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

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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 re-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 $Si_4O_6(OH)_4$*. 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).

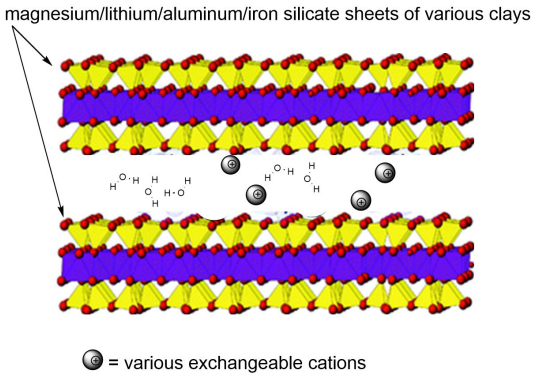

Figure 1. General structure of clay ingredients.[CIR Staff]

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 D_{50} 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 5µm, 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 ≤ 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 Attapulgite, 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] Attapulgite 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]

Attapulgite

Attapulgite 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 swine[36] 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 swine[36] 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 [^{134}Cs]-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 [^{134}Cs]-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 ^{134}Cs 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, [^{134}Cs]-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 µCi for 29 dogs and from 42 to 64 µCi lung burdens for the other 3 dogs. Effective translocation half-time of lung instillations was 390 d. The accumulation rate of [^{134}Cs]-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 LD_{50} 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 LD_{50} 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 LD_{50} 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 LC_{50} 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/m^3, ~ 10 µm 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 $\pm$ 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 µm mean diameter) in 300 µl of sterile phosphate-buffered saline (PBS).[39] The dust particles were injected into the trachea. Control animals (groups of 6) received either 300 µl of PBS, 50 mg of carbonyl iron in 300 µl PBS, or 50 mg of α-quartz in 300 µl 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 µm) 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 µm compared to 1225 µ in control animals. The corpus callosum was an average of 125 µm in thickness in the sheep compared to 475 µm 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 nucleoplastic 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/cm^2 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.

Attapulgite

In 2 studies looking at unscheduled DNA synthesis (UDS) in rat hepatocytes, Attapulgite 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, Attapulgite tested at 2, 4, or 10 µg/cm^2 produced a significant increase in UDS and inhibited cell growth at 10 µg/cm^2. Attapulgite 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 Attapulgite at 10 µg/cm^2 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 Attapulgite (IARC uses the mineralogical term "palygorskite" for Attapulgite).[49] 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 their carcinogenicity to humans (Group 3).

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

Inhalation

Attapulgite

In a rat inhalation study, groups of 40 (20 male and 20 female) SPF Fischer rats were exposed to samples of Attapulgite dust mined in Lebrija or Leicester in inhalation chambers at a concentration of 10 mg/m^3 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/m^3. 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 Attapulgite. Thirty-five rats had no proliferative changes. In rats treated with Leicester Attapulgite, 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/m^3 Kaolin for 6 h/d for 5 d/wk. Two bronchoalveolar hyperplasias were reported.

Parenteral

Attapulgite

In an intratracheal study, groups of 5 rats received a single instillation of Attapulgite 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 µm, and 100% of the fibers were less than 3 µm. 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 Attapulgite samples.[1] The samples were 90% pure with quartz being the other component. One dose consisted of fibers > 4 µm and the other contained no fibers > 4 µm. 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 Attapulgite. Of rats receiving the positive control, crocidolite, 14/29 developed pleural mesotheliomas.

Attapulgite (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 µm. 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 Attapulgite. Survival times between the Attapulgite-treated group and the controls were not statistically different.

Attapulgite 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 µm 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 Attapulgite-treated animals and 729 days for nontreated animals.

In another intrapleural study, injections of 20 mg of different Attapulgite 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 Attapulgite fibers in this experiment was 0.77 µm. 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 ± 155 days) was very similar to that of the control groups (809 ± 110 days). The incidence of mesothelioma was 0% for control groups and treated groups. The researchers concluded Attapulgite was not carcinogenic in this study.

Samples of Attapulgite 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 µm, for Lebrija Attapulgite, at most 0.54% > 6 µm for Torrejon Attapulgite, and 19% > 6 µm for Leicester Attapulgite. 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 Attapulgite, Torrejon Attapulgite, Leicester Attapulgite, and crocidolite, respectively. In the negative controls, no mesotheliomas were reported for the Kaolin and 1 mesothelioma was reported for the saline group. Lebrija Attapulgite dust extracted from the lung had fibers < 2 µm. Material examined from Torrejon Attapulgite was fibrous and had fiber length up to 8 µm. Leicester Attapulgite fibers from extracted lungs were up to 25 µm. The investigators considered these fibers to be tumorigenic.

Three samples of 25 mg of Attapulgite dust were injected intraperitoneally into 40 Wistar rats.[1] Electron microscopy of the sample revealed 37.5% of fibers < 2 µm long and 70.0% < 5 µm. 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 Attapulgite 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 Attapulgite 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 ± 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

Attapulgite

In an intratracheal study, groups of 5 rats received a single instillation of Attapulgite 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 Attapulgite 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, Attapulgite 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 Attapulgite, 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.

Attapulgite with a mean fiber length of 0.8 µm and diameter of 0.02 µm was delivered to the lungs of sheep by bronchioscopic cannulation.[1] The tracheal lobe of 16 sheep was subjected to a single exposure of 100 mg of Attapulgite 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 x 10^6 cells after instillation and started declining more slowly up to 4 d. At 7 d, the polymorphonuclear leukocyte numbers were 2.5 x 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 µg/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 µm. 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 μm 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 μm. 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 (kaolinosis) 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 (kaolinosis).

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

Attapulgite

A cohort of 2302 men employed for at least 1 mo between January 1, 1940 and December 31, 1975 at an Attapulgite 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/m^3 and respirable fraction - 5 mg/m^3.[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/m^3 and respirable fraction - 5 mg/m^3.

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 LD_{50} greater than 2000 mg/kg. The same product was tested in an acute inhalation study in rats and had an LC_{50} 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 μm) 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 nucleoplastic 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/cm^2 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/m^3 and respirable fraction - 5 mg/m^3. NIOSH lists the following REL for up to 10-h time weighted average for Kaolin: total dust - 10 mg/m^3 and respirable fraction - 5 mg/m^3. 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
Attapulgite 12174-11-7 1337-76-4	Attapulgite 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 $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$.	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
	Attapulgite	
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	$Mg(Al_{0.5\text{-}1}Fe_{0\text{-}0.5})Si_4O_{10}(OH)\cdot 4H_2O$	[1]
Density (g/ml)	2.2	[1]
Solubility	Insoluble in water	[1]
Particle Size/Length (µm)	< 5	[1]
	Bentonite	
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	$Al_2O_3\cdot 4SiO\cdot 2H_2O$	[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]
	Clay	
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]
	Fuller's Earth	
Physical Form	Non-plastic variety of Kaolin; sheet structure	[1]
	Hectorite	
Physical Form	Translucent colorless mineral when mined and turns white when dried; tridecahedral	[1]
Chemical Formula	$Na_{0.33}(Mg_{2.67}Li_{0.33})Si_4O_{10}(OH)_2$	[1]
Specific Gravity (g/ml)	2.65	[1]
	Illite	
Physical Form	Gray-white to silvery-white, greenish-gray claylike material; waxy, greasy, earthy, or dull luster; dioctahedral	[8]
Chemical Formula	$K_{0.65}Al_{2.0}[Al_{0.65}Si_{3.35}O_{10}](OH)_2$	[8]
Specific Gravity (g/ml)	2.79 - 2.80	[8]
	Kaolin	
Physical Form	White or yellowish white, earthy mass or white powder; unctuous when moist	[1]
Chemical Formula	$Al_2O_3\cdot 2SiO_2\cdot 2H_2O$	[1]
Formula Weight (Da)	258.2	[1]
Solubility	Insoluble in water, alcohol, dilute acids, and alkali solutions	[1,7]
	Montmorillonite	
Chemical Formula	$R^+_{0.33}(Al, Mg)_2Si_4O_{10}(OH)_2$, where $R^+ = Na^+, K^+, Mg^{2+}$ 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**
	Attapulgite				**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								
Eyeliner					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.

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

[b] 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

[c] 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 µg/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 µg/cm^2), 48 h (5 µg/cm^2), and 72 h (1 µg/cm^2); and with a quartz content of 1% for 72 h (1 µg/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 µg/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	*S. typhimurium* 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 µg/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 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	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 µg/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 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	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	*S. typhimurium* 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	*S. typhimurium* TA98 and TA100	*Salmonella*/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(α)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 ± 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 ± 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 *gpt* delta transgenic mice	*gpt* 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 *gpt* and Spi^- mutant frequencies observed in the lungs of the mice; mutation spectra analysis showed > 60% of the base substitution occurred in the *gpt* 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	KeratinoSens™ cells	KeratinoSens™ 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 erythemal dose of UVB; sites were evaluated at 24, 48, and 72 h after irradiation	Not a photosensitizer	[64]

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