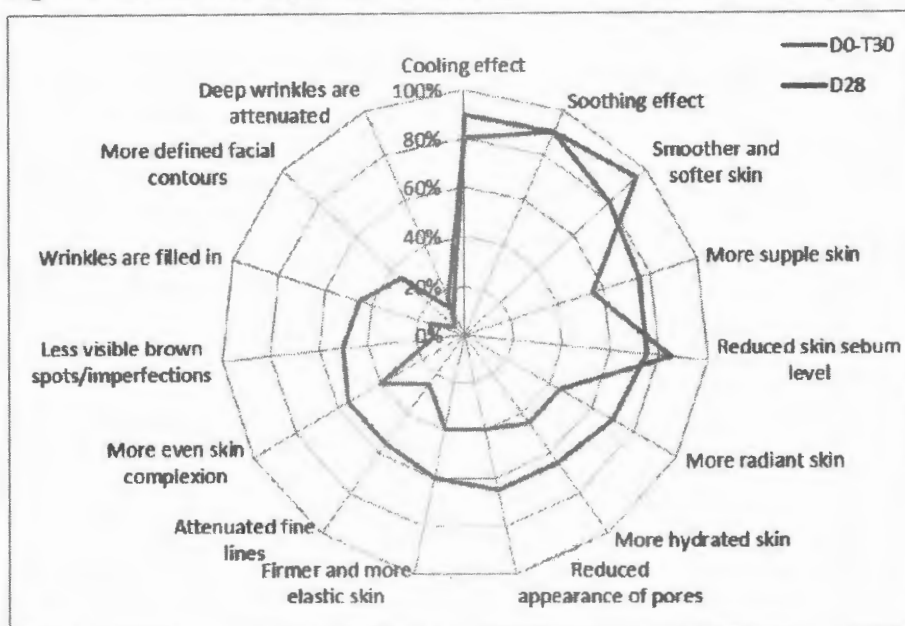
Criteria	"Positive Responses"	
	D0-T30	D28
Provides a cooling effect	80%	90%
Provides a soothing effect	90%	90%
Smoother and softer skin	95%	80%
More supple skin	55%	75%
Reduced skin sebum level	85%	75%
More radiant skin	45%	70%
More hydrated skin	45%	65%
Reduced appearance of pores	40%	65%
Firmer and more elastic skin	40%	60%
Fine lines are attenuated	25%	55%
More even skin complexion	40%	55%
Brown spots and imperfections are less visible	13%	50%
Wrinkles are filled in	15%	45%
More defined facial contours	5%	35%
Deep wrinkles are attenuated	17%	11%

After 28 days of treatment, the great majority (90%) of the participants declared that the tested mask provided cooling and soothing effects. Eighty percent (80%) of the participants found their skin smoother and softer. According to seventy-five percent (75%) of the participants, the test product provided a more supple skin and reduced their skin sebum level. A visible more radiant skin was observed by seventy percent (70%) of the participants. The effects of the test mask in improving skin hydration and reducing pores appearance received a score of sixty-five percent (65%).

The ability of the test product in reducing visible signs of aging received a total of positive responses varying from 60% to 11%. Indeed, sixty-percent (60%) of the participants found their skin firmer and more elastic at the end of the study. Fifty-five percent (55%) of the participants noticed their fine lines were attenuated and their skin complexion was more even. They were fifty percent (50%) of the participants who consider that their brown spots and imperfections were less visible. Forty-five percent (45%) of the participants noticed their wrinkles were filled in. The effect of the test product on facial contours received a score of thirty-five percent (35%). Finally, the effect of the tested clay mask on deep wrinkles was observed by only eleven percent (11%) of participants.

In figure 6 (next page) is represented an evolution of the performance of the tested mask, according to the participants' perception.

Figure 6. Evolution of the perception of mask performance from D0-T30 to D28



The average of performance scores was of forty-six percent (46%) at D0-T30, versus sixty-one percent (61%) at D28.

• Other Data

Responses to additional questions at the completion of the study (Day 28) pertained to suitability of the test product for people with mature and normal to oily skin types, global performance as well as readiness to purchase and recommendation. The results obtained from the self-evaluation questionnaires are presented in Table VI in the Appendices, and summarized below.

Eighty percent (80%) of the participants considered that the tested mask would be suitable for "mature and normal to oily skin types".

By combining the scores for "Excellent", "Very Good" and "Good", the global performance of the test product was rated as follows: eighty percent (80%) of the participants (30% "Excellent", 45% "Very Good" and 5% "Good") considered the test product as a performing facial mask.

Seventy-five percent (75%) of the participants would consider purchasing the test product. Among the participants who would consider purchasing the test product and who regularly use a facial mask, sixty-seven percent (67%) of them would replace their regular mask with the test product. Additionally, regarding the study sponsor's suggested retail price, eighty-seven percent (87%) of the participants who would consider purchasing the tested mask would be ready to pay 24.99\$.

Finally, eighty percent (80%) of the participants would recommend the test product to others.

• Comments

The complete list of all the comments expressed by the participants at D0-T30 and D28 about the test product is presented in Tables VII.A and VII.B in the Appendices. None of these comments need to be underscored or discussed at any length.

• Description of the Habitual Use

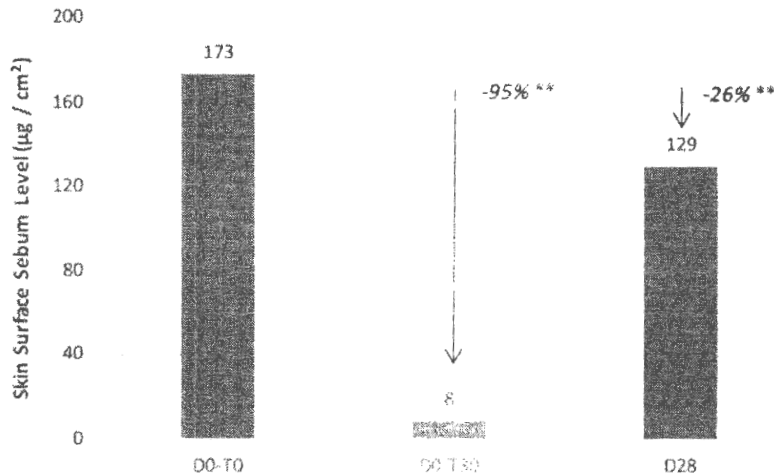
The description of the habitual use of a facial mask or a peeling product for each participant is presented in Table VIII in the Appendices. None of the descriptions presented in this table need to be underscored or discussed at any length.

3. Skin Surface Sebum Results (assessed via Sebumeter®)

The ability of the test product to reduce skin surface sebum level was assessed using the Sebumeter®. All individual data obtained during the study and statistical analysis are presented in the Appendices - Table IX.A for D0-T0, Table IX.B for D0-T30 and Table IX.C for D28.

The results obtained are summarized in Figure 7 below with a discussion.

Figure 7. Evolution of Skin Surface Sebum Level at D0-T30 and D28 compared to D0-T0



(Statistical Significance: ** = $p < 0.01$)

Statistical analysis of the data demonstrated that **skin surface sebum level was significantly reduced** ($p < 0.01$) after first application of the test mask as well as after 28 days of treatment.

A significant ($p < 0.01$) average reduction of 95% in skin surface sebum level was measured at D0-T30. In many cases, sebum was totally removed from the skin surface (reduction of 100% for participants #01-0627-006, #01-0627-018 and #01-0627-019).

At visit D28, a significant ($p < 0.01$) average improvement of 26% in skin surface sebum level was observed with effectiveness values up to 93% (participant #01-0627-006).

The test product may be considered as effective in reducing skin surface sebum.

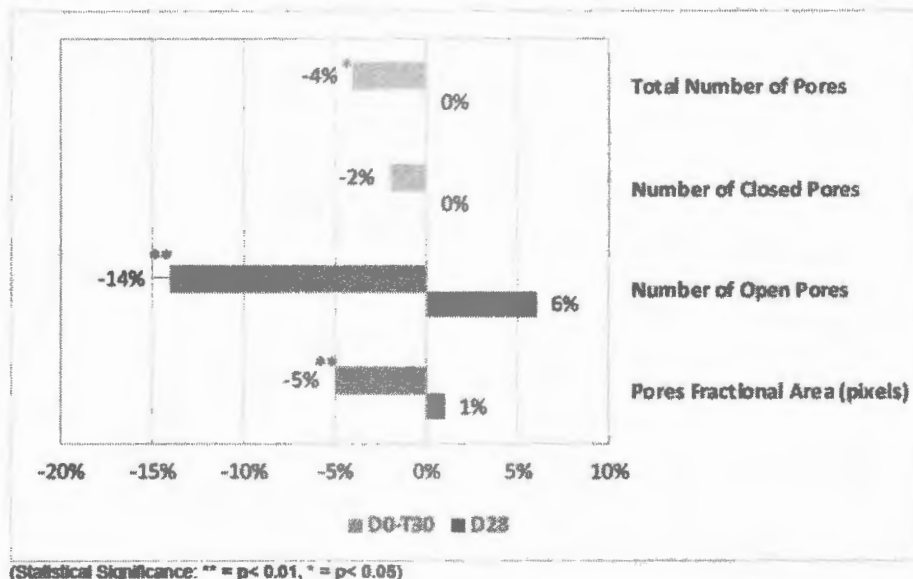
4. Pores Appearance (assessed via Image Analysis)

The ability of the test product to improve the appearance of pores on the face was assessed by image analysis based on photograph taken in standard conditions with Visia-CR® imaging system.

The summary of pores analysis expressed as the total number of pores, the number of closed pores, the number of open pores and pores fractional area, is provided in Figure 8. The data provided below for each parameter corresponds to the average obtained for the entire studied group.

The individual results for these parameters are presented in the Appendices – from Table X.A to Table X.D.

Figure 8. Evolution of Pores Parameters at D0-T30 and at D28 when compared to D0-T0



The total number of pores combines the number of barely perceptible pores, closed pores and open pores. Statistical analysis performed on the study results demonstrated a significant decrease ($p < 0.05$) in the total number of pores after first application (D0-T30) with an average reduction of 4%. The maximal improvement found in the test panel for this parameter was of -28% (participant #01-0627-020). No statistically significant changes were observed for this parameter at D28.

The number of closed pores represents the number of pores classified as non-dilated. Statistical analysis performed on the study results did not demonstrate any significant changes in the number of closed pores at D0-T30 nor at D28.

The number of open pores represents the number of pores classified as dilated. After first application (D0-T30), a statically significant ($p < 0.01$) reduction of 14% was observed for the number of open pores with effectiveness values up to 62% (participant #01-0627-006). No statistically significant changes were observed for this parameter at D28.

The fractional area corresponds to the total area occupied by pores relative to the area of the ROI (Region of Interest) for the analysis. Statistical analysis performed on the study results demonstrated a significant decrease ($p < 0.01$) in the pores fractional area at D0-T30 with an average reduction of 5%. The maximal improvement found in the test panel for this parameter was -24% (participant #01-0627-020). No statistically significant changes were observed for this parameter at D28.

The test product may be considered as having an immediate effect in reducing the appearance of pores, particularly dilated pores.

SUMMARY

In this study, the product "Glacial Oceanic Clay Mask" was evaluated for performance and tolerance over a period of 28 days by twenty (20) men and women healthy participants. The recruited participants applied the test product three (3) times per week over the entire face including wrinkles in the crow's feet area.

During the entire length of the study, the test product was very well accepted and tolerated by the participants. The tolerance and the perceived efficacy of the test product were evaluated via **self-evaluation questionnaires**.

There were reports of "Slight" to "Moderate" + "High" levels of tightness experienced by the participants while the tested mask was drying on their faces.

After 28 days of product use, the great majority (90%) of the participants declared that the tested mask provided cooling and soothing effects. Eighty percent (80%) of them noticed a smoother and softer skin. According to seventy-five percent (75%) of the participants, the tested mask reduced their skin sebum level. Additionally, sixty-five percent (65%) of the participants noticed the test product improved their skin hydration and reduced the appearance of their pores.

A significant proportion of participants (80%) considered that the test mask was suitable for people with mature and normal to oily skin types. Overall, the majority of the participants (80%) rated the test product as a performing facial mask.

Skin Surface Sebum was assessed with the Sebumeter® device.

Statistical analysis of the data demonstrated a significant ($p<0.01$) average reduction of 95% in skin sebum level ($p<0.01$) after first application (D0-T30) and an average reduction of 26% after 28 days of product application.

The effect of the test mask on the **appearance of pores** was assessed by image analysis.

After first application (D0-T30), statistical analysis conducted on the obtained results demonstrated a significant reduction of 4% ($p<0.05$) in the total number of pores, of 14% ($p<0.01$) in the number of open pores and of 5% ($p<0.01$) in the pores fractional area. There were no significant changes in the appearance of pores at visit D28. Additionally, no significant change in the number of closed pores was observed during the study.

CONCLUSION

Under the conditions of this study, the test product "Glacial Oceanic Clay Mask" developed by Ironwood Clay Company Inc. was well tolerated and well appreciated by the participants for various subjective parameters such as sensory attributes and improvement of overall skin condition. Ninety percent (90%) of the participants declared that the tested mask provided cooling and soothing effects along with a smoother and a softer skin (80%).

Furthermore, the test product provided a significant average reduction in skin surface sebum of 95%. The test product also provided an immediate effect in reducing the appearance of pores.

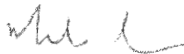
I, the undersigned, Marie-Laure Oula, declare that this study was conducted in accordance with the principles of "Good Clinical Practice" and that the information provided in this report reflects in a complete and exact manner the results obtained during the study.



Signature
Marie-Laure Oula, M. Sc.
Investigator, Laboratory Director

Revision Date: Mont-Royal – July 25th, 2018

I, the undersigned, Marilou Nadeau, declare having validated the information provided in this report.



Signature
Marilou Nadeau, B. Sc.
Quality Assurance Director

Revision Date: Mont-Royal – July 25th, 2018

APPENDICES

Table I – Participants' Profile

Participant #			Initials	Age	Sex
01	-0627-	001	LB	57	F
01	-0627-	002	AM	69	F
01	-0627-	003	FZA	68	F
01	-0627-	004	HC	51	F
01	-0627-	005	SR	60	F
01	-0627-	006	MF	73	F
01	-0627-	007	PL	66	F
01	-0627-	008	LL	51	F
01	-0627-	009	LMD	67	F
01	-0627-	010	ED	66	F
01	-0627-	011	AD	68	F
01	-0627-	012	MA	52	F
01	-0627-	013	MDL	66	F
02	-0627-	014	JPL	56	M
02	-0627-	015	AP	47	M
02	-0627-	016	RP	56	M
01	-0627-	017	SMH	48	F
01	-0627-	018	BFM	68	F
01	-0627-	019	AC	70	F
01	-0627-	020	AMF	65	F

Table II – Product's weight

Participant #	Weight (D0 to D28) - Glacial Oceanic Clay Mask		
	Before (D0)	After (D28)	Difference at D28
001	330.00	180.84	149.16
002	328.00	149.88	178.12
003	327.00	214.81	112.20
004	326.00	221.26	104.74
005	329.00	235.80	93.20
006	328.00	131.97	196.03
007	324.00	162.34	161.66
008	327.00	175.26	151.74
009	326.00	224.10	101.90
010	325.00	232.20	92.80
011	327.00	219.47	107.53
012	328.00	249.43	78.57
013	331.00	253.69	77.32
014	326.00	123.38	202.62
015	326.00	183.60	142.40
016	327.00	163.97	163.03
017	328.00	189.25	138.76
018	328.00	170.95	157.05
019	328.00	245.26	82.74
020	328.00	217.82	110.18
Average			130.09
Average use per application			9.89

Table IIIA – Scores for Tolerance at D0-T30

Participant #	Redness	Stinging	Tightness	Burning sensation	Ocular discomfort	Details
001	None	None	None	None	None	
002	None	None	Slight	None	None	
003	None	None	None	None	None	
004	Moderate	None	None	None	None	
005	None	Slight	None	None	Slight	Slight pressure on the eye contour area
006	None	None	Slight	None	None	When the mask was drying on the skin.
007	Moderate	None	None	Slight	None	Burning: when the mask was drying on the skin.
008	None	None	None	None	None	
009	None	None	Slight	None	None	
010	Slight	Moderate	Slight	None	None	When the mask was drying on the skin.
011	None	None	None	None	None	
012	None	None	None	None	None	
013	None	None	None	None	None	
014	None	None	None	None	None	
015	None	None	None	None	None	
016	None	Slight	Slight	None	None	When the mask was drying on the skin.
017	Slight	None	Slight	None	None	Around the nose area, when the mask was drying on the skin.
018	None	None	None	None	None	
019	None	None	None	None	None	
020	None	None	None	None	None	

Table III.B – Scores for Tolerance at D28

Participant #	Redness	Stinging	Tightness	Burning sensation	Ocular discomfort	Details
001	None	None	None	None	None	
002	None	None	None	None	None	
003	Slight	None	Moderate	None	None	Redness: During 10 minutes after mask removal / Tightness: When the mask was drying on the face.
004	None	None	None	None	None	
005	Slight	None	Moderate	None	None	During 2 first applications / When the mask was drying on the face.
006	None	None	High	None	None	After mask removal, until I applied my hydrating cream.
007	None	None	None	None	None	
008	None	None	Slight	None	None	When the mask was drying on the skin.
009	None	None	None	None	None	
010	None	Slight	Slight	None	None	When the mask was drying on the skin.
011	None	None	Slight	None	None	When the mask was drying on the skin.
012	None	None	Slight	None	None	When the mask was drying on the skin.
013	None	None	Slight	None	None	When the mask was drying on the skin.
014	None	None	None	None	None	
015	None	None	Slight	None	None	When the mask was drying on the skin.
016	None	None	Slight	None	None	When the mask was drying on the skin.
017	None	None	None	None	None	
018	None	None	Slight	None	None	When the mask was drying on the skin.
019	None	None	None	None	None	
020	None	None	None	None	None	

Table IV – Scores for Sensory Attributes at D0-T30

Participant #	Feeling after application	Hydrating effect after application
001	Indifferent	Indifferent
002	Highly Appreciated	Appreciated
003	Highly Appreciated	Appreciated
004	Highly Appreciated	Indifferent
005	Highly Appreciated	Appreciated
006	Highly Appreciated	Indifferent
007	Indifferent	Did not Appreciated
008	Highly Appreciated	Highly Appreciated
009	Appreciated	Indifferent
010	Highly Appreciated	Indifferent
011	Highly Appreciated	Appreciated
012	Highly Appreciated	Indifferent
013	Appreciated	Appreciated
014	Highly Appreciated	Appreciated
015	Highly Appreciated	Indifferent
016	Highly Appreciated	Appreciated
017	Appreciated	Appreciated
018	Highly Appreciated	Indifferent
019	Appreciated	Indifferent
020	Highly Appreciated	Appreciated

Table V.A – Scores for Performance at P0-T30

Participant #	More hydrated skin	More supple skin	Firmer and more elastic skin	Smoother and softer skin	More radiant skin
001	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
002	Somewhat Agree	Strongly Agree	Somewhat Agree	Strongly Agree	Strongly Agree
003	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Strongly Agree	Somewhat Agree
004	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
005	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Strongly Agree	Strongly Agree
006	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
007	Fully Disagree	Fully Disagree	Somewhat Agree	Somewhat Disagree	Fully Disagree
008	Strongly Agree	Somewhat Disagree	Somewhat Disagree	Strongly Agree	Somewhat Disagree
009	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
010	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
011	Somewhat Agree	Somewhat Agree	Somewhat Disagree	Strongly Agree	Somewhat Disagree
012	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
013	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
014	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
015	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
016	Somewhat Agree	Somewhat Agree	Somewhat Agree	Strongly Agree	Somewhat Agree
017	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
018	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
019	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
020	Somewhat Agree	Strongly Agree	Strongly Agree	Strongly Agree	Somewhat Agree

Table V.A – Scores for Performance at D0-T30 (cont'd)

Participant #	Fine lines are attenuated	Wrinkles are filled in	Deep wrinkles are attenuated	More defined facial contours	More even skin complexion
001	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree
002	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Disagree	Somewhat Agree
003	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Fully Disagree	Somewhat Disagree
004	Somewhat Disagree	Somewhat Disagree	Not applicable	Somewhat Disagree	Somewhat Disagree
005	Somewhat Disagree	Somewhat Disagree	Not applicable	Fully Disagree	Strongly Agree
006	Somewhat Disagree	Somewhat Disagree	Not applicable	Somewhat Disagree	Somewhat Disagree
007	Somewhat Agree	Somewhat Agree	Somewhat Agree	Fully Disagree	Fully Disagree
008	Somewhat Disagree	Somewhat Disagree	Not applicable	Somewhat Disagree	Somewhat Disagree
009	Somewhat Disagree	Somewhat Disagree	Not applicable	Somewhat Disagree	Somewhat Disagree
010	Somewhat Disagree	Somewhat Disagree	Fully Disagree	Somewhat Agree	Somewhat Agree
011	Somewhat Disagree	Somewhat Disagree	Fully Disagree	Somewhat Disagree	Fully Disagree
012	Somewhat Disagree	Fully Disagree	Not applicable	Somewhat Disagree	Somewhat Disagree
013	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree
014	Fully Disagree	Fully Disagree	Fully Disagree	Somewhat Disagree	Somewhat Agree
015	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree
016	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree
017	Somewhat Agree	Somewhat Agree	Not applicable	Somewhat Disagree	Somewhat Disagree
018	Fully Disagree	Fully Disagree	Fully Disagree	Fully Disagree	Somewhat Disagree
019	Somewhat Disagree	Somewhat Disagree	Fully Disagree	Somewhat Disagree	Somewhat Disagree
020	Somewhat Disagree	Somewhat Disagree	Not applicable	Somewhat Disagree	Somewhat Agree

Table V.A – Scores for Performance at D0-T30 (cont'd & end)

Participant #	Brown spots and imperfections are less visible	Reduced skin sebum level	Reduced appearance of pores	Provides a soothing effect	Provides a cooling effect
001	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
002	Somewhat Disagree	Strongly Agree	Somewhat Disagree	Strongly Agree	Strongly Agree
003	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Strongly Agree	Somewhat Agree
004	Somewhat Disagree	Strongly Agree	Somewhat Disagree	Somewhat Agree	Somewhat Agree
005	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Strongly Agree	Strongly Agree
006	Somewhat Disagree	Strongly Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
007	Fully Disagree	Strongly Agree	Somewhat Agree	Fully Disagree	Fully Disagree
008	Somewhat Disagree	Strongly Agree	Somewhat Disagree	Strongly Agree	Strongly Agree
009	Fully Disagree	Strongly Agree	Somewhat Agree	Somewhat Agree	Fully Disagree
010	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Strongly Agree
011	Fully Disagree	Somewhat Disagree	Somewhat Disagree	Strongly Agree	Strongly Agree
012	<i>Not applicable</i>	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree
013	Somewhat Agree	Strongly Agree	Somewhat Agree	Strongly Agree	Strongly Agree
014	<i>Not applicable</i>	Somewhat Agree	Somewhat Disagree	Strongly Agree	Strongly Agree
015	<i>Not applicable</i>	Somewhat Agree	Somewhat Disagree	Strongly Agree	Strongly Agree
016	<i>Not applicable</i>	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree
017	Somewhat Agree	Strongly Agree	Somewhat Agree	Strongly Agree	Strongly Agree
018	Fully Disagree	Somewhat Disagree	Somewhat Disagree	Strongly Agree	Strongly Agree
019	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Strongly Agree	Strongly Agree
020	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree

Table V.B – Scores for Performance at D28

Participant #	More hydrated skin	More supple skin	Firmer and more elastic skin	Smoother and softer skin	More radiant skin
001	Fully Disagree	Somewhat Disagree	Fully Disagree	Fully Disagree	Somewhat Disagree
002	Somewhat Agree	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree
003	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree
004	Fully Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree
005	Somewhat Agree	Somewhat Agree	Somewhat Disagree	Somewhat Agree	Strongly Agree
006	Fully Disagree	Somewhat Agree	Somewhat Disagree	Somewhat Agree	Somewhat Agree
007	Somewhat Agree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Somewhat Disagree
008	Strongly Agree	Strongly Agree	Somewhat Agree	Strongly Agree	Somewhat Disagree
009	Strongly Agree	Somewhat Agree	Strongly Agree	Strongly Agree	Strongly Agree
010	Somewhat Agree	Somewhat Agree	Somewhat Agree	Strongly Agree	Somewhat Agree
011	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Disagree
012	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Strongly Agree	Somewhat Agree
013	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree	Strongly Agree
014	Strongly Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
015	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Strongly Agree	Somewhat Agree
016	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
017	Strongly Agree	Strongly Agree	Somewhat Agree	Strongly Agree	Somewhat Agree
018	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
019	Somewhat Agree	Somewhat Agree	Somewhat Disagree	Strongly Agree	Somewhat Agree
020	Strongly Agree	Strongly Agree	Strongly Agree	Strongly Agree	Strongly Agree

Table V.B – Scores for Performance at D28 (cont'd)

Participant #	Fine lines are attenuated	Wrinkles are filled in	Deep wrinkles are attenuated	More defined facial contour	More even skin complexion
001	Somewhat Disagree	Somewhat Disagree	<i>Not applicable</i>	Fully Disagree	Somewhat Disagree
002	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
003	Somewhat Agree	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree
004	Somewhat Disagree	Somewhat Disagree	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree
005	Somewhat Agree	Somewhat Disagree	Fully Disagree	Fully Disagree	Somewhat Agree
006	Somewhat Disagree	Somewhat Disagree	<i>Not applicable</i>	Somewhat Disagree	Somewhat Agree
007	Somewhat Agree	Somewhat Agree	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree
008	Somewhat Disagree	Somewhat Disagree	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree
009	Strongly Agree	Strongly Agree	<i>Not applicable</i>	Strongly Agree	Strongly Agree
010	Somewhat Agree	Somewhat Agree	Fully Disagree	Strongly Agree	Somewhat Agree
011	Somewhat Disagree	Somewhat Disagree	Fully Disagree	Somewhat Disagree	Somewhat Disagree
012	Somewhat Disagree	Somewhat Disagree	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree
013	Somewhat Agree	Somewhat Agree	<i>Not applicable</i>	Strongly Agree	Somewhat Agree
014	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree
015	Somewhat Agree	Somewhat Agree	<i>Not applicable</i>	Fully Disagree	Somewhat Agree
016	Somewhat Agree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
017	Somewhat Agree	Somewhat Agree	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree
018	Somewhat Disagree	Somewhat Disagree	Fully Disagree	Somewhat Disagree	Somewhat Agree
019	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Disagree
020	Somewhat Agree	Somewhat Agree	<i>Not applicable</i>	Somewhat Agree	Strongly Agree

Table V.B – Scores for Performance at D28 (cont'd & end)

Participant #	Brown spots and imperfections are less visible	Reduced skin sebum levels	Reduced appearance of pores	Provides a soothing effect	Provides a cooling effect
001	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree
002	Somewhat Agree	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree
003	Somewhat Agree	Somewhat Agree	Strongly Agree	Somewhat Agree	Somewhat Agree
004	Somewhat Disagree	Somewhat Agree	Somewhat Disagree	Somewhat Agree	Somewhat Agree
005	<i>Not applicable</i>	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree
006	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Strongly Agree
007	<i>Not applicable</i>	Fully Disagree	Somewhat Disagree	Somewhat Disagree	Somewhat Disagree
008	Somewhat Disagree	Strongly Agree	Somewhat Disagree	Strongly Agree	Strongly Agree
009	Strongly Agree	Strongly Agree	Strongly Agree	Strongly Agree	Somewhat Agree
010	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree	Strongly Agree
011	Fully Disagree	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree
012	<i>Not applicable</i>	Somewhat Disagree	Somewhat Disagree	Somewhat Agree	Somewhat Agree
013	<i>Not applicable</i>	Strongly Agree	Strongly Agree	Somewhat Agree	Strongly Agree
014	<i>Not applicable</i>	Somewhat Disagree	Somewhat Agree	Strongly Agree	Strongly Agree
015	<i>Not applicable</i>	Somewhat Agree	Somewhat Disagree	Strongly Agree	Somewhat Agree
016	<i>Not applicable</i>	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree
017	Somewhat Disagree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
018	Fully Disagree	Somewhat Disagree	Somewhat Disagree	Strongly Agree	Strongly Agree
019	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree	Somewhat Agree
020	Somewhat Agree	Somewhat Agree	Somewhat Agree	Strongly Agree	Strongly Agree

Table VI – Scores for Overall Performance of the Test Product (data collected at Day 28)

Participants #	Suitability of the test product for mature and normal to oily skin types	Global performance : How would you rate this face mask product overall?	Would you recommend the test product?	Would you purchase the test product?	If yes, would you purchase the test product at 24.99\$?	If yes, would you replace your regular face mask product with the test product?
001	No	Bad	No	No		
002	Yes	Excellent	Yes	Yes	Yes	Yes
003	Yes	Very Good	Yes	Yes	No	No
004	No	Bad	No	No		
005	Yes	Very Good	Yes	Yes	Yes	Not Applicable
006	No	Acceptable	No	No		
007	No	Bad	No	No		
008	Yes	Very Good	Yes	Yes	Yes	Not Applicable
009	Yes	Excellent	Yes	Yes	Yes	Not Applicable
010	Yes	Excellent	Yes	Yes	Yes	Yes
011	Yes	Good	Yes	Yes	No	Not Applicable
012	Yes	Very Good	Yes	Yes	Yes	Not Applicable
013	Yes	Excellent	Yes	Yes	Yes	Yes
014	Yes	Excellent	Yes	No		
015	Yes	Very Good	Yes	Yes	Yes	Not Applicable
016	Yes	Very Good	Yes	Yes	Yes	Not Applicable
017	Yes	Very Good	Yes	Yes	Yes	No
018	Yes	Very Good	Yes	Yes	Yes	Not Applicable
019	Yes	Very Good	Yes	Yes	Yes	Not Applicable
020	Yes	Excellent	Yes	Yes	Yes	Yes

Table VII.A – Comments (collected from self-evaluation questionnaires at D0-T30)

Participant #	Positive comments	Negative comments
002	This is an excellent clay mask!	
004		I haven't noticed any "magical effect" after first application, it is a regular clay mask.
005	My skin tone is brighter.	
011		I have the impression that my dark circles under the eyes are more visible.

Table VII.B – Comments (collected from self-evaluation questionnaires at D28)

Participant #	Positive comments	Negative comments
001		The product dries too fast (after 10 minutes). I haven't noticed any positive effect of the mask. My skin is less soft than before.
002	This mask is fantastic! This is a very good product for oily and mature skin.	
003		I experienced slight redness on the cheeks and the nose after every application. Apply this type of mask 3 times per week is too much.
004	I would recommend this product for young people with oily skin and imperfections such as pimples, blackheads.	I think that apply this kind of product three times per week is too much.
005	The product dries very fast.	I experienced slight tightness during the first week of the study.
006	This product is very refreshing. This mask removes sebum well. I would recommend it to people suffering from acne. I think the product might be good for pimples or blackheads.	This product does not have any anti-aging effect, plus it dries out my skin a lot (maybe 3 times a week was too much).
007		I time, the product turns my skin dryer but more oily. The product is not practical to use and it is very messy.
009	I like the product! I think that it works for me. I receive a lot of compliments, people telling me that my skin is more radiant, smoother et more beautiful than before!	
010	I like the product.	
013	My pores look more tight. My skin is firmer. My skin tone is brighter. People even told me that my skin was smoother and that my facial contours were more defined. I feel that I have less sebum on my face. I would purchase this product as soon as it will be on the market!	
014	The product has a pleasant refreshing effect.	
016	Overall, I enjoyed the mask and the clean feeling I felt after application.	When the mask was drying up, it was a bit itchy.
017	Even if I think it is a good product for sebum and pores appearance.	This product does not hydrate the skin at all, this is why I would not purchase the product...
019	This is a very good product. My skin is smoother. Nice effect on the skin. The results are very good.	

Table VIII – Description of the Habitual Use of a Mask or Peeling Product

Participant #	Do you regularly use a face mask product?	If yes, Brand and Name of your regular product
001	No	
002	Yes	Homemade creamy mask - Coconut oil + Avocado
003	Yes	AslaVital - MineralActive Face Mask
004	Yes	Homemade mask - Salt + Honey
005	No	
006	Yes	Organic Dead Sea Mud Mask from Israel
007	No	
008	No	
009	No	
010	Yes	Homemade mask - Salt + Apricot oil
011	No	
012	No	
013	Yes	Organic Clay Mask
014	No	
015	No	
016	No	
017	Yes	Garnier PureActive - Self Heating Mask
018	No	
019	No	
020	Yes	Yves Rocher - Purifying Mask

Table IX.A – Individual Data for Skin Surface Sebum Level – D0-T0

D0-T0 - RESULTS	Measurements		
	Forehead		
Participant #	#1	#2	Mean
001	88	148	118
002	140	132	136
003	124	129	127
004	198	268	233
005	159	132	146
006	194	246	220
007	353	336	345
008	166	125	146
009	145	142	144
010	135	161	148
011	102	91	97
012	103	81	92
013	163	144	154
014	292	283	288
015	140	132	136
016	210	251	231
017	285	283	284
018	108	122	115
019	151	147	149
020	176	146	161
			173
			69

Table IX.B – Individual Data for Skin Surface Sebum Level – D0-T30

D0-T30 - RESULTS	Measurements		Mean	% Sebum Evolution
	Forehead			
Participant #	#1	#2		
001	4	7	6	-95%
002	2	0	1	-99%
003	2	2	2	-98%
004	21	18	20	-92%
005	3	6	5	-97%
006	2	0	1	-100%
007	30	39	35	-90%
008	11	17	14	-90%
009	1	2	2	-99%
010	21	6	14	-91%
011	3	1	2	-98%
012	2	1	2	-98%
013	1	1	1	-99%
014	18	17	18	-94%
015	2	1	2	-99%
016	10	14	12	-95%
017	18	24	21	-93%
018	0	0	0	-100%
019	1	0	1	-100%
020	4	2	3	-98%
			8	-95%
			9	
			P value	0.000
			Significance	S

(Statistical Significance: Blue = $p < 0.01$)

N.B. The t-Test result is expressed in p-value.

Decreases in Sebumeter® values correspond to improvements in skin surface sebum level.

Table IX.C – Individual Data for Skin Surface Sebum Level – D28

D28 - RESULTS	Measurements		Mean	% Sebum Evolution
	Forehead			
Participant #	#1	#2		
001	264	232	248	110%
002	58	53	55	-60%
003	116	98	107	-15%
004	231	161	196	-16%
005	47	57	52	-64%
006	13	16	15	-93%
007	259	327	293	-15%
008	113	87	100	-31%
009	78	67	73	-49%
010	84	78	81	-45%
011	36	34	35	-64%
012	132	132	132	43%
013	127	96	112	-27%
014	317	285	301	5%
015	67	71	69	-49%
016	98	122	110	-52%
017	276	290	283	0%
018	76	69	73	-37%
019	171	175	173	16%
020	71	67	69	-57%
			129	-26%
			89	
			P value	0.009
			Significance	S

(Statistical Significance: Blue = $p < 0.01$)

N.B. The t-Test result is expressed in p-value.

Decreases in Sebumeter® values correspond to improvements in skin surface sebum level.

Table X.A – Individual Data for Skin Pores Analysis at D0-T0, D0-T30 and D28 – Total Number of Pores

Participant #	Total Detected Pores			Evolution in Total Number of Detected Pores Compared to D0-T0	
	D0-T0	D0-T30	D28	D0-T30	D28
001	5498	4884	5407	-11%	-2%
002	6720	6680	6758	-1%	1%
003	5823	5366	6613	-8%	14%
004	5567	5290	5427	-5%	-3%
005	6050	6137	6291	1%	4%
006	5218	4872	5267	-7%	1%
007	8054	8116	8279	1%	3%
008	7489	7424	7741	-1%	3%
009	4011	3856	4030	-4%	0%
010	6515	6004	6438	-8%	-1%
011	4640	4479	4923	-3%	6%
012	7065	6584	6760	-7%	-4%
013	7460	7226	6951	-3%	-7%
014	5302	6806	5117	28%	-3%
015	6141	5965	5318	-3%	-13%
016	5161	4654	6334	-10%	23%
017	9118	8824	8268	-3%	-9%
018	6373	5755	5671	-10%	-11%
019	5832	5421	6189	-7%	6%
020	3401	2436	3588	-28%	5%
Average	6072	5839	6069	-4%	0%
St Dev				0.10	0.08
Min				28%	23%
Max				-28%	-13%
Significance				S	NS
P-value				0.044	0.976

(Statistical Significance: Red = $p < 0.05$)

N.B. The t-Test result is expressed in p-value.

Decreases in Total number of pores correspond to improvements in skin texture (appearance of pores)

Table X.B – Individual Data for Skin Pores Analysis at D0-T0, D0-T30 and D28 – Closed Pores

Participant #	Closed Pores			Evolution in Number of Closed Pores Compared to D0-T0	
	D0-T0	D0-T30	D28	D0-T30	D28
001	5182	4587	5051	-11%	-3%
002	5966	5960	5917	0%	-1%
003	5367	4878	6135	-9%	14%
004	5390	5121	5112	-5%	-5%
005	4994	4865	5220	-3%	5%
006	4338	4328	4779	0%	10%
007	6829	7113	6578	4%	-4%
008	6355	6485	6636	2%	4%
009	3176	3153	2991	-1%	-6%
010	5442	5187	5447	-5%	0%
011	4296	4256	4570	-1%	6%
012	5785	5773	6077	0%	5%
013	5994	6410	5783	7%	-4%
014	4489	5253	4328	17%	-4%
015	5311	4846	5109	-9%	-4%
016	5086	4420	6002	-13%	18%
017	7533	7726	6215	3%	-17%
018	4979	4818	4681	-3%	-6%
019	5348	4961	5724	-7%	7%
020	2158	2179	2164	1%	0%
Average	5201	5116	5226	-2%	0%
St Dev				0.07	0.08
Min				17%	18%
Max				-13%	-17%
Significance				NS	NS
P-value				0.292	0.814

N.B. The t-Test result is expressed in p-value

Table X.C – Individual Data for Skin Pores Analysis at D0-T0, D0-T30 and D28 – Open Pores

Participant #	Open Pores			Evolution in Number of Open Pores Compared to D0-T0	
	D0-T0	D0-T30	D28	D0-T30	D28
001	20	21	22	5%	10%
002	21	12	13	-43%	-38%
003	24	17	18	-29%	-25%
004	5	7	11	40%	120%
005	22	19	31	-14%	41%
006	21	8	14	-62%	-33%
007	59	58	69	-2%	17%
008	40	33	46	-18%	15%
009	21	23	23	10%	10%
010	47	38	43	-19%	-9%
011	9	14	19	56%	111%
012	12	11	11	-8%	-8%
013	35	29	39	-17%	11%
014	16	14	14	-13%	-13%
015	10	6	11	-40%	10%
016	14	18	9	29%	-36%
017	39	33	49	-15%	26%
018	28	20	27	-29%	-4%
019	9	10	5	11%	-44%
020	14	11	19	-21%	36%
Average	23	20	25	-14%	6%
St Dev				0.28	0.44
Min				56%	120%
Max				-62%	-44%
Significance				5	NS
P-value				0.008	0.325

(Statistical Significance: Blue = $p < 0.01$)

N.B. The t-Test result is expressed in p-value.

Decreases in number of open pores correspond to improvements in skin texture (appearance of pores)

Table X.D – Individual Data for Skin Pores Analysis at D0-T0, D0-T30 and D28 – Pores Fractional Area

Participants #	Pores Fractional Area			Evolution in Pores Fractional Area Compared to D0-T0	
	D0-T0	D0-T30	D28	D0-T30	D28
001	8.30	7.29	8.30	-12%	0%
002	7.94	7.79	7.73	-2%	-3%
003	8.97	7.91	10.30	-12%	15%
004	9.84	9.56	10.03	-3%	2%
005	8.93	8.41	9.48	-6%	6%
006	7.27	6.44	7.48	-11%	3%
007	12.89	13.36	12.88	4%	0%
008	11.74	11.50	11.98	-2%	2%
009	6.59	5.92	6.24	-10%	-5%
010	10.63	9.79	10.41	-8%	-2%
011	9.95	9.72	10.48	-2%	5%
012	10.30	9.64	10.70	-6%	4%
013	11.13	11.36	10.98	2%	-1%
014	6.10	7.58	5.95	24%	-3%
015	8.79	7.87	7.91	-11%	-10%
016	12.47	11.01	14.13	-12%	13%
017	13.11	12.66	11.34	-3%	-13%
018	9.30	8.42	8.86	-9%	-5%
019	8.54	7.68	9.00	-10%	5%
020	5.19	3.96	5.45	-24%	5%
Average	9.40	8.89	9.48	-5%	1%
St Dev				0.09	0.07
Min				24%	15%
Max				-24%	-13%
Significance				S	NS
P-value				0.003	0.612

(Statistical Significance: Blue = $p < 0.01$)

N.B. The t-Test result is expressed in p-value.

Decreases in pores fractional area correspond to improvements in skin texture (appearance of pores)

Project Final Report

Leaching of chemical elements from Canadian Natural Oceanic Clay

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Executive Summary

A review of previous studies commissioned by Ironwood Clay Company shows that the clay surface is negatively charged, which makes it an ideal ion exchanger. When the clay is applied to the skin, various cations and positively charged impurities on the skin can be removed by ion exchange mechanism. However, the clay materials can be both collectors and donors. It is likely that various elements are leached out from the clay material surfaces under different conditions that may interact with skin surfaces and cause irritation. For subsequent fully understanding of the interactions of the clay with skin surfaces, it is critical to firstly assess what elements can be released and under what conditions they can be released. The main conditions that affect the adsorption or release behavior of different elements on clay surfaces are pH, ionic strength, and temperature. The objective of this study was to investigate the effects of these factors on the leaching behaviour of the Natural Oceanic Clay harvested in British Columbia.

Using different techniques, we firstly characterized the mineralogy and chemical composition of the clay minerals, and then quantified the effects of pH, ionic strength, and temperature on the release of various elements from the clay materials. We divided the elements released into the solution into two categories: skin beneficial elements, including calcium (Ca), phosphorous (P), potassium (K), magnesium (Mg), selenium (Se), and zinc (Zn), and potential skin irritators, including cadmium (Cd), arsenic (As), chromium (Cr), and barium (Ba).

The main findings are: (1) increasing the pH resulted in a decrease in the concentrations of almost all elements released; (2) the increase in the ionic strength led to an increase in the dissolution of Zn, K and Ca, whereas there was no significant change in the concentrations of Mg, P and Se. For the potential skin irritators, the dissolution of Ba increased with increasing ionic strength, whereas Cr and As tended to be released more at lower ionic strength; (3) increasing temperature resulted in higher concentrations of K and Zn released, but did not have any significant effect on the dissolution of P, Mg, Se and Ca. Arsenic concentration increased with increasing leaching temperature from 21 °C to 30 °C. The concentration of Cr tended to increase continuously with increasing temperature. There was no change in the amount of Ba and Cd released from the clay with increasing temperature.

1. Introduction

A series of studies has been commissioned by Ironwood Clay to investigate the properties of the Natural Oceanic Clay harvested in British Columbia, Canada. Based on those previous studies (Garland, 1993; Klein, 1993), the clay is a phyllosilicate or layered silicate, mainly composed of mica and chlorite minerals that eroded from the land through glacial action. Minor amounts of calcite, quartz, feldspar and hornblende may be included with the clay. A variety of elements in the Northern British Columbia marine environment may be adsorbed to the clay surfaces and bonded with its molecular structure, providing beneficial effects to the skin.

The clay surface is negatively charged due to oxygen and silicon intrinsic to the layered molecular structure (Garland, 2008). These permanent negative electrical charges are intensified by pH-dependant negative charges (Sposito et al., 1999). The typical pH of clay is much higher than its isoelectric point, i.e., point of zero charge, which is the pH at which a particular molecule carries no net electrical charge. Predictable shifts in zero point of charge occur in response to specific adsorption and to changes in cation coordination (Parks, 1967). When applied to skin, the clay exchanges inorganic mineral nutrients such as calcium and phosphorous for inorganic and organic undesirable components such as dead cells, dust and dirt covering the skin surfaces. Although the clay is an ideal ion exchanger, the cleaning of the skin with the clay involves much more than an electrostatic interaction. Other forces, such as Van der Waals, and processes are also intimately involved (Garland, 1993; Burt, 1993).

The clay materials can be both collectors and donors. It is a structurally diverse mixture of inorganic complex minerals, trace elements and rare earth elements (Mackereth, 1966). On the one hand, this mixture provides the distinct and beneficial effects. On the other hand, various elements are likely to be adsorbed and leached out from the clay materials under different conditions (Manning and Goldberg, 1997), some of which may interact with skin surfaces and potentially cause skin irritation, such as arsenic. Clay minerals have high affinity for arsenic, a process important to the natural attenuation of arsenic in the subsurface environment (Lin and Puls, 2000).

For subsequent fully understanding of the interactions of the clay with skin surfaces, it is critical to firstly assess what elements can be released and under what conditions they can be released,

which is the focus of this study. The main conditions that affect the adsorption or release behavior of different elements on clay surfaces are pH, ionic strength, and temperature (Naidu, 1994). The objective of this study is to investigate the leaching behaviour of the Natural Oceanic Clay harvested in British Columbia under these conditions. As outlined in the original scope of work, the objectives of this study are:

- Objective 1: Characterize the mineralogy and chemical composition of the clay minerals
- Objective 2: Quantify the effect of pH, ionic strength, and temperature on the release of various elements from the clay materials.

2. Characterization of mineralogy and chemical composition

2.1. Sample Preparation and Moisture Content Analysis

Clay sample was received as a wet paste (Lot #: WPCH180607-A) and preserved with potassium sorbate 0.1658% and a blend of phenoxyethanol, benzoic acid and dehydroacetic acid 0.5526%. The as-received sample was gently stirred with an inert glass rod to ensure the homogeneity of the subsamples for subsequent testing. A quarter of the wet paste sample was collected (approx. 1.2 kg) from the main container and divided into petri dishes of known weight (Figure 1), which were weighted and placed inside an oven for 40 h with the temperature set at 110 °C for drying.

In the course of drying, samples were removed from the oven and weighted from time to time. Images were taken for comparison of the physical appearances of the sample during drying. Figure 2 shows the evolution of cracks on the surfaces of the clay samples at different intervals during the drying process. Large cracks formed in the course of drying due to increasing loss of moisture.

The moisture loss was calculated as the difference between the initial mass and the mass at any specific time. At the end of 40 h, the oven temperature was increased to 180 °C to remove the moisture possibly chemically bonded with the clay. However, by increasing the temperature to 180 °C, no further change in the mass was observed.



Figure 1. The as-received samples in wet paste form placed in petri dishes



Figure 2. Clay samples in the course of drying: a) 30 min, b) 1 h, c) 3 h, d) 6 h, e) between 20 and 40 h, and f) dried clay

The clay moisture content at different intervals was calculated as Eq. 1:

$$MC_i = \frac{W - D_i}{W} \times 100 \quad \text{Eq (1)}$$

Where MC_i is the moisture content in percentage at time i , W is the initial mass (g), and D_i is the mass of the clay at a specific drying time i (g). The calculated moisture content was plotted versus the drying time (Figure 3). Figure 3 shows that the clay sample used in this study had a moisture content of approximately 35.5%.

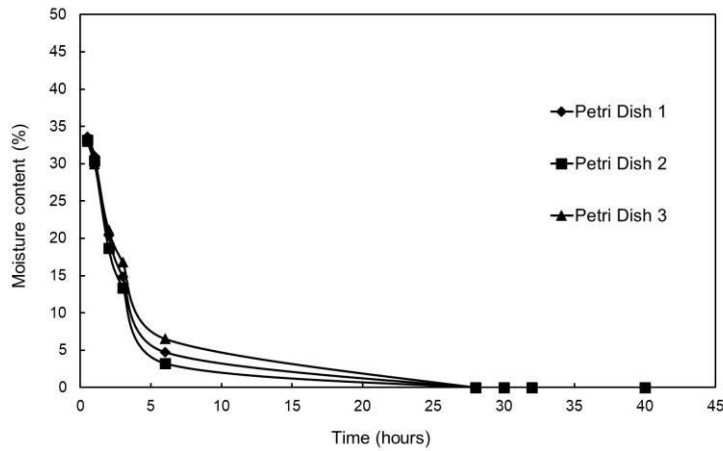


Figure 3. The calculated moisture content of the clay sample versus drying time

2.2. Clay Chemical Composition

For characterization of the chemical composition of the clay, a subsample of the disaggregated dried sample was sent to ALS Laboratory for ICP-AES multi-element analysis. Table 1 shows the concentrations of the chemical elements in the clay sample. Based on the results, the elements found in the clay structure were divided into three different categories: skin beneficial elements, skin irritator elements, and rare earth elements. Table 2 summarizes these elements and their concentrations in the clay structure.

The chemical composition of the supernatant was also measured by multi-element ICP-MS. The as-received wet clay was filtered by a filter paper (0.45 μm) and the supernatant was sent out for analysis of the concentration of the beneficial and the skin irritator elements in the solution (Table 3). The pH and ORP of the supernatant were measured as 6.4 and 334 mV (versus SHE), respectively.

Table 1. Chemical composition of the clay analyzed by ICP-AES

Element unit	Al %	Fe %	Ca %	Na %	Mg %	K %	Ti %	S %	
Replicate 01	8.47	4.71	3.73	3.17	2.13	1.91	0.503	0.12	
Replicate 02	8.09	4.63	3.64	3.13	2.06	1.87	0.491	0.12	
Element unit	P ppm	Mn ppm	Ba ppm	Sr ppm	V ppm	Zn ppm	Cu ppm	Rb ppm	Ni ppm
Replicate 01	1080	922	830	47.9	525	150	118	72.5	56.5
Replicate 02	1060	921	820	516	148	116	72.3	46.7	45.7
Element unit	Li ppm	Cr ppm	Ce ppm	Ga ppm	Co ppm	Sc ppm	Nd ppm	Y ppm	La ppm
Replicate 01	41.1	38	27.5	21.8	19.5	15.7	15.1	14.1	11.9
Replicate 02	38	38	23	21.2	19.5	14.7	13.3	12.6	9.9
Element unit	Zr ppm	Pb ppm	Nb ppm	As ppm	Pr ppm	Sm ppm	Gd ppm	Th ppm	Cs ppm
Replicate 01	11.3	9.1	4.7	2.69	4.6	3.58	3.55	3.02	2.88
Replicate 02	10.6	9.2	4.5	4.5	3.13	3.19	2.95	2.55	2.59
Element unit	Dy ppm	U ppm	Er ppm	Mo ppm	Yb ppm	Be ppm	Sn ppm	Eu ppm	W ppm
Replicate 01	2.64	1.7	1.5	0.6	1.49	1.35	1.15	1.1	0.96
Replicate 02	2.53	1.5	1.41	1.46	1.29	1.11	1	0.9	0.6
Element unit	Hf ppm	Ho ppm	Sb ppm	Tb ppm	Tl ppm	Ta ppm	Tm ppm	Lu ppm	Ge ppm
Replicate 01	0.6	0.54	0.49	0.16	0.43	0.36	0.3	0.22	0.21
Replicate 02	0.5	0.5	0.44	0.43	0.36	0.28	0.21	0.2	0.16
Element unit	Cd ppm	Bi ppm	Ag ppm	Te ppm	In ppm	Re ppm	Se ppm		
Replicate 01	0.13	0.11	0.1	<0.05	0.049	<0.002	<1		
Replicate 02	0.13	0.1	0.1	<0.05	0.058	<0.002	<1		

2.3. Clay Mineralogy

The sample was reduced to the optimum grain size range for quantitative X-ray analysis (<10 μ m) by grinding under ethanol in a vibratory McCrone Micronizing Mill for 10 min. Step-scan X-ray powder diffraction data were collected over a range 3-80°2 θ with CoK α radiation on a Bruker D8 Advance Bragg-Brentano diffractometer equipped with an Fe monochromator foil,

0.6 mm (0.3°) divergence slit, incident- and diffracted-beam Soller slits and a LynxEye-XE detector. The long fine-focus Co X-ray tube was operated at 35 kV and 40 mA, using a take-off angle of 6°.

The X-ray diffractogram was analyzed using the International Centre for Diffraction Database PDF-4 and Search-Match software by Bruker. X-ray powder-diffraction data of the sample were refined with Rietveld program Topas 4.2 (Bruker AXS). The results of quantitative phase

analysis by Rietveld refinements are given in Table 4. These amounts represent the relative amounts of crystalline phases normalized to 100%.

Table 2. Beneficial, skin irritator and rare earth elements in the clay structure

Skin irritator elements									
Element	pb	Cd	As	Cr	Tl	Ba	Sr		
unit	ppm	ppm	ppm	ppm	ppm	ppm	ppm		
Replicate 01	9.1	0.13	4.6	38	0.36	830	526		
Replicate 02	9.2	0.13	4.5	38	0.36	820	516		
Beneficial elements									
Element	Se	Zn	P	K	Mg	Na	Ca		
unit	ppm	ppm	ppm	%	%	%	%		
Replicate 01	<1	118	1080	1.91	2.13	3.17	3.73		
Replicate 02	<1	116	1060	1.87	2.06	3.13	3.64		
Rare earth elements									
Element	Ce	Sc	Nd	Y	La	Pr	Sm	Gd	Dy
unit	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Replicate 01	27.50	15.70	15.10	14.10	11.90	3.58	3.55	3.02	2.64
Replicate 02	23	14.70	13.30	12.60	9.90	3.13	3.19	2.95	2.53
Element	Er	Yb	Eu	Ho	Tb	Tm	Lu		
unit	ppm	ppm	ppm	ppm	ppm	ppm	ppm		
Replicate 01	1.50	1.35	0.96	0.60	0.43	0.22	0.21		
Replicate 02	1.41	1.29	0.90	0.50	0.43	0.21	0.20		

Table 3. Concentrations of elements in the clay supernatant

Skin irritator elements								
Element	Tl	Hg	Pb	Cd	As	Cr	Ba	Sr
unit	ppb	ppb	ppb	ppb	ppb	ppb	ppm	ppm
Replicate 01	0.09	<0.2	0.3	1.7	20	22	1.0	8.9
Beneficial elements								
Element	Zn	Se	P	Mg	Ca	K	Na	
unit	ppb	ppb	ppb	ppm	ppm	ppm	ppm	
Replicate 01	4	50	700	508	780	1500	5500	

Table 4. Mineralogy of the clay analyzed by QXRD

Mineral	Ideal Formula	Wt.%
Actinolite	$\text{Ca}_2(\text{Mg}, \text{Fe}^{2+})_5\text{Si}_8\text{O}_{22}(\text{OH})_2$	11.6
Biotite	$\text{K}(\text{Mg}, \text{Fe}^{2+})_3\text{AlSi}_3\text{O}_{10}(\text{OH})_2$	9.0
Clinocllore	$(\text{Mg}, \text{Fe}^{2+})_5\text{Al}(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_8$	3.6
Halite	NaCl	0.6
Illite-Muscovite 2M1	$\text{K}_{0.65}\text{Al}_{2.0}\text{Al}_{0.65}\text{Si}_{3.35}\text{O}_{10}(\text{OH})_2/\text{KAl}_2\text{AlSi}_3\text{O}_{10}(\text{OH})_2$	2.9
K-feldspar (microcline)	KAlSi_3O_8	6.8
Plagioclase (albite calcian)	$\text{NaAlSi}_3\text{O}_8 - \text{CaAlSi}_2\text{O}_8$	48.6
Quartz	SiO_2	17.0
Total		100.0

2.4. Particle Size Distribution Analysis

The particle size distribution of the clay sample was analyzed by laser diffraction using the Mastersizer 2000. A suspension of the representative sample in deionized water was prepared and placed on a stirrer to disperse the solids. Ultrasonic was used in one test to ensure the dispersion of the clay particles. Table 5 shows the particle size analysis results obtained from the laser diffraction method. The analysis was carried out in duplicate. A major fraction of the particles is in the size range of 2 to 20 μm and approximately 80% of the particles are finer than 24.74 μm .

Table 5. Summary of laser diffraction particle size data (wt%)

Dry clay	0.01-2 μm	2-20 μm	20-50 μm	50-64 μm	64-200 μm	200-2000 μm
no ultrasound	10.71	62.15	23.64	2.25	1.24	0.0
ultrasound	10.96	59.99	23.94	2.29	1.26	0.0

2.5. Surface Charge Analysis

To measure the surface charge of the clay, 30 mg of the dried sample was suspended in a 200 mL 0.01 mol/L NaCl solution. The suspension was analysed by a zeta potential analyser. The surface charge for the clay sample was measured as -45 mV. Table 6 shows the stability behavior of colloids with different surface charges. For colloidal particles, a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. The measured surface charge shows that the clay sample has a good stability in aqueous solution and the solid particles will remain dispersed in the solution. To confirm the stability of the solids, a suspension of the clay sample in deionized water was left in the laboratory for about two weeks. Figure 4 shows that the suspension after about two weeks maintained good stability.

Table 6. Stability behavior of colloids at different surface charges

Zeta potential [mV]	Stability behavior of the colloid
from 0 to ± 5 ,	Rapid coagulation or flocculation
from ± 10 to ± 30	Incipient instability
from ± 30 to ± 40	Moderate stability
from ± 40 to ± 60	Good stability
more than ± 61	Excellent stability



Figure 4. The clay suspension in deionized water after two weeks

The effect of different pH and ionic strengths on the surface charge of the clay was investigated. The same amount of dried clay (30 mg) was added into deionized water. pH was adjusted using sodium hydroxide and citric acid for alkaline and acidic pH, respectively. Sodium chloride was used to adjust the ionic strength of the solution.

Figure 5 shows that within the ranges of pH and ionic strength, the clay surfaces were negatively charged. By increasing pH the surface became more negatively charged. In terms of ionic strength, there was a more pronounced change in the clay surface charge in response to the variation in the ionic strength. With increasing ionic strength of the solution, the clay surface became more negatively charged.

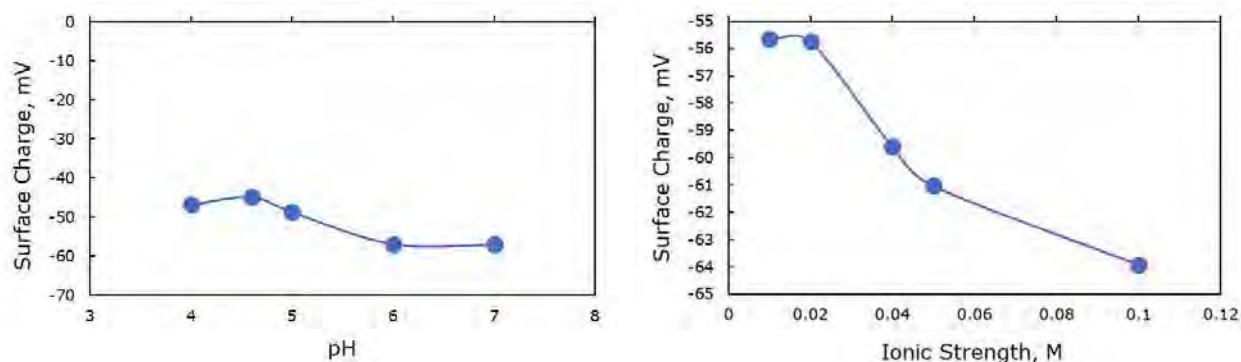


Figure 5. Clay surface charge in response to changes in ionic strength (left) and pH (right)

3. Quantify the effect of pH, ionic strength, and temperature on the release of various elements from the clay materials

Leaching experiments were carried out to investigate the effect of pH, ionic strength and temperature on the release of different elements from clay materials. The batch reactor employed in the study was a 500 mL jacketed reactor). Temperature was kept constant using a water bath to circulate water at a constant temperature through the jacketed wall. 250 mL of leaching solution was used in each experiment. Mechanical stirrer was used to keep the solids suspended in the reactor. pH electrode connected to a pH meter was used to measure the pH of the leaching solution. Figure 6 shows the leaching setup used for the experiments.



Figure 6. Leaching experimental setup

Leaching experiments were carried out in the setup mentioned above. 25 g of solid samples was added to 250 mL solution. 20 mL of aqueous samples was taken from the leaching solution at specific time intervals for ICP-OES analysis. Leaching experiments were continued for 1 h and for each experiment 4 samples were taken at 1, 15, 30 and 60 min. The solution samples taken (leachates) were filtered and analyzed by ICP-OES for determination of the total concentrations of different cations in the leachates. 30-elements standard solution was used to calibrate ICP and obtain the calibration curve.

3.1 Effect of pH on the element release from the clay

The pH of the leaching solution was changed based on the range of skin pH values reported in the literature. Five different pH levels (4.0, 5.0, 6.0, 6.4 (clay pH) and 7.0) were used. To maintain a constant pH during leaching, citric acid ($C_6H_8O_7$) and sodium hydroxide (NaOH) were used as acid and base for pH adjustment, respectively.

Figure 7 (left) shows the effect of pH on the dissolution of skin beneficial elements from the clay at 30 min of leaching. As it is shown in the graph, increasing the pH resulted in a decrease in the concentrations of almost all elements. In the more acidic environments (pH 4.0 and 5.0) more beneficial elements were released from the clay sample. By increasing the pH to 6.0, 6.4 and 7.0, the release process significantly slowed down and there was no significant change in the concentrations of elements at pH 6.0 and 7.0.

Figure 7 (right) shows the effect of pH change on the release behavior of potential skin irritating elements at 30 min of leaching. Increasing the pH had a similar effect to what was observed with the skin beneficial elements. In other words, increasing pH led to a decrease in the concentrations of the potential skin irritating elements in the solution.

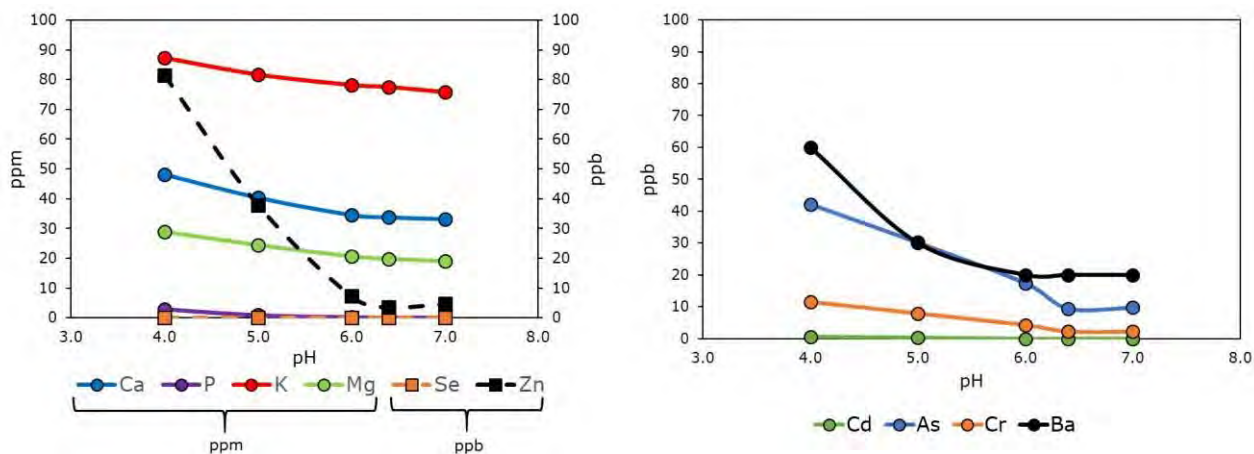


Figure 7. Effect of pH on the dissolution of elements for 30 min of leaching at 21 °C: (left) beneficial elements; (right) potential skin irritating elements

Figure 8 shows the effect of leaching time on the dissolution of skin beneficial elements at different pH. Extending the leaching time to 1 h resulted in an increase in the concentration of

Mg and P in the solution. These increases were more pronounced in a more acidic environment. After 1 h of leaching at pH of 4.0, the concentrations of Mg and P were detected to be 31.3 ppm and 3.6 ppm, respectively. The concentrations of other skin beneficial elements did not show significant changes with increasing leaching time.

Figure 9 shows the release behavior of the potential skin irritating elements as a function of time at different pH. Increasing the leaching time resulted in higher concentrations of these elements released from the clay surfaces. Similar to the leaching behavior of the skin beneficial elements, the dissolution was accelerated in a more acidic environment.

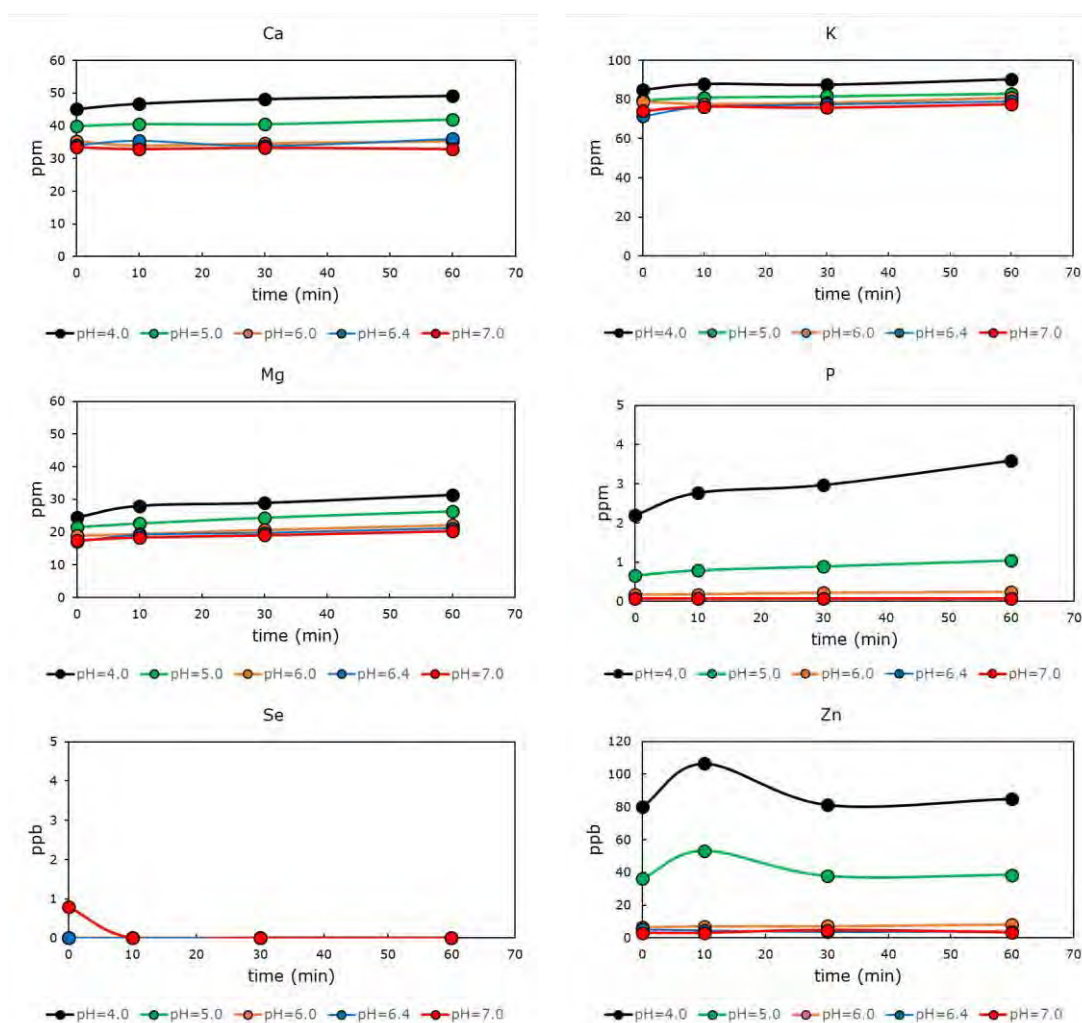


Figure 8. Effect of leaching time on the release of skin beneficial elements at different pH

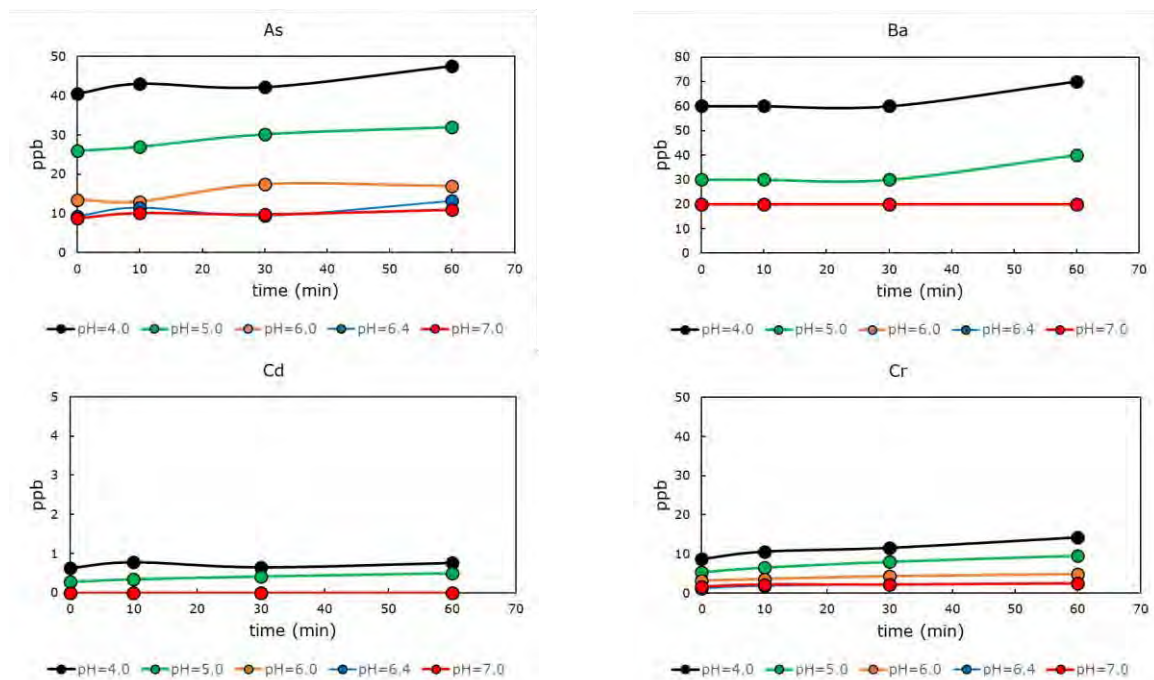


Figure 9. Effect of leaching time on the release of potential skin irritating elements at different pH

3.2 Effect of ionic strength on the element release from the clay

These tests were done to investigate the effect of ionic strength resulting from skin sweating on the release behavior of different elements from clay. Despite some variations, the typical measured concentrations are: sodium (0.9 g/L), potassium (0.2 g/L), calcium (0.015 g/L), and magnesium (0.0013 g/L). Using these typical concentrations, the ionic strength of skin was estimated to be around 0.02 mol/L. To investigate the effect of different ionic strength on the release of elements from clay surfaces, ionic strength was varied from 0.0 (no reagent added) to 0.1 mol/L using sodium chloride to control the ionic strength of the leaching environment.

Figure 10 (left) shows the release of skin beneficial elements for 30 min of leaching at different ionic strength. The increase in the ionic strength led to an increase in the dissolution of Zn, K and Ca, whereas there was no significant change in the concentrations of Mg, P and Se. For the potential skin irritators, as shown in Figure 10 (right), an increase was observed in the concentration of Ba with increasing ionic strength and there seemed to be no significant change in the concentrations of Cr, As and Cd.

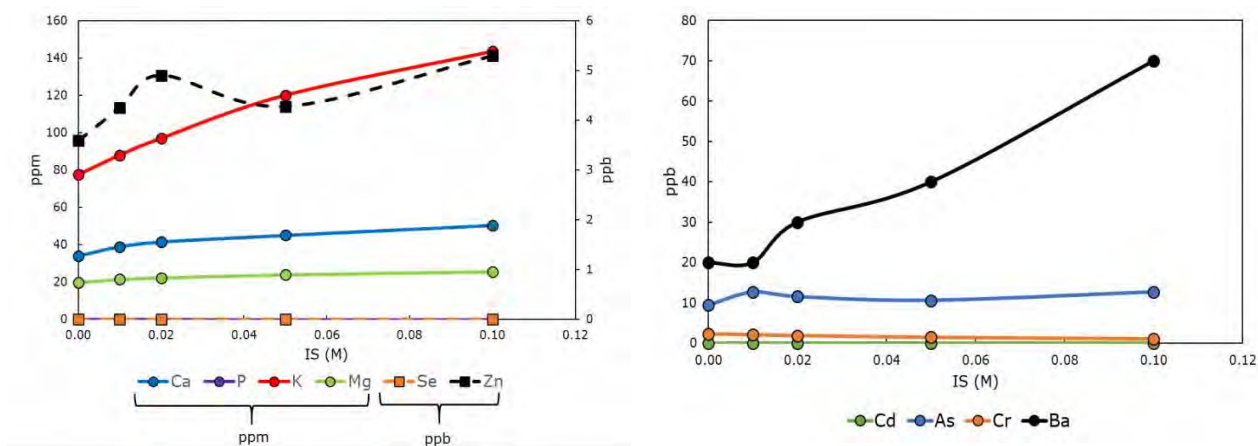
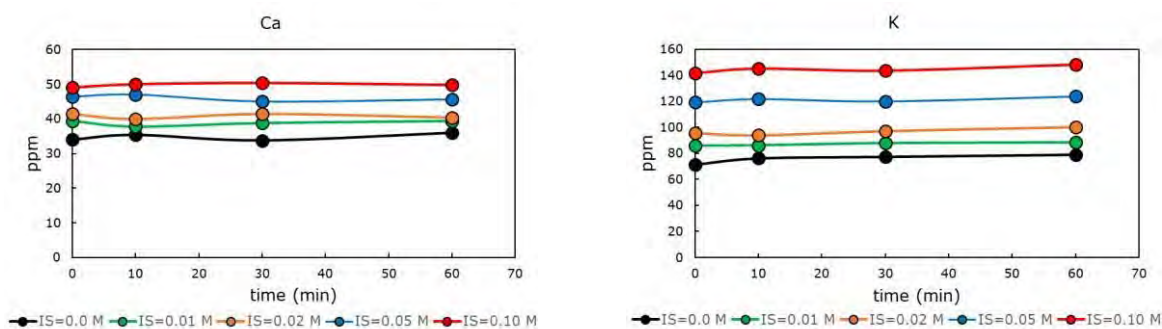


Figure 10. Effect of ionic strength on the dissolution of elements for 30 min of leaching at 21 °C: (left) beneficial elements; (right) potentially skin irritating elements

Figure 11 illustrates the effect of leaching time on the release of skin beneficial elements at different ionic strength. A slight increase in the dissolution was observed with increasing leaching time. In general, higher amounts of these elements were released into the solution with increasing ionic strength. However, P showed a different behavior, i.e., lower ionic strength was associated with higher concentrations of P released from the clay.

Figure 12 shows the dissolution of the potential skin irritating elements for 1 h of leaching at different ionic strength. Generally, a slight increase in the dissolution was observed with increasing leaching time. Increasing ionic strength led to an increase in the dissolution of Ba, while higher concentration of As and Cr tended to be released in lower ionic strength.



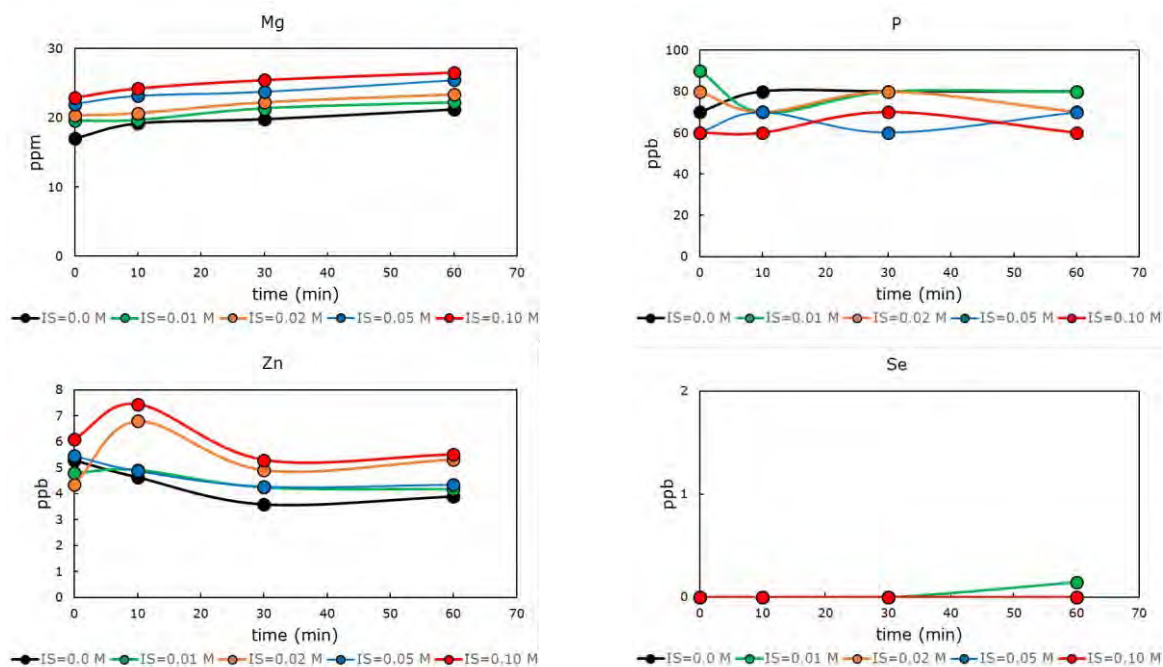


Figure 11. Effect of leaching time on the release of skin beneficial elements at different ionic strength

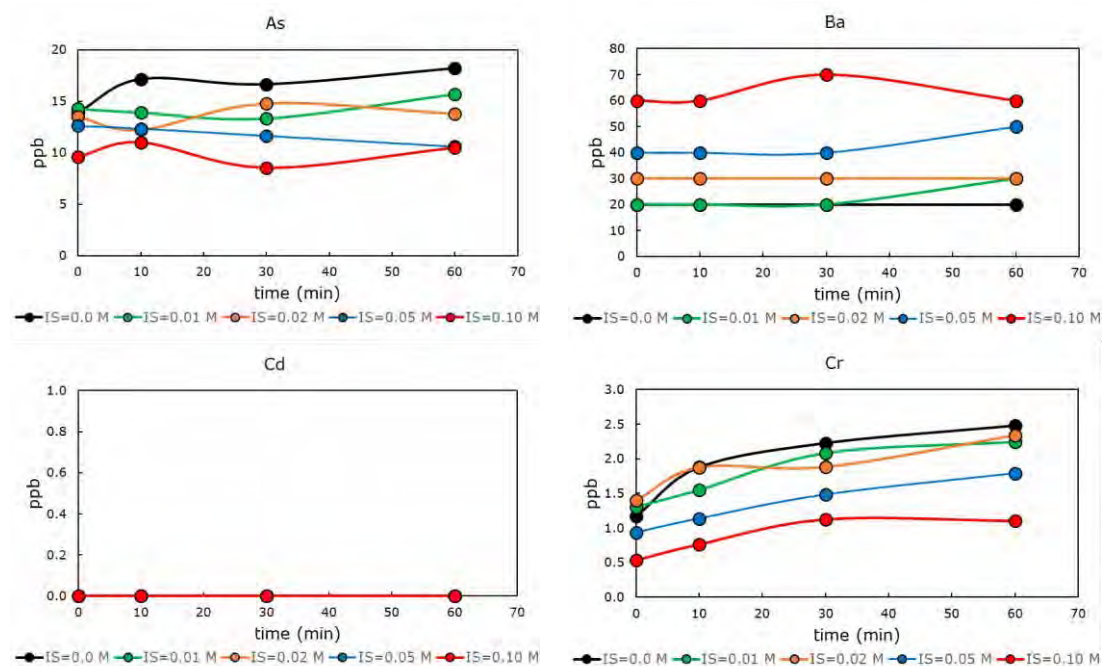


Figure 12. Effect of leaching time on release of potentially skin irritating elements at different ionic strength

3.3 Effect of temperature on the element release from the clay

Skin temperature depends on air temperature and time spent in that environment. Such weather factors as wind chill and humidity cause changes in skin temperature. The normal temperature of skin is about 33 °C. In order to study the effect of different temperatures in different weather conditions on the leaching behavior of the clay, four different temperatures from ambient temperature (~21 °C) to 40 °C was used in the leaching experiments.

Figure 13 (left) shows the effect of temperature on the release behavior of skin beneficial elements for 30 min of leaching. Increasing temperature from 21 °C to 40 °C did not have any significant effect on the dissolution of P, Mg, Se and Ca. However, increasing the system temperature from 21°C to 30 °C resulted in higher concentrations of K and Zn and further increase in temperature from 30 °C to 40 °C did not result in any change.

For the potentially irritating elements, shown in Figure 13 (right), arsenic concentration increased with increasing leaching temperature from 21 °C to 30 °C, followed by a decrease in the concentration with a further increase in the temperature to 40 °C. The concentration of Cr tended to increase continuously with increasing system temperature. There was no change in the amount of Ba and Cd released from the clay by changing the temperature.

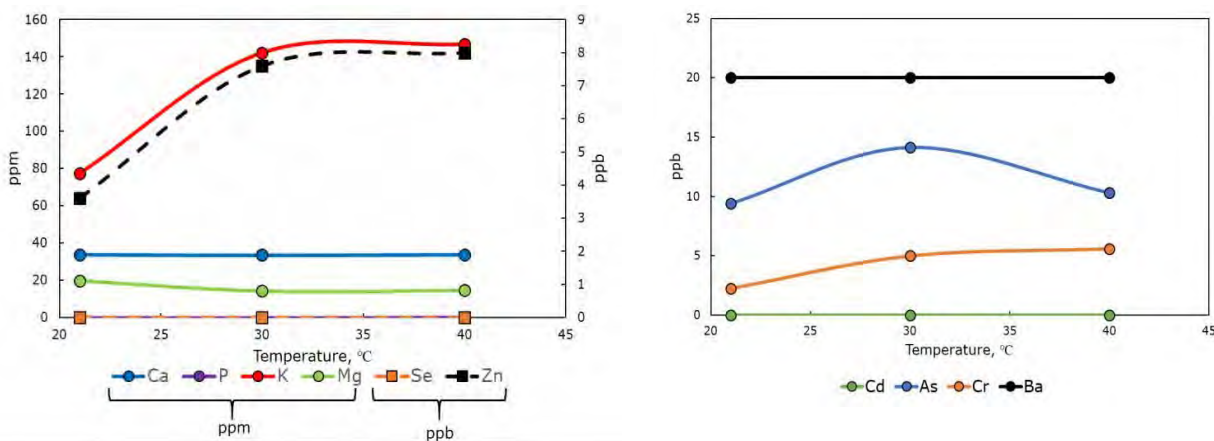


Figure 13. Effect of temperature on the dissolution of elements for 30 min of leaching (pH6.4 and no adjustment on ionic strength): (left) beneficial elements; (right) potentially skin irritating elements

Figure 14 shows the effect of leaching time on the dissolution of the skin beneficial elements at different temperature. When the leaching time was extended to 1 h, only K and Zn showed an increase in their dissolved concentrations. There was no significant difference in the

concentrations of Ca, P and Se in response to the changes in the system temperature. The dissolution of Mg was inversely proportional to temperature, with less amount of magnesium released at higher temperatures.

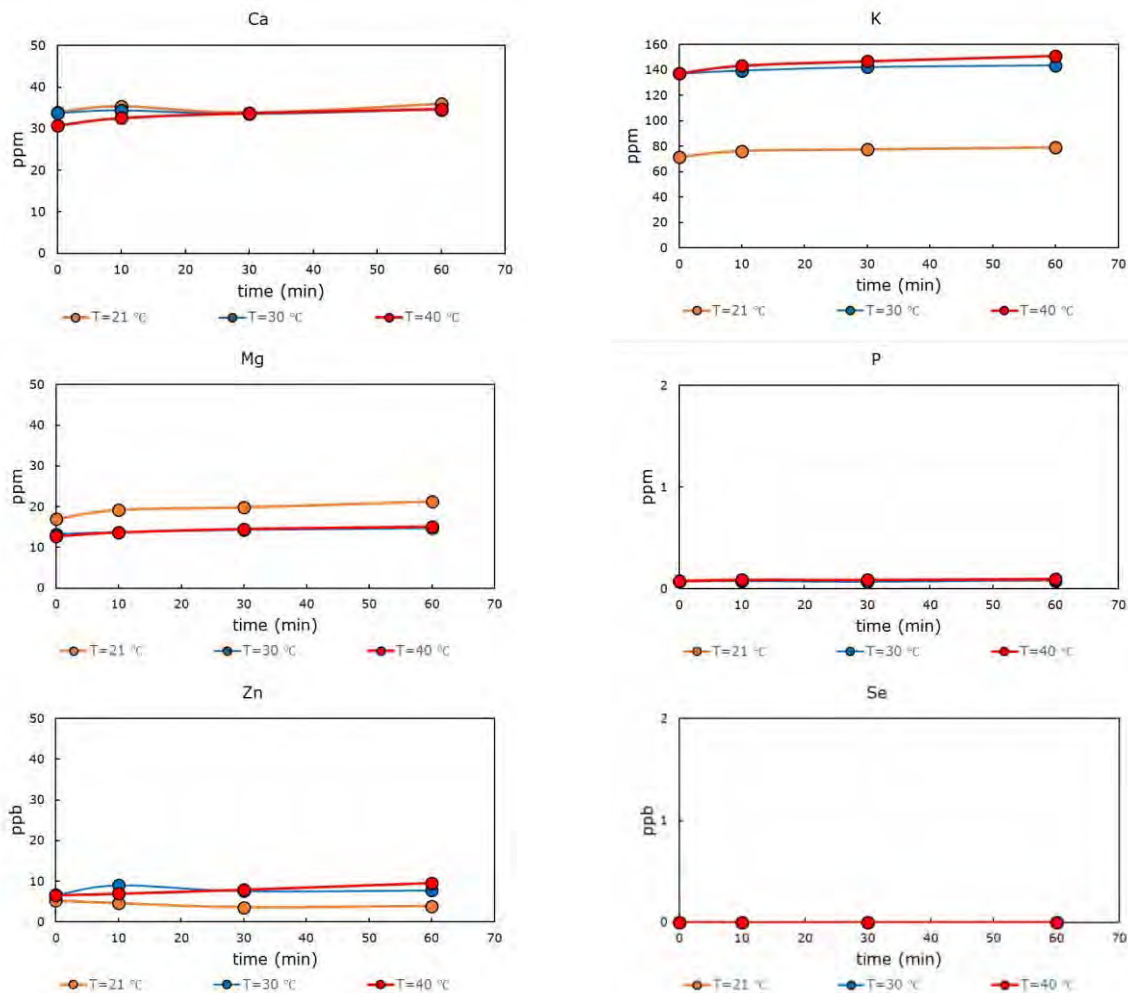


Figure 14. Effect of leaching time on the release of skin beneficial elements at different temperatures

Figure 15 illustrates the leaching behavior of the potentially irritating elements versus the leaching time at different temperatures. Increasing the leaching time resulted in an increase in the concentrations of these elements dissolved to the solution. Increasing the temperature from 21 °C to 40 °C can increase the dissolution of Ba and Cr, whereas more arsenic tended to be released at 30 °C.

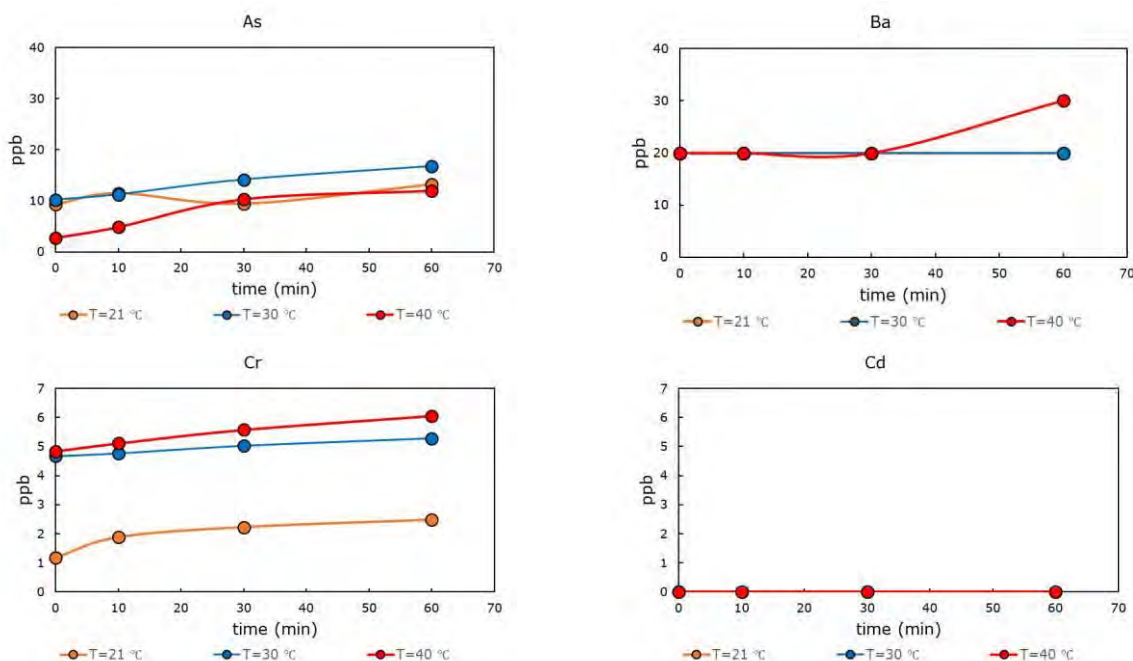


Figure 15. Effect of leaching time on the release of potentially skin irritating elements at different temperatures

4. Conclusions

The main conditions that affect the adsorption or release behavior of different elements on clay surfaces are pH, ionic strength, and temperature. Using different techniques, we firstly characterized the mineralogy and chemical composition of the clay minerals, and then quantified the effect of pH, ionic strength, and temperature on the release of various elements from the clay materials. The clay sample used in this study has the following characteristics: a moisture content of approximately 35.5%, approximately 80% of the particles finer than 24.74 μm , and the zeta potential (surface charge) being -45 mV.

For the subsequent leaching tests, we divided the elements released into two categories: skin beneficial elements, including calcium (Ca), phosphorous (P), potassium (K), magnesium (Mg), selenium (Se), and zinc (Zn), and potential skin irritators, including cadmium (Cd), arsenic (As), chromium (Cr), and barium (Ba).

The main findings are: (1) increasing the pH resulted in a decrease in the concentrations of almost all elements released; (2) the increase in the ionic strength led to an increase in the dissolution of Zn, K and Ca, whereas there was no significant change in the concentrations of

Mg, P and Se. For the potential skin irritators, the dissolution of Ba increased with increasing ionic strength, whereas Cr and As tended to be released more at higher ionic strength; (3) increasing temperature resulted in higher concentrations of K and Zn released, but did not have any significant effect on the dissolution of P, Mg, Se and Ca. Arsenic concentration increased with increasing leaching temperature from 21 °C to 30 °C. The concentration of Cr tended to increase continuously with increasing temperature. There was no change in the amount of Ba and Cd released from the clay with increasing temperature.

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The Effect of Ironwood Clay on Skin Fibroblasts and Immune Cells

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Abstract

Clay has been extensively in use around the world for cosmetic and medicinal purposes. However, there are limited number of studies investigating how the clay works at molecular and cellular levels. The purposed of this project was to study the effect of Ironwood Glacial clay at a cellular level with regard to inflammation, skin cell viability, and collagen production. In this study, we have shown that Ironwood Glacial Clay has effect on inflammatory response/proliferation of immune cells, as well as collagen production of skin fibroblasts.

Introduction

There is no study or data available to indicate the effect of the clay on a cellular level with regard to inflammation or collagen production. Burn and Wound Healing Research Lab, Department of Surgery, Division of Plastic Surgery at UBC, received Ironwood clay and compared it with another commercially available product for testing. Our initial working hypothesis was that Ironwood Glacial Clay will a) decrease the inflammatory response/proliferation of immune cells, and b) will alter proliferation rate /collagen production of fibroblasts. This was investigated through following objectives:

- 1- To investigate anti-inflammatory effects of Ironwood Glacial Clay
- 2- To investigate effects of Ironwood Glacial Clay on fibroblasts viability and collagen production

Materials and Methods

Cell Culture:

The Jurkat cells, immortalized line of T-lymphocyte, were purchased from American Type Culture Collection (ATCC, Manassas, Virginia, United States). Cells were cultured in culture medium in 12-well flat-bottom plates (Costar; Corning Incorporated) and maintained in a 37°C humidified incubator containing 5% CO₂. Fibroblasts were cultured in culture medium and fibroblasts at passages 4–7 were used in all the experiments.

Establishing the experimental model:

In order to investigate whether Ironwood Glacial Clay has effect on inflammatory response/proliferation of immune cells, as well as proliferation and collagen production of skin fibroblasts, first we established the experimental model that mimics skin. Our experimental model was developed based on three facts, first: not all molecules can pass through the skin, second: skin serves as the body's outermost protective layer by limiting the movement of molecules into and out of the body and third: smaller than a few micrometers (10-1000nm) (0.01- 1µm)¹. As such, in this model, we used double chamber with 0.1µm pore size and apply the thin layer of clay on the top of the chamber. Chamber limits large clay particles to pass through the filter and mix with cells.

Fibroblasts Live/dead, viability/cytotoxicity assay

Fibroblasts were plated in 12-well plates (50,000 cells/well) and after 24 hours chambers (0.1 µm pore size) were placed in the wells. A day after 50 and 100% of Ironwood Clay or French Clay were applied on the chamber. Control samples (0% treated) were treated with dH₂O. After 24 hours of incubation cells viability was evaluated by fluorescence microscopy using the Live/Dead, Viability/ Cytotoxicity assay kit for mammalian cells (Invitrogen). In this assay ethidium homodimer (EthD-1) (4 mL/mL), a red fluorescent nucleic acid dye, stains dead cells and those undergo apoptosis; while, Calcein (1 mL/mL) is converted to a green fluorescent compound by active intracellular esterase in live cells. Percentage of dead cells with damaged (porous) cellular membrane was quantitatively evaluated according to the manufacturer instruction (Molecular Probes, Invitrogen, Mississauga Canada). Briefly, for each well, one photograph with 100X magnification was taken by Zeiss fluorescent microscope. On each image, four non-overlapping randomly selected high-power fields (HPF) were used, coded, and the number of the red nuclei (dead cell) was counted manually. The average number of these four HPFs was reported as the cell number/HPF for each slide.

Fibroblast Cell Proliferation Assay

Dermal fibroblasts were plated in 12-well plates (50,000 cells/well) and after 24 hours chambers (0.1 µm pore size) were placed in the wells. A day after 50 and 100% of Ironwood Clay

or French Clay were applied on the chamber. Subsequently, after 24 hours of incubation, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) at 5 mg/ml was added to each well and incubated for 4 hours in the dark. The MTT/ medium was carefully removed, and 100 μ l dimethyl sulfoxide (DMSO, Sigma-Aldrich) was added, followed by incubation at 37°C for 10 minutes. The absorbance at 570 nm was measured using a BioTek Microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT).

Collagen production using Immunofluorescence microscopy

In order to evaluate the effect of Ironwood clay and French clay on collagen production, fibroblasts were seeded at 2×10^5 cells per well on 12-well plates and after 24 hours chambers (0.1 μ m pore size) were placed in the wells. A day after, 50 and 100% of Ironwood Clay or French Clay were applied on the chambers. After 24 hours, treated cells were fixed in 4% paraformaldehyde for 15 minutes, and then washed. In case of immunofluorescence microscopy, staining was carried out using primary collagen monoclonal antibody at a 1:5 dilution and incubated overnight in a humidified chamber at 4°C. After three washing steps with PBS-T for 5 minutes each, samples were incubated with FITC-conjugated secondary antibody at 1 : 1,000 dilution for 60 minutes under dark conditions. Finally, after washing with PBS-T three times for 5 minutes each DAPI was added to the wells for nuclei staining. Wells were viewed using a Zeiss Axiovert microscope and Axiovision 4.8 software. Image analysis was conducted using Image J (NIH software).

Quantitative real-time PCR analysis

In order to evaluate the effect of Ironwood clay and French clay on collagen gene expression, fibroblasts were seeded at 2×10^5 cells per well on 12-well plates and after 24 hours chambers (0.1 μ m pore size) were placed in the wells. A day after, 50 and 100% of Ironwood Clay and French Clay were applied on the chambers. Cells harvested after 24 hours and total RNA was extracted, using GeneJET RNA purification Kit (Thermo Scientific) , after 24 hours.

RNA (5 μ g) was then transcribed into cDNA using the Superscript III First Strand cDNA Synthesis Kit. Procollagen mRNA levels in isolated fibroblasts was measured by Applied Biosystems 7500 qPCR machine using fast start universal SYBR green master mix (Rox). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the house keeping gene. 2- $\Delta\Delta$ CT method was used to calculate the gene expression fold change, relative to that of control.

The sequences of collagen and GAPDH (Forouzandeh et al., 2010) primers were:

ColA1: Forward 5'- AGACGAAGACATCCCACCAATC -3', Reverse 5'- AGATCACGTCATCGCACAACA-3';

GAPDH: Forward 5'- GCGGGGCTCTCCAGAACATCAT-3', Reverse 5'- GACGCCTGCTTACCACCTTCTT-3';

T-cell survival evaluation

The survival of Jurkat (T cells) cells were compared by 7-AAD staining. 7-AAD intercalates into double-stranded nucleic acids. It is excluded by viable cells but can penetrate cell membranes of dying or dead cells. After each treatment, cells were harvested, washed in PBS, stained with 7-AAD for 10 minutes and then examined using FACS analysis, as per the manufacturer's protocol (BD).

T-cell Proliferation rate

To investigate whether Ironwood clay or French clay change the T-cell proliferation, a series of experiments was conducted. Jurkat cells were stained with 2.5 µg/ml of carboxyfluorescein succinimidyl ester (CFSE) and plated in 12-well plates (200,000 cells/well). After 24 hours chambers (0.1 µm pore size) were inserted. Cells were treated with 50 and 100% of Ironwood Clay or French Clay by applying them on the chambers. After 24 hours of incubation, Jurkat cells proliferation was assessed using FACS analysis.

Production of pro-inflammatory cytokines using flow cytometric intracellular staining assay.

Jurkat cells were plated in 12-well plates (200,000 cells/well) and after 24 hours they were stimulated with 70ng/ml phorbol 12-myristate 13-acetate (PMA) and 2µM Ionomycin. Chambers (0.1 µm pore size) were placed in the wells and after 24 hours 50 and 100% of Ironwood Clay or French Clay were applied on the chamber. Subsequently, after 24 hours' cells were harvested and stained for CD4, IL-12 and IFN-Gamma. All flow cytometry analyses were performed with a BD Accuri C6 Flow Cytometer (BD Biosciences, San Jose, CA). All the antibodies were bought from eBioscience, San Diego, USA. For characterization of Jurkat cells, they were stained with FITC-conjugated anti-human CD4 (1:1000), PE-conjugated anti-human INF-Gamma (1:1000) and APC-conjugated anti-human IL-12 (1:1000) antibodies. Cells were analyzed by a BD Accuri™ C6 Flow Cytometer, gates on physical parameters. A total of 50,000 events were recorded for each assay.

Statistical analysis

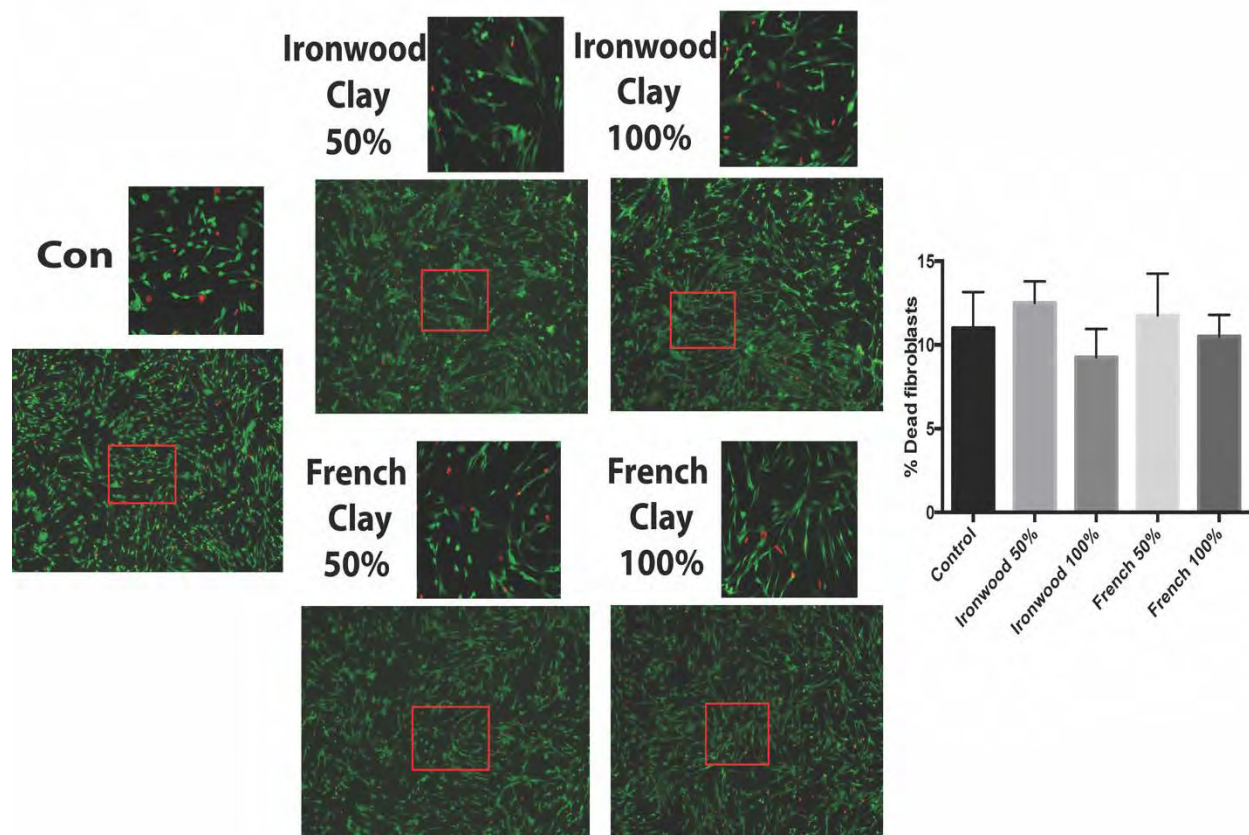
Data are reported as a mean ± SEM of three or more independent set of experiments. The statistical differences of mean value among treated and control groups were tested with one-way ANOVA followed by Post hoc comparisons using Bonferroni correction. A p-value less than 0.01 was considered statistically significant.

Results

Fibroblasts Live/dead, viability/cytotoxicity assay:

Determination of cellular cytotoxicity by fluorescent microscope using live/dead, viability/cytotoxicity assay kit. In this assay clacein AM is converted to a green fluorescent compound by active intracellular esterase in live cells with intact membranes (FITC positive cells); while, ethidium homodimer (EthD-1), a membrane impermeable, red fluorescent nucleic acid dye, stains dead cells and those undergo apoptosis (PE positive cells). Fibroblast cells were either treated with dH₂O, 50 and 100% of Ironwood Clay or French Clay. Fibroblast cell cytotoxicity is represented by the percentage of dead cells (red cells) in the total cell population. (*P-value<0.01, n=4). It was shown that exposure to 50 and 100% of Ironwood Clay did not significantly increase the number of dead cells (Fig. 1), indicating that Ironwood Clay is non-toxic to fibroblast cells.

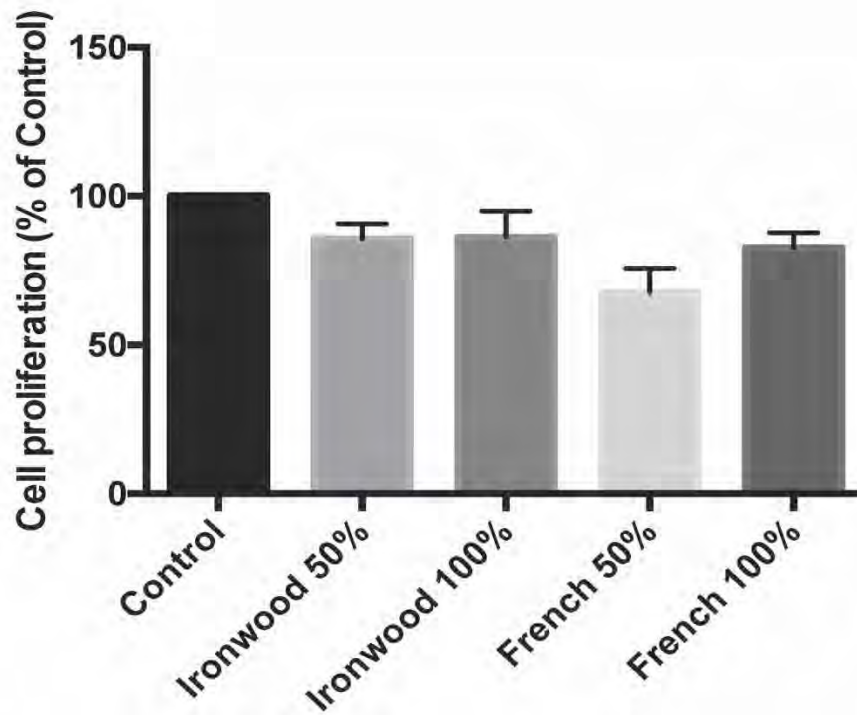
Figure 1



Fibroblast Cell Proliferation Assay:

The effect of Ironwood Clay on fibroblast proliferation. In order to determine the effect of Ironwood Clay on fibroblasts proliferation, an MTT assay was done on fibroblast cells cultured in either treated with dH₂O, 50 or 100% of Ironwood Clay or French Clay. As shown in Figure 2, no significant changes were observed in cellular proliferation of fibroblast cells cultured in 50 or 100% of Ironwood clay as compared with control (treated with dH₂O).

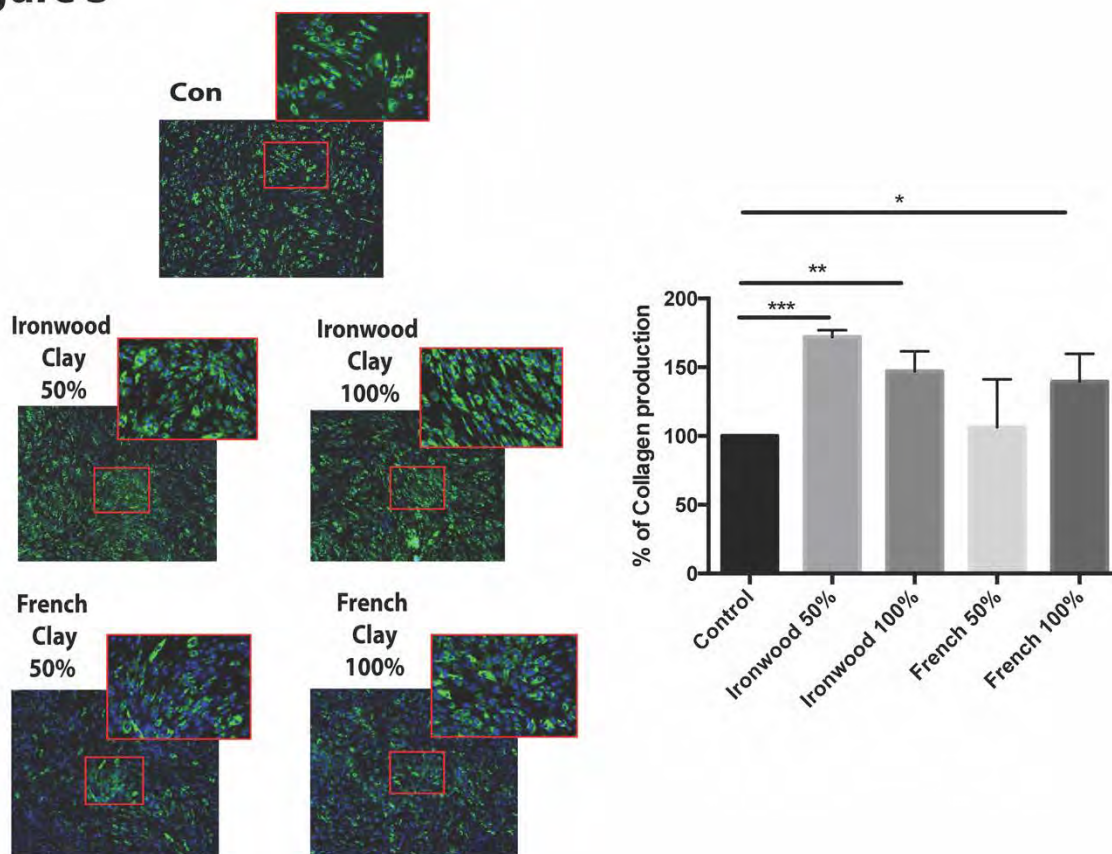
Figure 2



Collagen production using Immunofluorescence microscopy:

The effect of Ironwood Clay on collagen production. Collagen production was also evaluated using Immunofluorescence microscopy in which collagen is stained green and cell nuclei are stained blue (Fig. 3). Ironwood Clay 50% (171.8 ± 2.1), 100% (106.3 ± 14.2) and French Clay 100% (139.6 ± 8.1) significantly increased the production of collagen in fibroblasts after 24 hours compared to control (*P-value<0.01, **P-value<0.001 and ***P-value<0.0001, n=6).

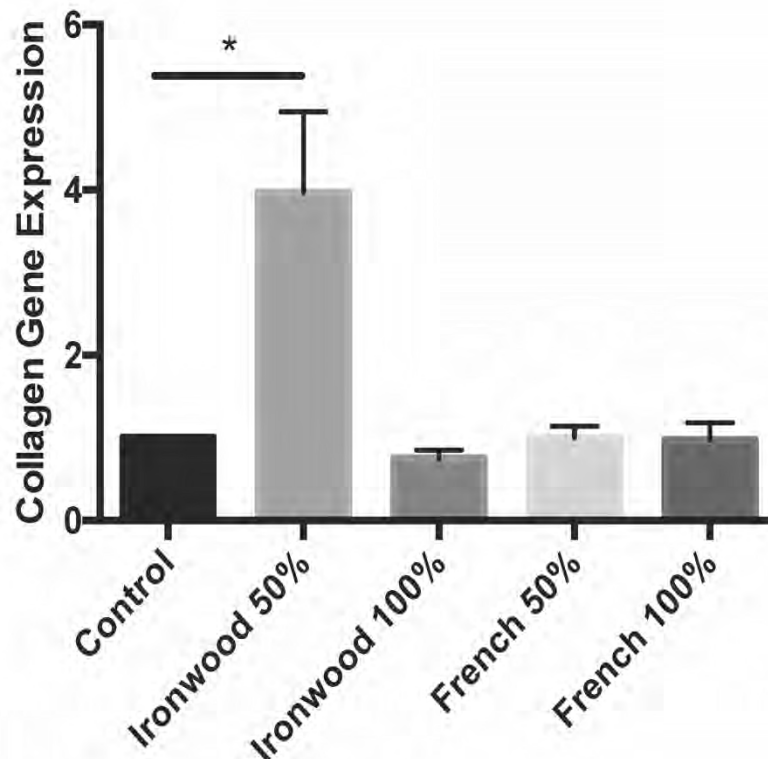
Figure 3



Quantitative real-time PCR analysis:

Type-I collagen mRNA expression in treated fibroblasts. GAPDH was used as the reference gene. The results obtained from the Q-PCR analysis demonstrated that 50% of Ironwood Clay (3.97 ± 0.48) increased the collagen mRNA (Fig. 4). Although our result on Fig. 3 showed significant increase in protein level of collagen by ironwood Clay 100%, it does not have any noticeable stimulatory effect on collagen expression by dermal fibroblasts, (0.75 ± 0.05 , *P-value < 0.01 , $n=4$). This could be due to the fact that after translation, the particular mRNA is prone to degradation while its protein has higher half-life and remains in the cellular pool. In any case, the level of collagen protein is more important than gene expression level. Therefore both 50 and 100% clay can increase collagen level in the dermal fibroblasts.

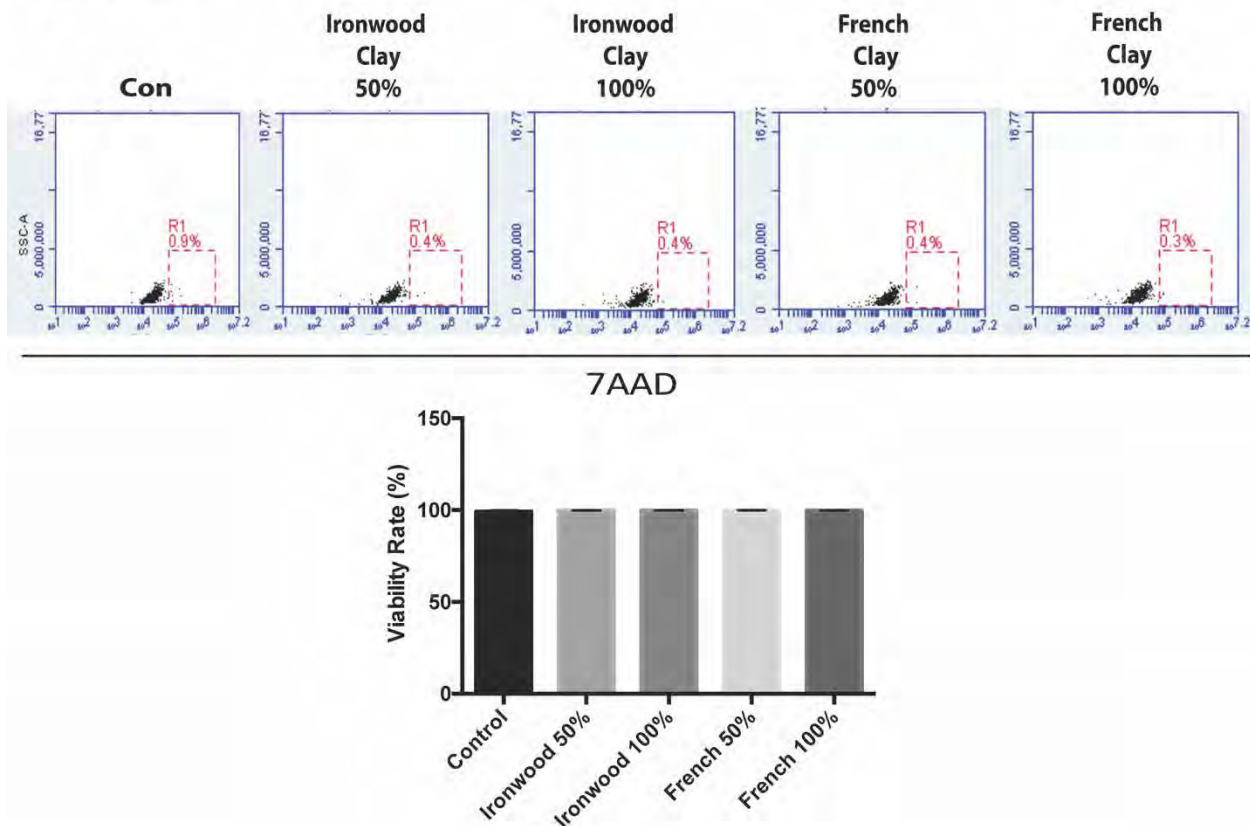
Figure 4



T-cell survival evaluation

The effect of 50 and 100% of Ironwood Clay on T-cell survival was evaluated by flowcytometry using 7-AAD staining. Jurkat cells were either treated with dH₂O, 50 and 100% of Ironwood Clay or French Clay. Jurkat cell cytotoxicity is represented by the percentage of live cells in the total cell population. (*P-value<0.01, n=3). It was shown that exposure to 50 and 100% of Ironwood Clay did not decrease the number of live cells (Fig. 5), indicating that Ironwood Clay is non-toxic to T-cells.

Figure 5

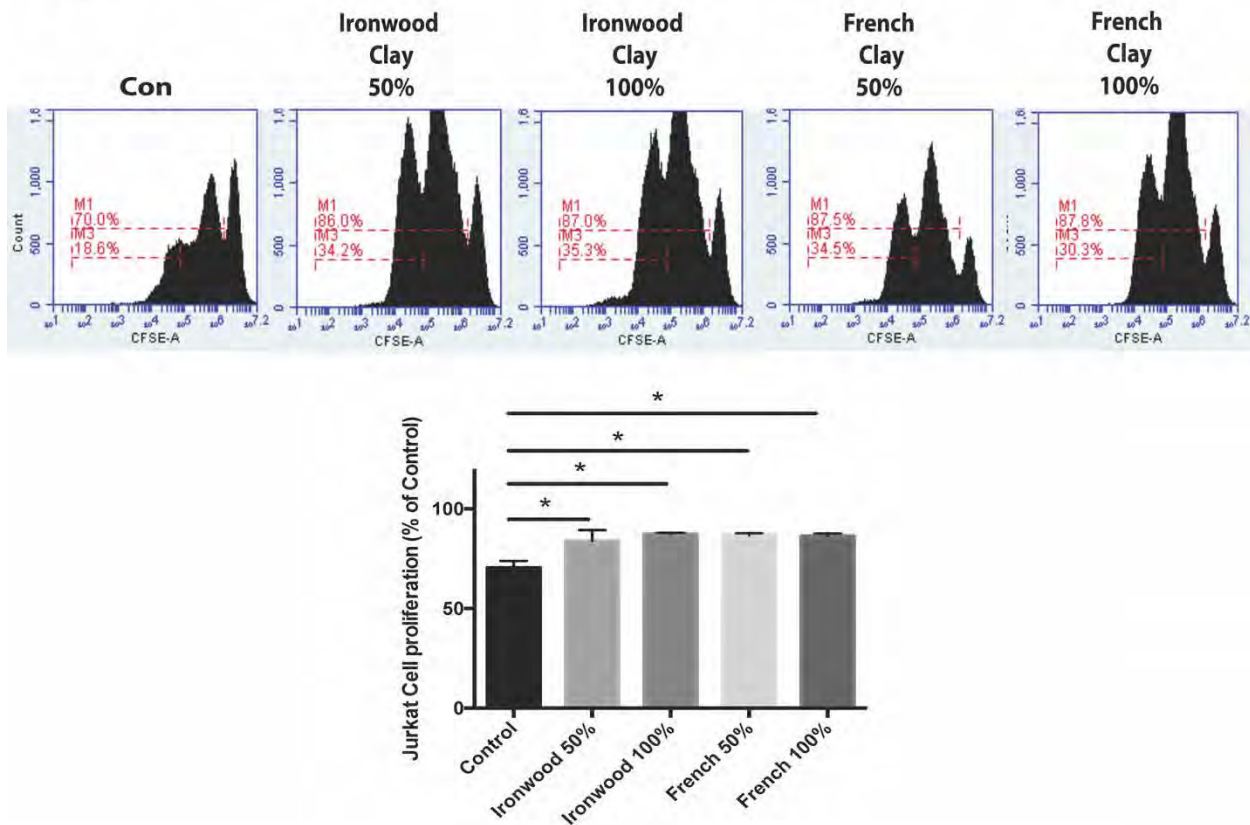


T-cell Proliferation rate

To determine the proliferation of the T-cell population, Jurkat cells were stained with CFSE, then CFSE labeled jurkat cells were planted and treated with dH₂O, 50 or 100% of Ironwood Clay or French Clay. Carboxyfluorescein succinimidyl ester (CFSE) is a standard and common method to measure immune cell proliferation. The fluorescent dye in CFSE covalently binds to intracellular molecules. When a CFSE-labeled cell divides, each of two newly generated cells contain almost half the number of CFSE-tagged molecules and thus the intensity of fluorescent is decreased in these new cells. Each cell division cycle can then be assessed by measuring the corresponding decrease in cell fluorescence via flow cytometry. As such, each cell division will be detected as a new peak in the left side of the original peak in the flow cytometry plot.

Our results have shown significant increase in proliferation of the jurkat cells in the Ironwood Clay 50% (83.7 ± 3.2), 100% (87.3 ± 0.3), French Clay 50% (86.7 ± 0.5) and French Clay 100% (86.2 ± 0.8) compared to the control group (70.4 ± 1.9 , *P-value<0.01, n=3) (Fig. 6).

Figure 6



Production of pro-inflammatory cytokines (IL-12 & IFN-Gamma) using flow cytometric intracellular staining assay.

Flow cytometry analysis of IL-12⁺ and IFN-Gamma⁺ T-cells. As IL-12 and IFN-Gamma are key pro-inflammatory cytokines in inflammation, we wanted to see whether the number of IL-12 or IFN-Gamma producing cells is influenced with Ironwood Clay. In these experiments, Jurkat cells were stimulated with 70ng/ml phorbol 12-myristate 13-acetate (PMA) and 2uM Ionomycin and after 24 hours cells treated either with dH₂O, 50 or 100% of Ironwood Clay or French Clay. Cells were then harvested and stained for anti-human cell-membrane CD4 and anti-human intra-cellular IL-12 and IFN-Gamma. Figure 7-a, shows that the frequency of IL-12⁺ T cells markedly decreased in response to 100% Ironwood Clay compared with control (1.9%±0.1, 7.6%±0.3, **p < 0.001, n = 3). Ironwood Clay 50% (3.6±0.3) did not significantly decrease the frequency of IL-12 producing T-cells.

Figure 7-a

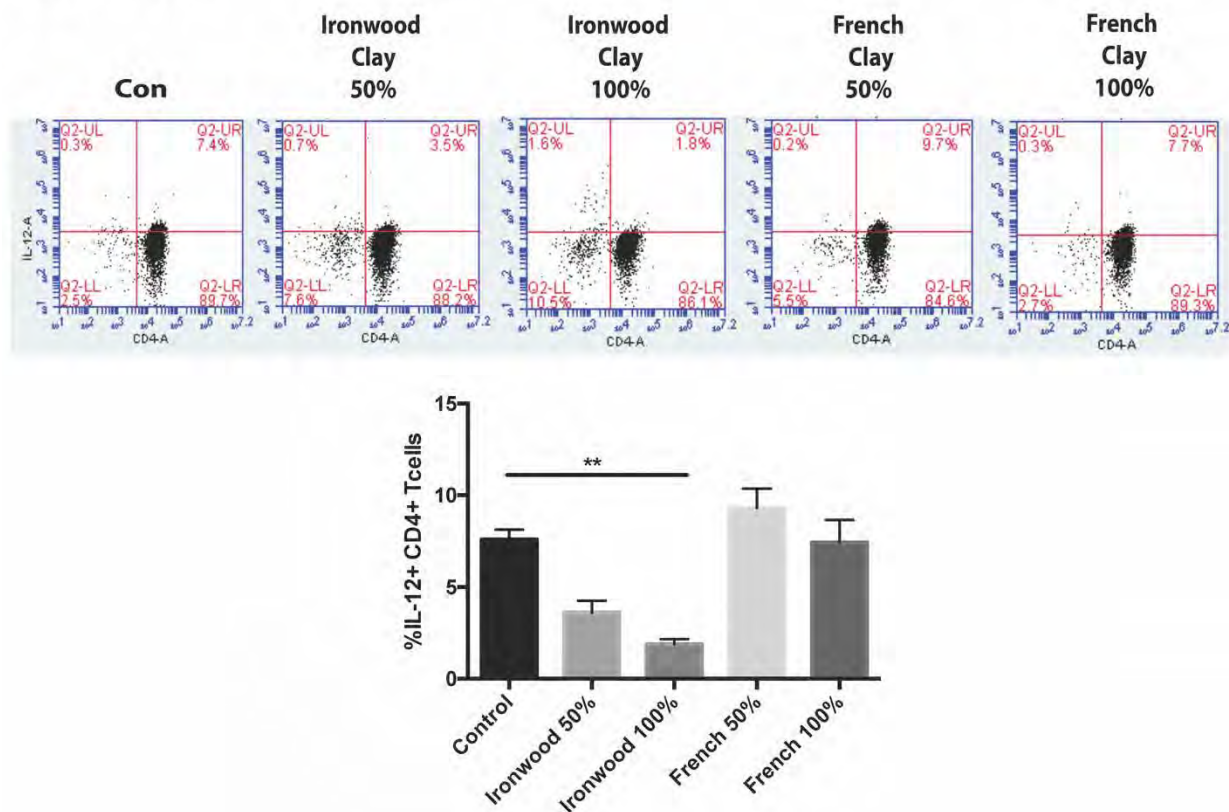
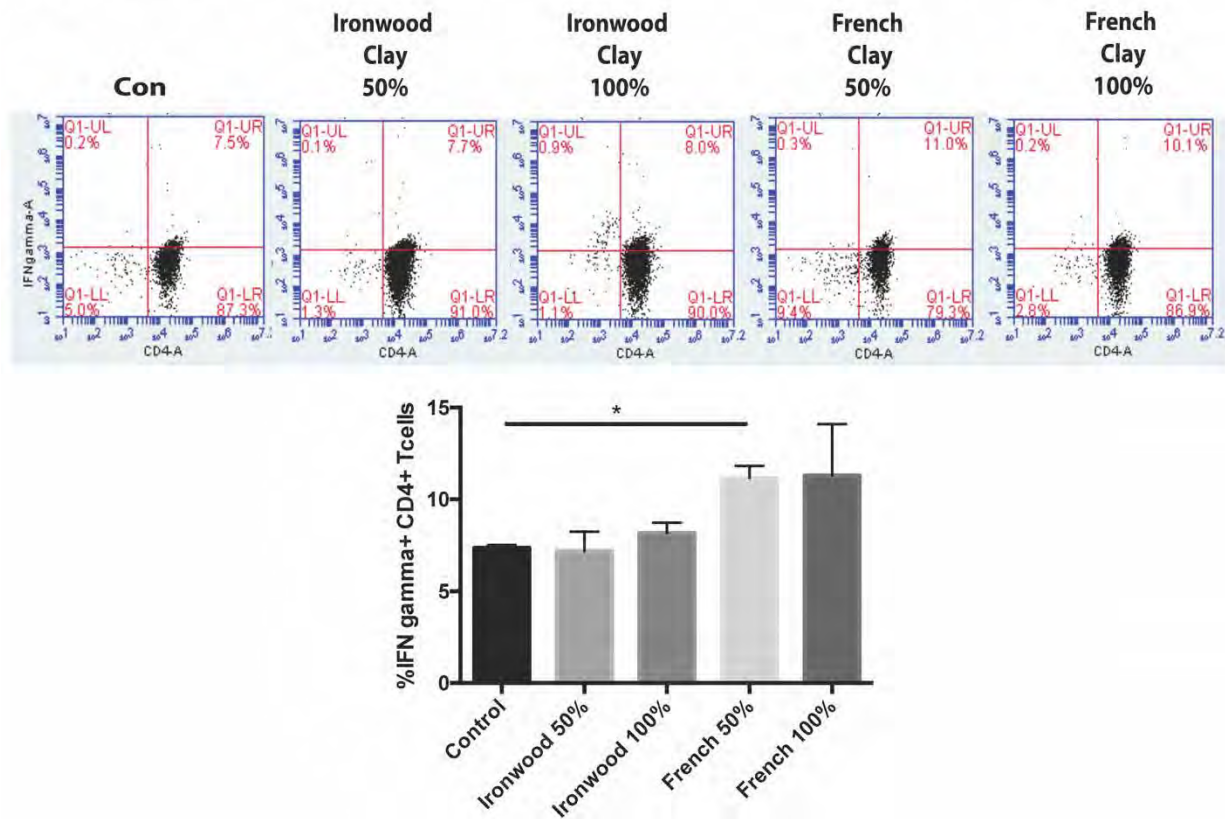


Figure 7-b, shows that under a similar experimental condition, the number IFN-Gamma producing T cells significantly did not change with 50% and 100% of Ironwood Clay. However, 50% French clay significantly increased the frequency of IFN-Gamma producing T cells compared to control ($11.1\% \pm 0.3$, $7.3\% \pm 0.1$, $*p < 0.01$, $n = 3$).

Figure 7-b



Acknowledgments

This work was supported and funded with support from Ironwood Clay Company and MITACS canada.

References

1. Uchechi O, Ogbonna JDN, Attama AA. Nanoparticles for Dermal and Transdermal Drug Delivery. *Application of Nanotechnology in Drug Delivery*. 2014;(Chapter 6):193-235. doi:<http://dx.doi.org/10.5772/57353>

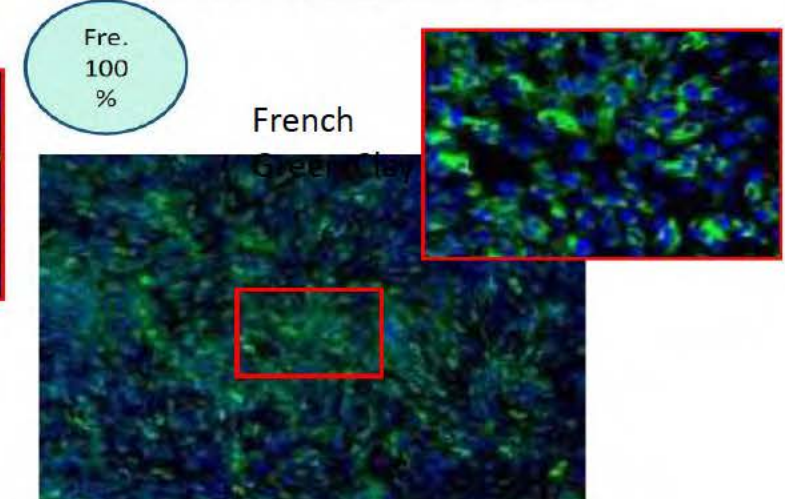
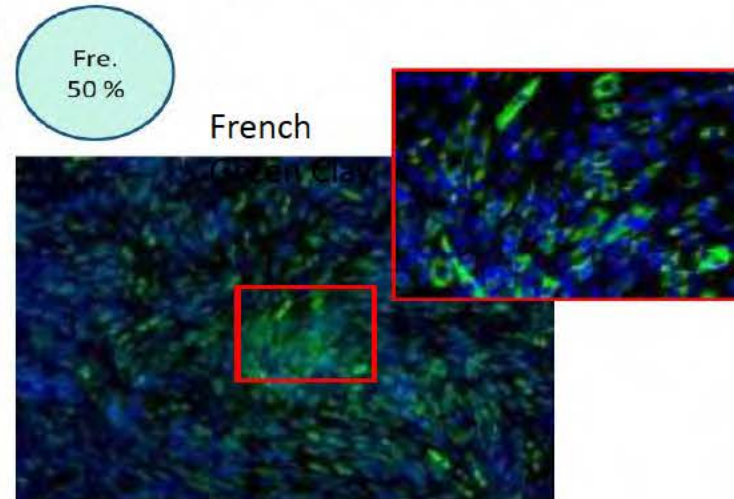
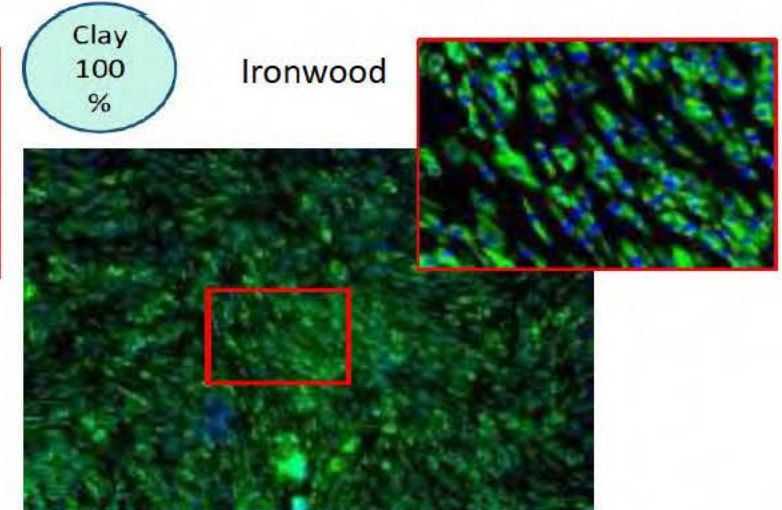
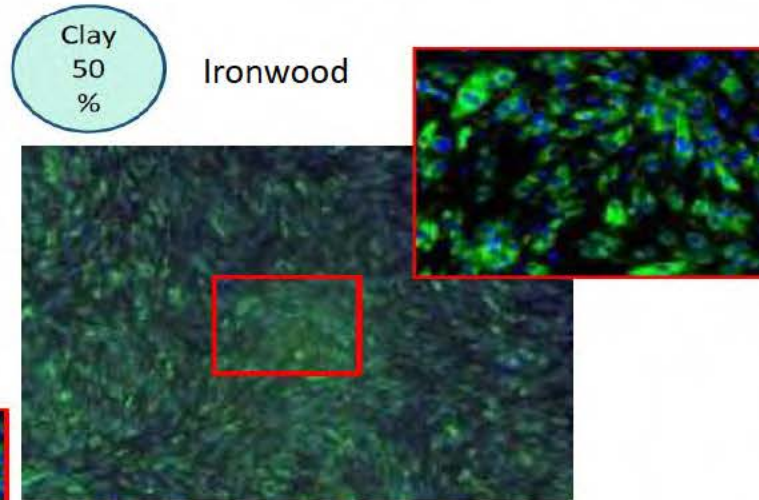
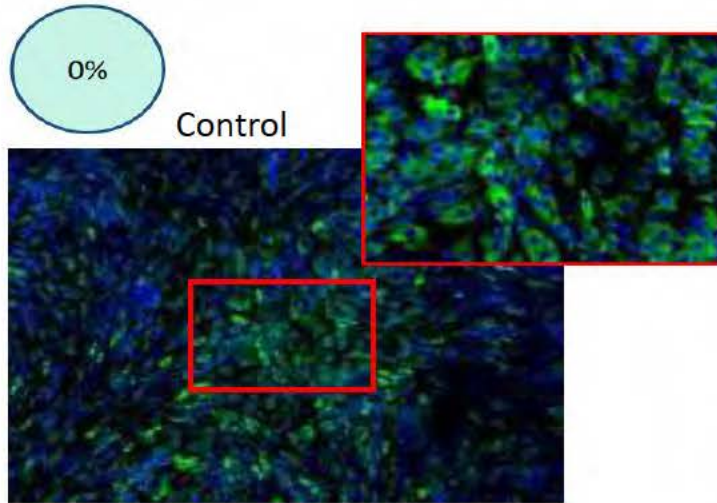


Effect of Glacial Oceanic Clay tested against French
Green Clay on Collagen and Pro-inflammatory
Protein Production

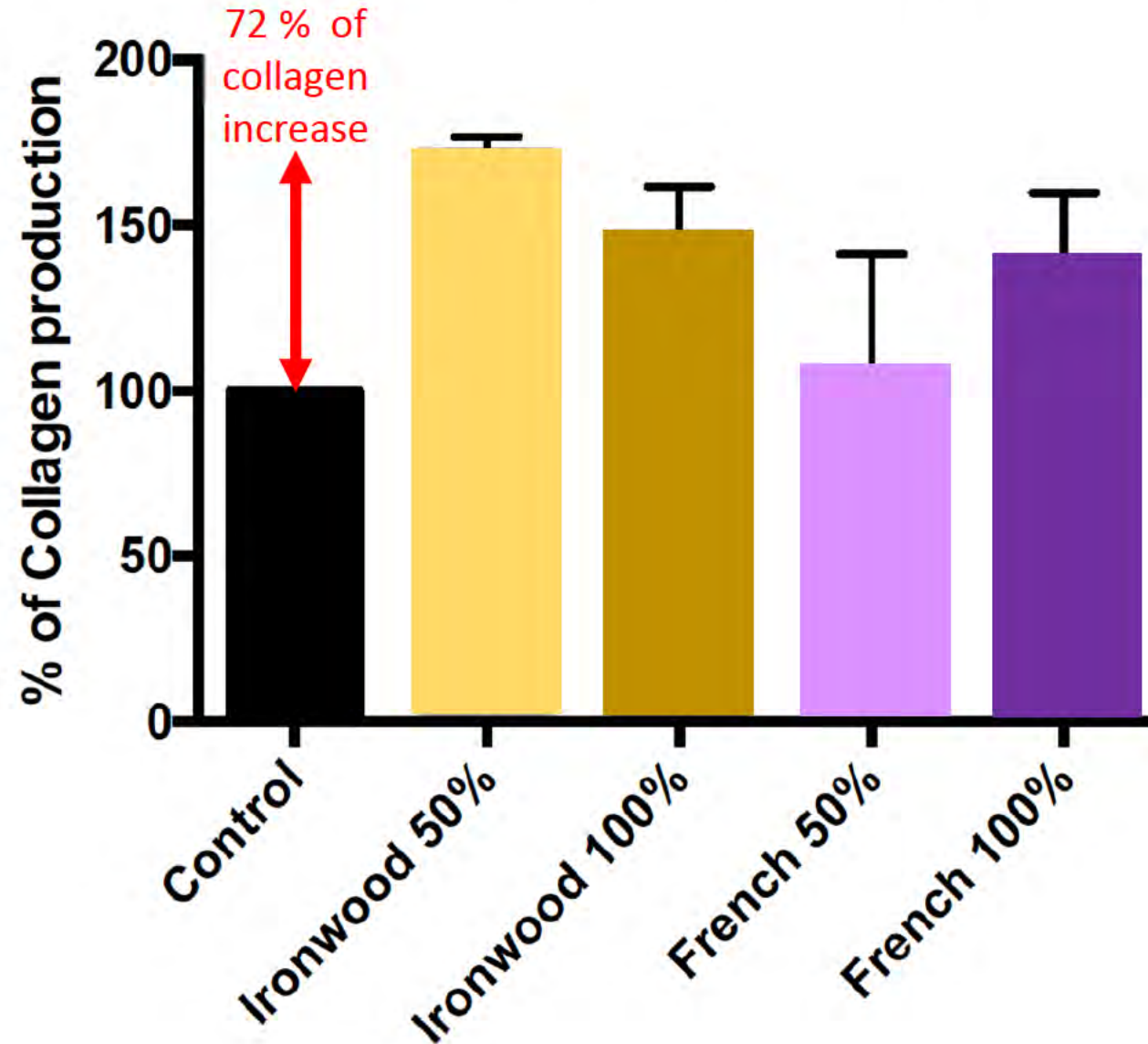
Effect of Glacial Oceanic Clay on Collagen Production

Immunofluorescence microscopy

Green fluorescence shows collagen production which compares with French Green Clay is more prominent.



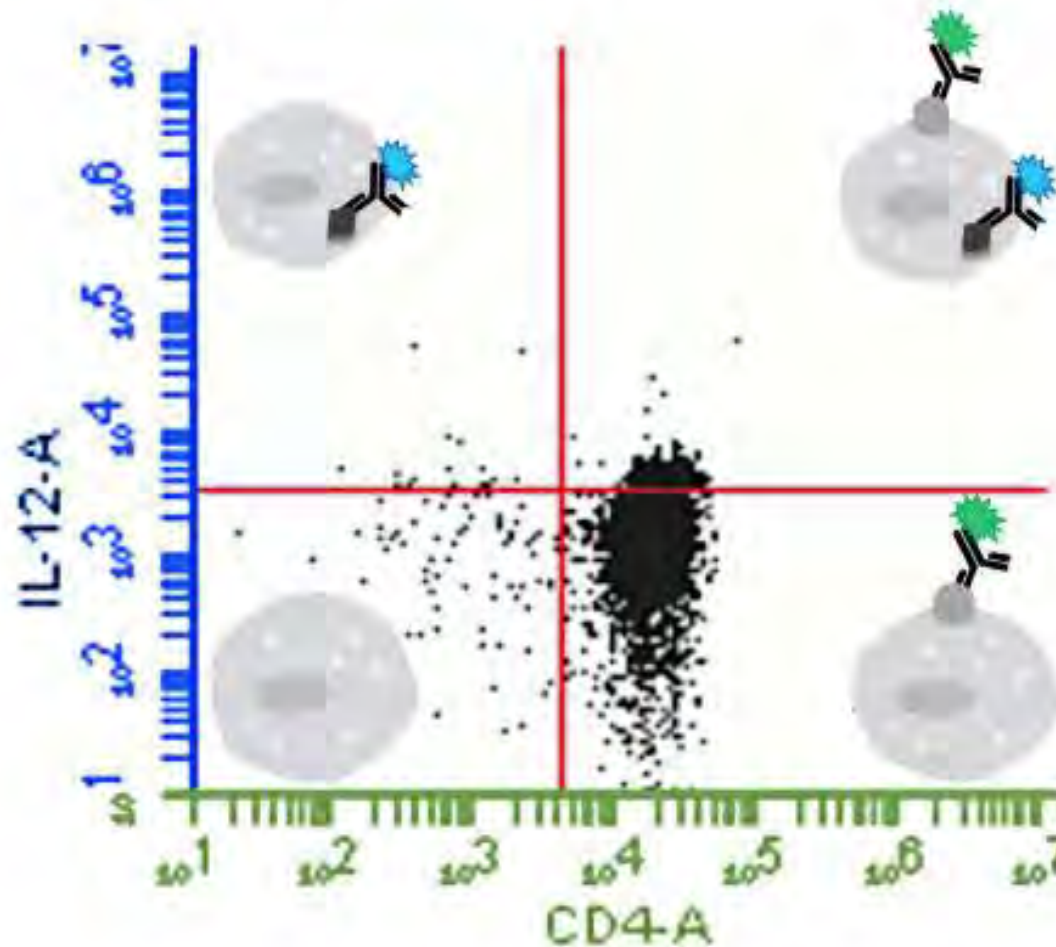
Effect of Glacial Oceanic Clay on Collagen Production



Glacial Oceanic Clay with 50% concentration increase collagen production by 72%.

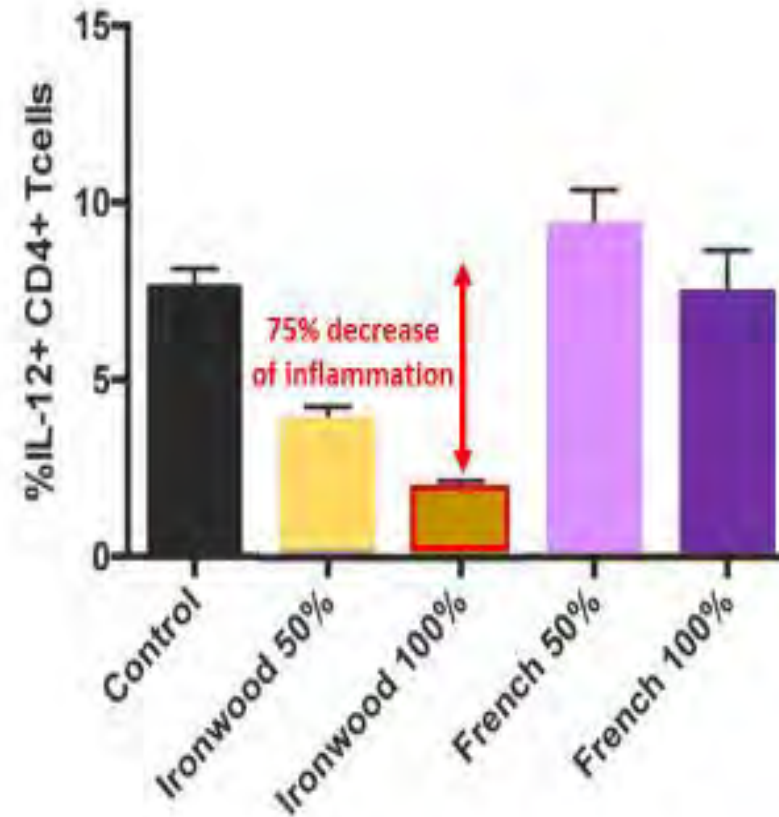
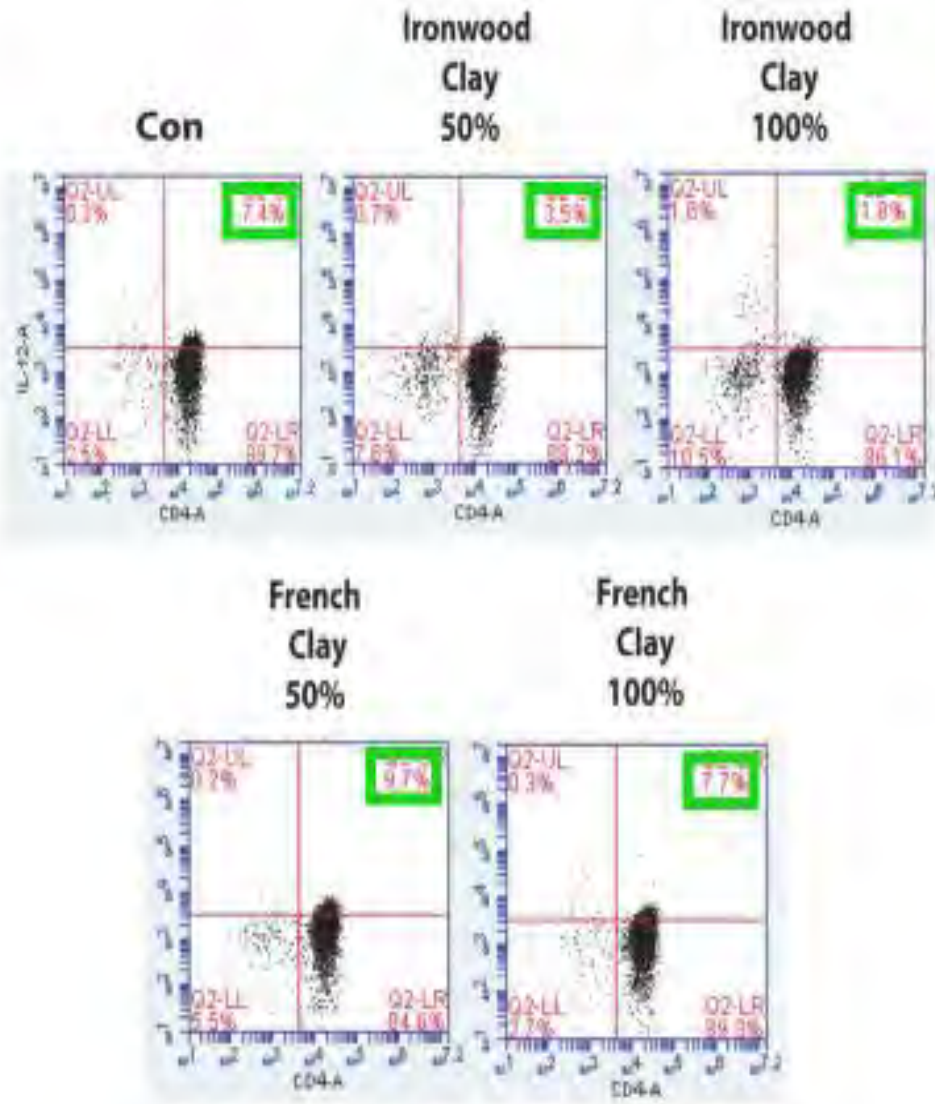
Effect of Glacial Oceanic Clay on pro inflammatory proteins

Flow Cytometry Results Interpretation



Detection of
FEWER number of
pro-inflammatory cells
in the top right corner
of the graphs means:
LESS INFLAMMATION

Effect of Glacial Oceanic Clay on pro inflammatory proteins



Glacial Oceanic Clay
decrease
inflammation by
75%.

The conclusion of the in vitro study in few words :

- Immunofluorescence microscopy shows that 50% of Glacial Oceanic Clay can increase the collagen production by 70% compare to control.
Ironwood Glacial Oceanic Clay helps with anti-aging.
- The production of pro-inflammatory cytokines IL-12 decreases by 75% in response to 100% Glacial Oceanic Clay.
Ironwood Glacial Oceanic Clay has a soothing effect.

***Comparative study for the determination of In Vivo
SPF (Sun Protection Factor) of two topical products
according to FDA Method 21 CFR 201.327(i)***

***Study N° 16AU-0620
Final Report***

Study Sponsor:

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This report is composed of 11 pages including appendices (2 pages).

July 29th, 2016

**Comparative study for the determination of In Vivo SPF
(Sun Protection Factor) of two topical products
according to
FDA Method 21 CFR 201.327(i)**

Study Report #16AU-0620

- **Tested Products:**
Glacial Oceanic Mineral Sunscreen – V3
batch #: BN3440
with Clay (Evalulab Lot #: 160610.EA.02)

Mineral Sunscreen - V1
batch #: BN3264
with purified water (Evalulab Lot #: 160610.EA.01)
- **Reference product:**
2011 FDA Standard SPF (Padimate O/Oxybenzone)
Lot 11-289-179
Evalulab Lot # 110726.AI.02
- Sponsor:**
Ironwood Clay Company Inc.
Represented by Holly Xing, Director of R&D
Unit 450 – 3771 Jacombs Road
Richmond, BC, V6V 2L9
Canada
Tel: 604 279-9498 ext. 122
- **Study duration:**
June 27th to July 14th, 2016
- **Location Study:**
Evalulab Inc.
5475 Paré, suite 206
Mont-Royal, QC, H4P 1P7
Canada
Tel: 514-343-0001
- **Date of Report submission:**
July 29th, 2016

Summary

1. Method.....	4
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1. Method

Study Objective

The purpose of the study described herein is to determine the "in vivo" Sun Protection Factor (SPF) of two test products against UVB radiation, based on the FDA's final monograph 21 CFR 201.327 (i), published on June 17th, 2011.

Experimental Design

A monocentric, open-label, randomized study involving a comparative assay of non-treated sites against sites treated with each test product.

Method Principle

The method consists of exposing the skin to a series of controlled but increasing doses of UVB radiation that will induce delayed erythematic response (redness of the skin due to superficial vasodilatation) which intensity is observed 24 hours after irradiation. The least amount of irradiation producing a marked redness on the skin is called MED (Minimal Erythema Dose).

Dividing the tpMEDp (Minimal Erythema Dose producing a marked redness on the protected skin with the test product, tp) by the MEDu (Minimal Erythema Dose producing a marked redness on the unprotected skin) will allow obtaining the SPF of the test product.

A FDA reference (SPF Standard, ss) is also tested to validate the method (i.e. dividing ssMEDp by MEDu).

Material

A Multiport, 150 W, Model 601 UV simulator, equipped with Xenon Arc lamp (Solar Light Company, Philadelphia, PA) is used. In accordance with the FDA requirements, the simulator is equipped with a UG-11 filter and a UV cut off filter (UVB or UVA), producing a spectrum of UV rays (290-400 nm) similar to natural sunlight impacting the surface of the earth, with an aperture of approx. 1 cm². The intensity, time, and therefore the total dose of UV exposure is measured and controlled by a dosimeter, Model PMA 2100 (Solar Light Company, Philadelphia, PA).

Selection of Volunteers

For this study, ten (10) volunteers were selected.

Volunteer Inclusion Criteria

- Men or Women,
- 18 years old or older,
- In good health,
- With skin phototype I, II or III based on the Fitzpatrick classification (Appendix),
- Without any skin anomalies on his/her back,
- Fully cooperating, after reading and signing the Informed Consent Form.

Volunteer Exclusion Criteria

- Those with a history of cutaneous sensitization,
- Those with a history of asthma, diabetes, psoriasis, or cutaneous anomalies in the tested zones,
- Those having significantly pigmented skin,
- Those who are pregnant or breastfeeding,
- Those with an existing or evolving serious illness,
- Those undergoing treatment or taking medication that may influence the outcome of the study.

Test Products

At the request of the sponsor, the following products have been tested:

Product name: Glacial Oceanic Mineral Sunscreen – V3
Category product: Sunscreen
Sponsor Batch #: BN3440
Evalulab lot#: 160610.EA.02
Received quantity: 2 containers of 200 g
Active ingredients used: Zinc Oxide 20%

Product name: Mineral Sunscreen - V1
Category product: Sunscreen
Sponsor Batch #: BN3264
Evalulab lot#: 160610.EA.01
Received quantity: 2 containers of 200 g
Active ingredients used: Zinc Oxide 20%

To validate the accuracy of the results, a FDA standard (7% Padimate O / 3% Oxybenzone) with an established SPF of $16.3 \pm$ Standard Deviation 3.43, was tested simultaneously with the test products.

Storage: Upon reception, the test products details were recorded and a number was assigned to each product. The samples were then stored at ambient temperature and humidity in an area designated for this purpose, until use.

Application: 0.1 g of each product is applied evenly to individual test sites, each 50 cm², to achieve a ratio of 2 mg/cm² on the respective sites. UV exposures to each zone begin 15 minutes after the application of the test product(s).

Adverse Events: Adverse events and/or severe adverse events, as well as concurrent treatments, if any, are documented in case report forms and mentioned in the final report submitted to the sponsor.

Determination of MEDu (Minimal Erythema Dose for unprotected skin):

A series of five UV exposures involving consecutive dose increments of 1.25 are administered to the non-protected skin to determine the MEDu.

Determination of MED_p (Minimal Erythema Dose for protected skin) for the SPF Standard (ssMED_p) and the test product (tpMED_p):

Depending on the expected SPF of the test product, the protected skin is exposed to varying doses of UVB radiation:

If the SPF is less than 8:

MED times 0.64X, 0.80X, 1.00X, 1.25X and 1.56X.

If SPF is between 8 and 15:

MED times 0.69X, 0.83X, 1.00X, 1.20X and 1.44X.

If SPF is greater than 15:

MED times 0.76X, 0.87X, 1.00X, 1.15X and 1.32X.

where "X" is the expected SPF value of the test product.

Calculations:

1) Individual SPF for each volunteer:

$$\text{Individual SPF} = \frac{\text{MED protected (MED}_p\text{)}}{\text{MED unprotected (MED}_u\text{)}}$$

2) Mean SPF:

$$\text{Mean SPF} = \bar{x} = \frac{\text{Sum of individual SPF values}}{n}$$

Where "n" is the total number of volunteers participating in the study.

The standard deviation (Stdev=s) of the mean SPF is also calculated.

3) Calculation of the Standard Error (SE):

$$\text{SE} = \frac{s}{\sqrt{n}}$$

Where "n" is the number of volunteers.

4) Value of "t".

For a statistical significance of >95%, (standard error ≤ 5 %) the "t" value is obtained from the t Distribution Table for n-1 degrees of freedom.

5) Determination of the labeled SPF value:

Labeled SPF = largest whole number less than $\bar{x} - (t \cdot \text{SE})$

2. Results

The following table presents the individual SPF values for the two test products and the FDA Standard as obtained for each volunteer. Individual data including UV doses in mJ/cm^2 was used for the calculation of MED_u, ssMED_p and tpMED_p:

Sunscreen products with expected SPF 30
Active: Zinc Oxide 20%

					Standard FDA		Sunscreen V3		Sunscreen V1	
Volunteer	Initials	Gender	Age	MED _u	ssMED _p	Results	Test Product tpMED _p	Results	Test Product tpMED _p	Results
02-0620-001	KM	M	24	14,0	228	16,3	358	25,6	252	18,0
01-0620-002	KM	F	22	12,0	196	16,3	338	28,2	307	25,6
01-0620-003	GH	F	68	14,5	236	16,3	334	23,0	242	16,7
01-0620-004	LB	F	57	13,0	248	19,1	348	26,8	172	13,2
01-0620-005	CG	F	65	13,0	212	16,3	366	28,2	218	16,8
01-0620-006	JW	F	66	13,5	191	14,1	188	13,9	217	16,1
01-0620-007	AG	F	41	20,0	326	16,3	493	24,7	239	12,0
01-0620-008	CM	F	63	15,0	245	16,3	258	17,2	212	14,1
01-0620-009	LM	F	29	11,0	179	16,3	164	14,9	258	23,5
01-0620-010	ML	F	58	17,0	277	16,3	419	24,6	233	13,7
				Mean	49,3	14,3	Mean	22,7	Mean	17,0
				Stdev	18,5	2,6	Stdev	5,4	Stdev	4,4

For the test products:

Glacial Oceanic Mineral Sunscreen – V3

The Mean SPF is $x = 22.7$

The Standard Deviation s is 5.4

$n = 10$ $df = n-1 = 10-1 = 9$ t (5% one tail) = 1.833

$SE = s/\sqrt{n} = 5.4/\sqrt{10} = 1.700$

$x - (t*SE) = 22.7 - 3.1 = 19.6$

Labeled SPF = Largest whole number less than $x - (t*SE) = 19$

Mineral Sunscreen - V1

The Mean SPF is $x = 17.0$

The Standard Deviation s is 4.4

$n = 10$ $df = n-1 = 10-1 = 9$ t (5% one tail) = 1.833

$SE = s/\sqrt{n} = 4.4/\sqrt{10} = 1.399$

$x - (t*SE) = 17.0 - 2.564 = 14.4$

Labeled SPF = Largest whole number less than $x - (t*SE) = 14$

For the 2011 FDA Standard: The mean SPF obtained was 16.4 ± 1.2 . Our result falls within the expected SPF for the FDA standard (i.e. 16.3 ± 3.43), and hence validates the test method.

Comparison

#Volunteer	SPF Sunscreen Results	
	V3	V1
001	25,6	18,0
002	28,2	25,6
003	23,0	16,7
004	26,8	13,2
005	28,2	16,8
006	13,9	16,1
007	24,7	12,0
008	17,2	14,1
009	14,9	23,5
010	24,6	13,7
Average	22,7	17,0
Stdev	5,4	4,4
t-Test		0,03

(Statistical Significance - Red: $p < 0.05$)

N.B. All t-Test results are expressed in p-values.

In addition, the comparison of SPF between **Glacial Oceanic Mineral Sunscreen – V3** and **Mineral Sunscreen - V1** indicated a significant difference between the two formulas suggesting a SPF boosting effect for the formula containing the glacial oceanic clay.

3. Tolerance

No cutaneous intolerance or adverse events were observed for the test products nor reported by the volunteers during the study.

4. Quality Assurance

No deviation from the protocol was observed.

This study was conducted under my responsibility in accordance with experimental protocols and the principles of "*Good Clinical Practice*".

All observations and data collected during the study have been reported in this report.

This standard procedure and associated documents were reviewed and approved by an Ethics Committee prior to the beginning of the study.

The records obtained during the study will be kept by Evalulab Inc. for a period of 2 years.



Signature
Elisabeth Fiquet, M. Sc.
Investigator

Date: July 29th, 2016

After reviewing this document, I the undersigned, Linh Nguyen, declare that the information provided in this report reflects in a complete and exact manner the results obtained during the study.



Signature
Linh Nguyen, M. Sc.
Quality Assurance Director

Date: July 29th, 2016

5. APPENDICES

Table I: Fitzpatrick Classification for Skin Phototypes

Phototype	Type	Hair	Complexion	Erythema Potential	Tanning Potential
0	Caucasian	White	Albinos	Burns	Does not tan
I	Caucasian	Red	Off white	Burns easily	Does not tan
II	Caucasian	Blond	Very Clear White	Burns easily	Tans slightly
III	Caucasian	Blond to brown	Clear Matte	Burns moderately	Tans gradually
IV	Mongolian, Asian, Mediterranean, Arab	Light Brown	Matte	Burns rarely	Tans well
V	Mongolian	Dark Brown	Matte	Burns rarely	Tans very well
VI	African / Black	Black	Black	Does not burn	Highly pigmented

Table II: Ingredients list of each test product provided by the sponsor

Glacial Oceanic Mineral Sunscreen – V3

PRODUCT Sunscreen SPF 30 – V3			
Note:	Evaluate, w clay		
Batch Number:	3440		
Date issued:	June 8, 2018		
Note:			
INGREDIENTS	CTFA/INCI NAME	Supplier	% W/W
Montanov 68	Cetearyl Alcohol & Cetearyl Glucoside	CHARLES TENNANT & COMPANY	3.00
Crodacol 595	Stearyl Alcohol	Croda	2.50
OLWAX® LC	Cetyl Palmitate, Sorbitan Palmitate, Sorbitan Olivat	CAMBRIAN / HALLSTAR	2.00
Shea Butter - Organic	Shea Butter - Organic	AZELIS CANADA CHEMICALS LTD	0.50
Cetiol C5	Coco-caprylate	PACHEM DISTRIBUTION	8.00
Xiameter PMS 200 Silicon Fluid 100 CS	Dimethicone	UNIVAR CANADA LTD / DOW CORNING	8.00
G-Block DZ 370 CCT	Zinc Oxide (and) Caprylic/Capric Triglyceride (and) Polyhydroxystearic Acid (and) Polyglyceryl-3 Polyricinoleate (and) Isostearic Acid (and) Lecithin	AZELIS CANADA CHEMICALS LTD	28.57 (20% ZnO)
Purified Water	Aqua	IC	44.77
Natural Oceanic Clay	See SR	IC	1.66
Optiphen ND	Phenoxethanol (and) Benzoic Acid (and) Dehydroacetic Acid	Ashland	1.00

Mineral Sunscreen - V1

PRODUCT Sunscreen SPF 30 - V1			
Note:	Evaluate, w P Water		
Batch Number:	3284		
Date issued:	14-Oct-15		
Note:			
INGREDIENTS	CTFA/INCI NAME	Supplier	% W/W
Montanov 68	Cetearyl Alcohol & Cetearyl Glucoside	CHARLES TENNANT & COMPANY	3.00
Crodacol 595	Stearyl Alcohol	Croda	2.50
OLWAX® LC	Cetyl Palmitate, Sorbitan Palmitate, Sorbitan Olivat	CAMBRIAN / HALLSTAR	2.00
Shea Butter - Organic	Shea Butter - Organic	AZELIS CANADA CHEMICALS LTD	0.50
Cetiol C5	Coco-caprylate	PACHEM DISTRIBUTION	8.00
Xiameter PMS 200 Silicon Fluid 100 CS	Dimethicone	UNIVAR CANADA LTD / DOW CORNING	8.00
G-Block DZ 370 CCT	Zinc Oxide (and) Caprylic/Capric Triglyceride (and) Polyhydroxystearic Acid (and) Polyglyceryl-3 Polyricinoleate (and) Isostearic Acid (and) Lecithin	AZELIS CANADA CHEMICALS LTD	28.57 (20% ZnO)
Purified Water	Aqua	IC	46.43
Optiphen ND	Phenoxethanol (and) Benzoic Acid (and) Dehydroacetic Acid	Ashland	1.00

MINERAL BENEFITS IN GLACIAL OCEANIC CLAY

Calcium

The human body contains more calcium than any other mineral -about 90% of our bone structure is made up of it. It plays a major role in providing firmness and elasticity for the skin as well as all tissues and cells of the body. A calcium deficiency will result in fragile and thin skin, loss of hair, weak bones and brittle nails.

Magnesium

Magnesium provides the power needed for enzymes to carry out the antioxidant processes necessary for maintaining good skin health. Antioxidant protection helps to neutralize free-radicals present in the environment. Studies show that magnesium also possesses good water-binding molecules. As a result, magnesium skin care products help to enhance moisture-retention and hydration. This allows for an improvement in the skin's overall appearance and texture.

Potassium

Potassium, helps regulate the amount of water in your body's cells, so a lack of potassium in the diet can lead to dry skin and other skin disorders. Potassium is an electrolyte, which means it holds an electric charge, which is needed for cells to function normally. Most people get enough potassium through their daily diet, but if you've been sick with diarrhea or lost a lot of fluid through perspiration, you may need to take an electrolyte powder to top up your stores. The health benefits of potassium are vital for skin care, along with maintaining overall good health, but try to avoid going over the recommended daily amounts.

Copper:

Copper plays a key role in the synthesis and stabilization of skin proteins. It stimulates dermal fibroblasts proliferation, upregulates collagen (types I, II, and V) and elastin fiber components (elastin, fibrillin) production, serves as a cofactor of LOX enzyme responsible for cross-linking of extracellular matrix (ECM) proteins and stabilization of the skin ECM once formed, inhibition of cellular oxidative effects such as membrane damage and lipid peroxidation. Copper is a very weak sensitizer as compared with other metal compounds, and the risk of adverse reactions, due to dermal contact with copper, is exceptionally low.

Zinc:

Abundant evidence demonstrates the antioxidant role of zinc. Zinc has anti-inflammatory properties and increases reepithelialization supporting its use for treating eczemas. It also prevents recurrence of flaking, itching, and irritation associated with dandruff and its antifungal activity has been attributed to its ability to disrupt fungal membrane transport. Its use has also expanded manifold over the years for a number of dermatological conditions including infections, inflammatory dermatoses (acne vulgaris, rosacea, pigmentary disorders).