Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite[1]

This report reviews the safety of Aluminum, Calcium, Lithium Magnesium, Lithium Magnesium Sodium, Magnesium Aluminum, Magnesium, Sodium Magnesium, and Zirconium Silicates, Magnesium Trisilicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite as used in cosmetic formulations. The common aspect of all these claylike ingredients is that they contain silicon, oxygen, and one or more metals. Many silicates occur naturally and are mined; yet others are produced synthetically. Typical cosmetic uses of silicates include abrasive, opacifying agent, viscosity-increasing agent, anticaking agent, emulsion stabilizer, binder, and suspending agent. Clay silicates (silicates containing water in their structure) primarily function as adsorbents, opacifiers, and viscosity-increasing agents. Pyrophyllite is also used as a colorant. The International Agency for Research on Cancer has ruled Attapulgite fibers >5 μm as possibly carcinogenic to humans, but fibers <5 μm were not classified as to their carcinogenicity to humans. Likewise, Clinoptilolite, Phillipsite, Mordenite, Nonfibrous Japanese Zeolite, and synthetic Zeolites were not classified as to their carcinogenicity to humans. These ingredients are not significantly toxic in oral acute or short-term oral or parenteral toxicity studies in animals. Inhalation toxicity, however, is readily demonstrated in animals. Particle size, fibrogenicity, concentration, and mineral composition had the greatest effect on toxicity. Larger particle size and longer and wider fibers cause more adverse effects. Magnesium Aluminum Silicate was a weak primary skin irritant in rabbits and had no cumulative skin irritation in guinea pigs. No gross effects were reported in any of these studies. Sodium Magnesium Silicate had no primary skin irritation in rabbits and had no cumulative skin irritation in guinea pigs. Hectorite was nonirritating to the skin of rabbits in a Draize primary skin irritation study. Magnesium Aluminum Silicate and Sodium Magnesium Silicate caused minimal eye irritation in a Draize eye irritation test. Bentonite caused severe iritis after injection into the anterior chamber of the eyes of rabbits and when injected intralamellarly, widespread corneal infiltrates and retrocorneal membranes were recorded. In a primary eye irritation study in rabbits, Hectorite was moderately irritating without washing and practically nonirritating to the eye with a washout. Rats tolerated a single dose of Zeolite A without any adverse reaction in the eye. Calcium Silicate had no discernible effect on nidation or on maternal or fetal survival in rabbits. Magnesium Aluminum Silicate had neither a teratogenic nor adverse effects on the mouse fetus. Female rats receiving a 20% Kaolin diet exhibited maternal anemia but no significant reduction in birth weight of the pups was recorded. Type A Zeolite produced no adverse effects on the dam, embryo, or fetus in either rats or rabbits at any dose level. Clinoptilolite had no effect on female rat reproductive performance. These ingredients were not genotoxic in the Ames bacterial test system. In primary hepatocyte cultures, the addition of Attapulgite had no significant unscheduled DNA synthesis. Attapulgite did cause significant increases in unscheduled DNA synthesis in rat pleural mesothelial cells, but no significant increase in sister chromosome exchanges were seen. Zeolite particles (<10 μm) produced statistically significant increase in the percentage of aberrant metaphases in human peripheral blood lymphocytes and cells collected by peritoneal lavage from exposed mice. Topical application of Magnesium Aluminum Silicate to human skin daily for 1 week produced no adverse effects. Occupational exposure to mineral dusts has been studied extensively. Fibrosis and pneumoconiosis have been documented in workers involved in the mining and processing of Aluminum Silicate, Calcium Silicate, Zirconium Silicate, Fuller's Earth, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the extensive pulmonary damage in humans was the result of direct occupational inhalation of the dusts and noted that lesions seen in animals were affected by particle size, fiber length, and concentration. The Panel considers that most of the formulations are not respirable and of the preparations that are respirable, the concentration of the ingredient is very low. Even so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation. With this admonition to the cosmetics industry, the CIR Expert Panel concluded that these ingredients are safe as currently used in cosmetic formulations.

Received 4 December 2002; accepted 18 March 2003.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Amy R. Elmore, former Scientific Analyst and Writer. Address correspondence to F. Alan Andersen, Cosmetic Ingredient Review Director, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

International Journal of Toxicology, 22(Suppl. 1):37–102, 2003

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810390204890

The Panel did note that the cosmetic ingredient, Talc, is a hydrated magnesium silicate. Because it has a unique crystalline structure that differs from ingredients addressed in this safety assessment, *Talc is not included in this report.*

INTRODUCTION

Various silicates and silicate clays are used in cosmetics, largely for their adsorbent, anticaking, bulking, and other similar properties. They are created synthetically in some cases, e.g., Lithium Magnesium Silicate, or are refined from naturally occurring minerals, e.g., Magnesium Aluminum Silicate. In either case, variations in composition occur. Thus the Zeolite group of hydrated aluminosilicates has forms that are crystalline or fibrous, and contain interchangeable cations.

This report reviews the safety of these ingredients. Because the issues of safety are likely to be similar, many ingredients have been grouped. Although there are not data on each and every ingredient, it is expected that the data will be broadly applicable among the following ingredients: Aluminum Silicate (CAS no. 1327-36-2); Calcium Silicate (CAS no. 1344-95-2); Magnesium Aluminum Silicate (CAS no. 12199-37-0, 1327-43-1, 12511-31-8); Magnesium Silicate (CAS no. 1343-88-0); Magnesium Trisilicate (CAS no. 14987-04-3); Sodium Magnesium Silicate; Zirconium Silicate (CAS no. 14940-68-2); and the silicate clays/clay minerals: Attapulgite (CAS no. 1337-76-4, 12174-11-7); Bentonite (CAS no. 1302-78-9); Fuller's Earth (CAS No. 8031-18-3); Hectorite (CAS no. 12173-47-6); Kaolin (CAS no. 1332-58-7); Lithium Magnesium Silicate; Lithium Magnesium Sodium Silicate (CAS no. 53320-86-8); Montmorillonite (CAS no. 1318-93-0); Pyrophyllite (CAS no. 12269-78-2); and Zeolite (CAS no. 1318-02-1) used in cosmetics.

It is important to note that the cosmetic ingredient, Talc, is not included in this safety assessment. Talc is a hydrated magnesium silicate with the CAS no. 14807-96-6, but it should not be confused with any of the silicates in this report. Talc is differentiated by its definition, a hydrated magnesium silicate, and its unique crystalline form.

The safety of Quaternium-18 Hectorite and Quaternium-18 Bentonite have been previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel; the final conclusion indicated that "Quaternium-18 Hectorite and Quaternium-18 Bentonite are safe as cosmetic ingredients in the present practices of use and concentration" (CIR 1980).

CHEMISTRY

Given the large number of ingredients, a tabular presentation of basic information concerning the chemical description has been provided (Table 1).

Zeolites

The Zeolite group is very diverse. Over 100 structural types of Zeolites, both natural and synthetic, have been reported, 40 of which are natural Zeolites (IARC 1997). Even though these Zeolites are considered to be a group, the formulas of the most common are listed in tabular form in Table 2 so the reader can understand the diversity in this category.

Physical and Chemical Properties

In alphabetical order according to the cosmetic ingredient name as specified in the *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger et al. 2000), Table 3 provides information on the various synonyms used to describe each cosmetic ingredient, lists the available information on physical properties, and, if available, provides the specifications for the cosmetic grade of the ingredient.

Clay Structure

According to Grim (1967), clays in general have atomic lattices consisting of two structural units. One unit consists of two sheets of closely packed oxygens or hydroxyls as shown in Figure 1. 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 as shown in Figure 2. 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 (Grim 1967).

Attapulgite

The general attributes of structure and composition of the minerals are not very well known. The structurally important element is the amphibole double silica chain oriented with its long direction parallel to the *c* axis as shown in Figure 3. Attapulgite

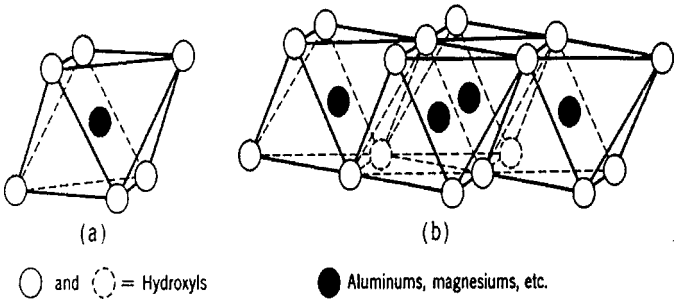

FIGURE 1
(*a*) Single octahedral unit; (*b*) Sheet of units (taken from Grim 1967 with permission).

TABLE 1
Chemical formulas and compositions of Silicates and Silicate Clays used in cosmetics

Ingredient	Description	Reference
Aluminum Silicate	$Al_2O_3 \cdot SiO_2$	Wenninger et al. 2000
	Complex inorganic salt that has a composition of consisting generally of 1 mole of alumina and 1 to 3 moles of silica	Wenninger et al. 2000
Calcium Silicate	Varying CaO and SiO_2	Wenninger et al. 2000
	Hydrous or anhydrous silicate with varying proportions of calcium oxide and silica	Wenninger et al. 2000
Magnesium Aluminum Silicate	$Al_2MgO_8Si_2$	Budavari 1989
	Complex silicate refined from naturally occurring minerals	Wenninger et al. 2000
Magnesium Silicate	$MgO \cdot SiO_2 \cdot xH_2O$	Wenninger et al. 2000
	Inorganic salt of variable composition	Wenninger et al. 2000
Magnesium Trisilicate	$2MgO_3 \cdot SiO_2 \cdot xH_2O$	Wenninger et al. 2000
	Inorganic compound	Wenninger et al. 2000
Zirconium Silicate	$ZrSiO_4$	Wenninger et al. 2000
	Inorganic compound	Wenninger et al. 2000
	Zircon sand or flour; specially sized grades of the mineral zircon—a naturally occuring zirconium silicate	American Minerals, Inc. 1998
Attapulgite	$[Mg(Al_{0.5-1}Fe_{0-0.5}]Si_4O_{10}(OH) \cdot 4H_2O$	IARC 1997
	Variety of Fuller's Earth (q.v.) found typically near Attapulgas, Georgia. It is characterized as having a chain structure rather than the usual sheet structure of other clays	Wenninger et al. 2000
	Hydrated magnesium aluminum silicate with magnesium partially replaced by aluminum, or to a lesser extent, iron	IARC 1997
	Purified native magnesium aluminum silicate	Barr and Arnista 1957
Bentonite	$Al_2O_3 \cdot 4SiO \cdot 2H_2O$[a] (empirical formula)	Informatics, Inc. 1974
	$Na_{0.33}[Al_{1.67}Mg_{0.33}]Si_4[OH]_2$	Rheox Inc. 1999
	Native hydrated colloidal aluminum silicate clay	Wenninger et al. 2000
	Commercial term for clays containing montmorillonite type minerals formed by the alteration of volcanic ash	Gamble 1986
Fuller's Earth	No specific formula	Wenninger et al. 2000
	Nonplastic variety of kaolin containing an aluminum magnesium silicate	Wenninger et al. 2000
	Porous colloidal aluminum silicate, a catch-all phrase for clay or other fine-grained earthy material suitable for use as an absorbent and bleach	Gamble 1986
Hectorite	$Na_{0.67}(Mg,Li)_6Si_8O_{20}(OH,F)_4$[a]	Budavari 1989
	$Na_{0.33}[Mg_{2.67}Li_{0.33}]Si_4O_{10}[OH]_2$	Rheox Inc. 1999
	Montmorillonite mineral that is the principle constituent of bentonite clays	Wenninger et al. 2000
	Fluorine-bearing magnesium rich montmorillonite	Grim 1972
	Almost a complete substitution of aluminum in the lattice structure of bentonite by magnesium in hectorite and the presence of lithium and flourine	United States Pharmacopeial Convention, Inc. 1994
Kaolin/Kaolinite	$Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$	Wenninger et al. 2000
	Native hydrated aluminum silicate	Wenninger et al. 2000
	Kaolinite is the mineral that characterizes most Kaolins	Ross and Kerr 1931
Lithium Magnesium Silicate	No specific formula	Wenninger et al. 2000
	Synthetic clay consisting of mainly lithium and magnesium silicates	Wenninger et al. 2000

(Continued on next page)

TABLE 1
Chemical formulas and compositions of Silicates and Silicate Clays used in cosmetics *(Continued)*

Ingredient	Description	Reference
Lithium Magnesium Sodium Silicate	No specific formula	Wenninger et al. 2000
	Synthetic clay consisting mainly of lithium, magnesium, and sodium silicates	Wenninger et al. 2000
Montmorillonite	$R^{+}_{0.33}(Al,Mg)_2Si_4O_{10}(OH)_2$, where $R^{+} = Na^{+}$, K^{+}, Mg^{2+}, or Ca^{2+}	Budavari 1989
	Complex aluminum/magnesium silicate clay	Wenninger et al. 2000
	Term used to describe a group of minerals with an expanding lattice, except vermiculite and also a specific mineral with a high-alumina end member of the montmorillonite group with some slight replacement of Al^{3+} by Mg^{++} and substantially no replacement of Si^{4+} by Al^{3+}	Grim 1972
Pyrophyllite	$Al_2O_3 \cdot 4SiO \cdot 2H_2O$	Wenninger et al. 2000
	Naturally occurring mineral substance consisting predominantly of a hydrous aluminum silicate	Wenninger et al. 2000
Sodium Magnesium Silicate	No specific formula	Wenninger et al. 2000
	Synthetic silicate clay with a composition mainly of magnesium and sodium silicate	Wenninger et al. 2000
Zeolite	$M_{2/n}O \cdot Al_2O_3 \cdot ySiO_2 \cdot xH_2O$(M = a group IA or IIA element; n = cation valence; y = 2 or greater; x = the number of water molecules within the molecule)	IARC 1997
	Hydrated alkali aluminum silicate	Wenninger et al. 2000
	Group of hydrated, crystalline aluminosilicates containing exchangeable cations of group IA and IIA elements such as sodium, potassium, magnesium, and calcium	IARC 1997

TABLE 2
Zeolites (IARC 1997)

Zeolite	CAS no.	Chemical formula
Clinoptilolite	12173-10-3 (general)	Not given
	12271-42-0	$Na(AlSi_5O_{12} \cdot xH_2O)$
	67240-23-7	$AlNaH_{16}(SiO_4 \cdot 4H_2O)$
Mordenite	12173-98-7 (general)	Not given
	12445-20-4	$AlNaH_6(SiO_3)_5$
	66732-10-3	$Al_2CaH_{12}(SiO_3)_{10} \cdot H_2O$
	68652-75-5	$Na(AlSi_5O_{12})$
Phillipsite	12174-18-4 (general)	Not given
	61027-84-7	$CaK[Al_3O(SiO_3)_5] \cdot 6H_2O$
	66733-09-3	$AlNa(SiO_4) \cdot 6H_2O$
Zeolite A	68989-22-0	$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}] \cdot 27H_2O$
Zeolite X	68989-23-1	$Na_{86}[(AlO_2)_{86}(SiO_2)_{106}] \cdot 264H_2O$
Zeolite Y	Not specified	$Na_{56}[(AlO_2)_{56}(SiO_2)_{136}] \cdot 250H_2O$
Zeolite L	Not specified	$K_9[(AlO_2)_9(SiO_2)_{27}] \cdot 22H_2O$
ZSM-5	79982-98-2	$(NaTPA)_3[(AlO_2)_3(SiO_2)_{93}] \cdot 16H_2O$*

*TPA = tetrapropylammonium.

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 is considered probable (Grim 1967).

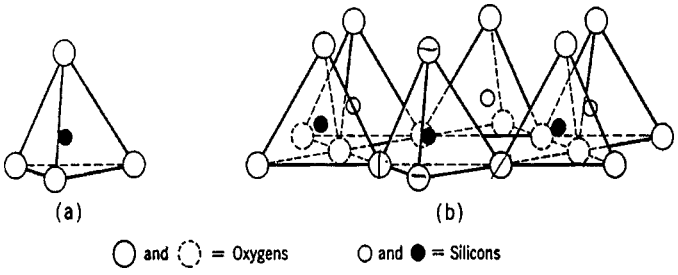

FIGURE 2
(*a*) Single tetrahedral unit; (*b*) Sheet of units (taken from Grim 1967 with permission).

TABLE 3
Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics

Item	Description	Reference
	Aluminum Silicate	
Synonyms	Anhydrous aluminum silicate, china clay, natural aluminum silicate, pyrophyllite, synthetic aluminum silicate, willinite	Wenninger et al. 2000
	Kaolin	Budavari 1989
	Aluminosilicate	Syracuse Research Corp. 1974
Form/description	Generally consisting of 1 mole of alumina and 1 to 3 moles of silica	Wenninger et al. 2000
	Four naturally occurring minerals (andalusite, cyanite, sillimainte, mullite); other associated minerals: anauxite, dickite, kaolinite, kochite, newtonite, pyrophyllite, takizolite, termierite, and ton	Budavari 1989
Molecular weight	Variable: ranging from 162.05 to 426.05 Da	Lide 1993
Density	Variable: 3.156, 3.247	Lide 1993
Solubility	Insoluble in water	Syracuse Research Corp. 1974
	Calcium Silicate	
Synonyms	Silicic acid, calcium salt	Wenninger et al. 2000
Form/description	Hydrous or anhydrous silicate with varying proportions of calcium oxide and silica	Wenninger et al. 2000
	White or slightly cream colored free-flowing powder	Budavari 1989
Molecular weight	116.16 Da	Lide 1993
Solubility	Insoluble in water	Budavari 1989
pH	8.0–10.0 (aqueous slurry)	Budavari 1989
	Magnesium Aluminum Silicate	
Synonyms	Aluminum magnesium silicate, magnesium aluminosilicate, complex colloidal, *Carrisorb*, Gelsorb, VEEGUM	Palmieri 1994
	Aluminosilicic acid, magnesium salt, aluminum magnesium silicate	Wenninger et al. 2000
Form/description	Complex silicate refined from naturally occurring minerals	Wenninger et al. 2000
	Off-white to creamy white small flakes or micronized powder	Palmieri 1994
Molecular weight	262.4 Da	Budavari 1989
Solubility	Insoluble in water, alcohol, and organic solvents	Palmieri 1994
pH	9.0–10.0 (5% aqueous solution)	Nikitakis and McEwen 1990b
Viscosity	225–2200 mPa	Palmieri 1994
CTFA specifications	Arsenic (as As), 3 ppm maximum	Nikitakis and McEwen 1990a
	Lead (as Pb), 10 ppm maximum	Nikitakis and McEwen 1990a
	Magnesium Silicate	
Synonyms	Silicic acid, magnesium salt (1:1)	Wenninger et al. 2000
Form/description	Fine, white, odorless, tasteless, powder, free from grittiness	United States Pharmacopeial Convention, Inc. 1994
Solubility	Insoluble in water and alcohol	United States Pharmacopeial Convention, Inc. 1994
CTFA specifications	Arsenic (as As), 3 ppm maximum	Nikitakis and McEwen 1990a
	Lead (as Pb), 20 ppm maximum	Nikitakis and McEwen 1990a
	Magnesium Trisilicate	
Synonyms	Silicic acid, magnesium salt (1:2)	Wenninger et al. 2000
Form/description	Fine, white, odorless, tasteless powder, free form grittiness	United States Pharmacopeial Convention, Inc. 1994
Solubility	Insoluble in water and alcohol	United States Pharmacopeial Convention, Inc. 1994
	Sodium Magnesium Silicate	
Synonyms	Synthetic sodium magnesium silicate	Wenninger et al. 2000
Form/description	Synthetic silicate clay with a composition mainly of magnesium and sodium silicate	Wenninger et al. 2000

(Continued on next page)

TABLE 3
Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics *(Continued)*

Item	Description	Reference
	Zirconium Silicate	
Synonyms	Silicic acid, zirconium salt (1:1)	Wenninger et al. 2000
	Zircon, zirconium orthosilicate	Budavari 1989
	Zirconium (IV) silicate (1:1)	Lewis 1993
Form/description	Bipyramidal crystals, colorless unless has impurities and radioactive bombardment	Budavari 1989
	Red or various colored crystals	Lewis 1993
Molecular weight	183.31 Da	Budavari 1989
Solubility	Insoluble in alcohol, aqueous solution, and alkali	Lide 1993
Density	4.56	Lide 1993
pH	6–7.5 (10% aqeous slurry)	American Minerals 1998
CTFA specifications	Arsenic (as As), 3 ppm maximum	Nikitakis and McEwen 1990a
	Lead (as Pb), 20 ppm maximum	Nikitakis and McEwen 1990a
	Attapulgite	
Synonyms	Activated attapulgite, Attaclay, Attagel, Attasorb, Min-u-gel, palygorskit, Permagel, Zeogel	Registry of Toxic Effects of Chemical Substances (RTECS) 1999
	Palygorskite	IARC 1997
Form/description	Variety of Fuller's Earth; characterized by a chain structure rather than the sheet structure of other clay minerals	Wenninger et al. 2000
	White, gray, or transparent, dull, elongated, lath-shaped crystals in bundles that comprise thin sheets of minute interlaced fibers; surface is protonated and hydrated	IARC 1997
Density	2.2	IARC 1997
Solubility	Insoluble in water	United States Pharmacopeial Convention, Inc. 1994
	Bentonite	
Synonyms	CI 77004, soap clay	Wenninger et al. 2000
	Albagel Premium USP 4444, Bentonite magma, Hi-gel, Imvite I.G.B.A., Magbond, montmorillonite, Tixoton, Volclay, Wilkinite	RTECS 1999
	BentoPharm, E558, mineral soap, soap clay, taylorite, Veegum HS, wilkinite	Belmonte 1994
Form/description	Native hydrated colloidal aluminum silicate clay	Wenninger et al. 2000
	Crystalline, claylike material, available as an odorless, palebuff or cream to grayish-colored fine powder, which is free from grit	Belmonte 1994
	Dioctahedral	Rheox Inc. 1999
Molecular weight	359.16 Da	Belmonte 1994
Solubility	Practically insoluble in ethanol, fixed oils, glycerin, propan-2-ol and water	Belmonte 1994
pH	9.5–10.5 for a 2% aqueous solution	Belmonte 1994
Particle size	Mainly 50–150 μm along with 1–2 μm particles	Belmonte 1994
	$0.8 \times 0.8 \times 0.01$ μ	Rheox Inc. 1999
Color	Grey to green	Rheox Inc. 1999
Swelling ability	15×	Rheox Inc. 1999
Iron	2.3%	Rheox Inc. 1999
	Fuller's Earth	
Synonyms	English Fuller's earth	Wenninger et al. 2000
Form/description	Nonplastic variety of kaolin	Wenninger et al. 2000
	Sheet structure	Gamble 1986

(Continued on next page)

TABLE 3
Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics *(Continued)*

Item	Description	Reference
	Hectorite	
Synonyms	Macaloid, Ben-A-Gel	Barr 1963
	Bentone and Bentone Gel	Rheox Inc. 1999
Form/description	Translucent colorless mineral when mined and turns white when dried	Barr 1963
	Tridecahedral	Rheox Inc. 1999
Particle size	$0.8 \times 0.08 \times 0.01\ \mu$	Rheox Inc. 1999
pH	8.5 (5% slurry)	Rheox Inc. 1999
Iron	0.2% (typical)	Rheox Inc. 1999
Color	Light pink to tan; off-white	Rheox Inc. 1999
Swelling ability	35×	Rheox Inc. 1999
Odor	None	Rheox Inc. 1999
Specific gravity	2.65	Rheox Inc. 1999
	Kaolin	
Synonyms	Bolbus Alba, China Clay, CI 77004, Kolite, Pigment White 19	Wenninger et al. 2000
	Altowhites, Argilla, Bentone, China Clay, Emathlite, Fitrol, Glomax, Hydrite, Kaopaous, Langford, Mcnamee, Parclay, Porcelin Clay, Snow tex	RTECS 1999
	Bolbus alba, China clay, white bole, argilla, terra alba, porcelin clay	Informatics, Inc. 1974
	White or yellowish white, earthy mass or white powder; unctous when moist	Budavari 1989
Form/description	Native hydrated aluminum silicate	Wenninger et al. 2000
Molecular weight	258.2 Da	Budavari 1989
Solubility	Insoluble in water, cold acids, or in alkali hydroxides	Budavari 1989
Cation exchange capacity	3–15 mEq/100 g	Carrol 1959
CTFA specifications	Arsenic (as As), 3 ppm maximum	Nikitakis and McEwen 1990a
	Lead (as Pb), 20 ppm maximum	Nikitakis and McEwen 1990a
	Lithium Magnesium Silicate	
Synonyms	Silicic acid, lithium magesium salt	Wenninger et al. 2000
Form/description	Synthetic silicate clay consisting mainly of lithium and magnesium silicates	Wenninger et al. 2000
	Lithium Magnesium Sodium Silicate	
Synonyms	Magnesium lithium sodium silicate; silicic acid, lithium, magnesium, and sodium salt	Wenninger et al. 2000
Form/description	Synthetic silicate clay consisting mainly of lithium, magnesium and sodium silicates	Wenninger et al. 2000
	Montmorillonite	
Synonyms	Smectite	Grim 1972
Form/description	Complex aluminum/magnesium silicate clay	Wenninger et al. 2000
Cation exchange capacity	80–150 mEq/100 g	Carrol 1959
	Pyrophyllite	
Synonyms	Pyrophyllite clay	Wenninger et al. 2000
Form/description	Naturally occurring mineral—predominantly hydrous aluminum silicate	Wenninger et al. 2000
	Sodium Magnesium Silicate	
Synonyms	Synthetic sodium magnesium silicate	Wenninger et al. 2000
Form/description	Synthetic silicate clay with a composition mainly of sodium and magnesium silicate	Wenninger et al. 2000
pH	8.5–10.5 of 2% aqueous dispersion	Nikitakis and McEwen 1990b
Solubility	Insoluble in organic solvents and disperses in water	Nikitakis and McEwen 1990b

(Continued on next page)

TABLE 3
Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics *(Continued)*

Item	Description	Reference
	Zeolite	
Synonyms	Aluminosilicates, Bacterkiller, CS100, Sitton, Zeokar, Zeolith, Zeolum, Zeostar	Wenninger et al. 2000
	Clinoptilotile, Mordenite, Phillipsite, Zeolite A, Zeolite X, ZSM-5, Non-fibrous Japanese Zeolite	IARC 1997
Form/description	Crystalline, hydrated alkali-aluminum silicates	Budavari 1989; Wenninger et al. 2000

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 as shown in Figure 4. 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 (Grim 1967).

Smectites (Montmorillonites, Hectorite, and Bentonite)

Smectite units comprise of two silica tetrahedral sheets with a central alumina octahedral sheet as shown in Figure 5. 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 become 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 smectites is the ability of water and other 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 smectites have substitutions within their lattices: aluminum or phosphorous for

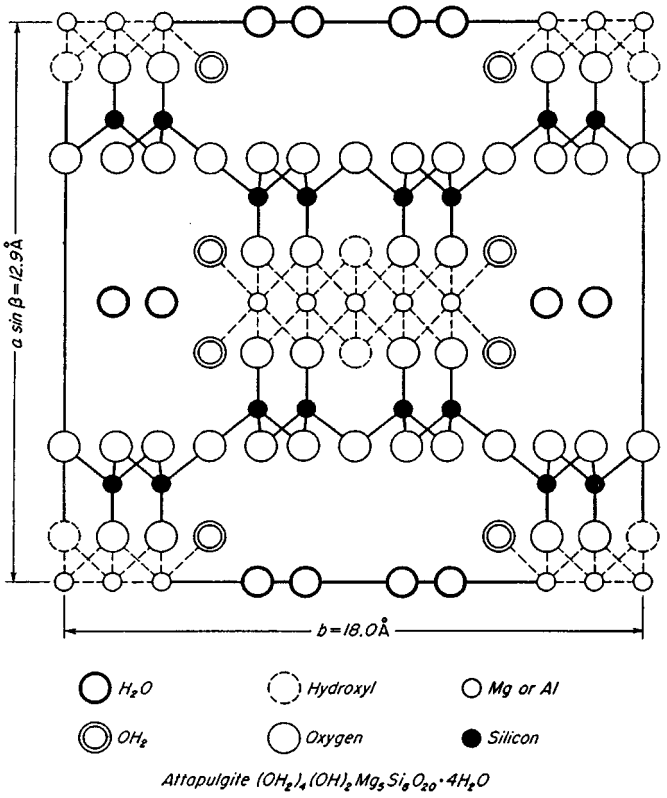

FIGURE 3
Attapulgite structure (taken from Grim 1967 with permission).

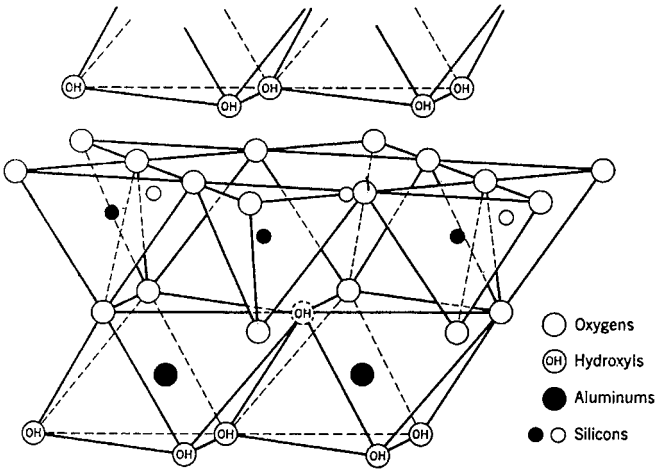

FIGURE 4
Kaolin layer (taken from Grim 1967 with permission).

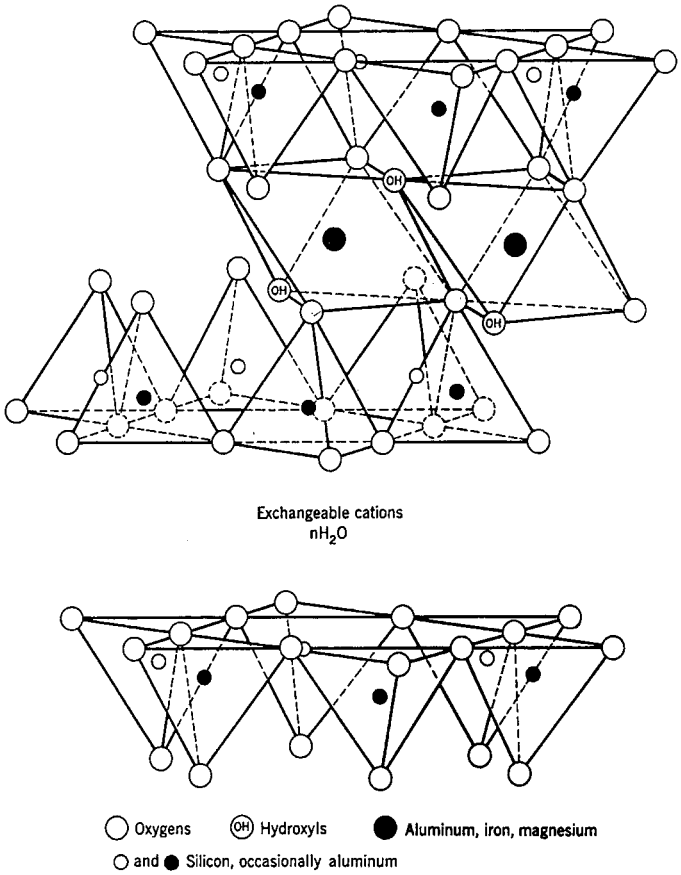

FIGURE 5
Smectite structure (taken from Grim 1967 with permission).

silicon in the tetrahedral coordination and/or magnesium, iron, zinc, nickel, lithium, etc. for aluminum in the octahedral sheet (Grim 1967).

Natural Occurrence of Clays

Aluminum Silicate

Natural Aluminum Silicates are reportedly being mined in India, California, North Carolina, and Georgia (Gamble 1986).

Attapulgite

Attapulgite is mined in 10 countries: Australia, China, France, India, Russia, Senegal, South Africa, Spain, Turkey, and the United States (Informatics, Inc. 1974).

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 former Soviet Union, and the United States (Informatics, Inc. 1974).

Kaolin

Deposits of Kaolin have been found in England, the United States, France, Czechoslovakia, Germany, and Japan (Informatics, Inc. 1974).

Pyrophyllite

Gamble (1986) reported Pyrophyllite being mined primarily in North Carolina.

Zeolite

Natural Zeolites are mined in Japan, the United States, Hungary, Bulgaria, Cuba, Italy, and South Africa (Roskill Informations Services Ltd. 1988).

Method of Manufacture

Aluminum Silicate

Aluminum Silicate is a naturally occurring mineral as well as artificially produced. The naturally occurring Aluminum Silicate minerals are know as andalusite, sillimanite, and cyanite. Natural Aluminum Silicate is mined from an ore and synthetic Aluminum Silicate is formed by heating compositions of controlled proportions of silica, alumina, and alkalis under conditions to promote the specific structure (Syracuse Research Corp. 1981).

Attapulgite

Hevilin and Murray (1994) describe the mining process of Attapulgite as an opencast technique, stripping layers with heavy machines such as bulldozers, backhoes, and excavators. The clay is then transported to a processing plant where crushing, drying, classification, and pulverizing takes place. High-heat drying to remove water may occur to enhance absorbent qualities.

Bentonite

The mined ore of Bentonite is processed to remove grit and nonswelling materials (Belmonte 1994).

Kaolin

In a process described by Wells, Bhatt, and Flanagan (1985), Kaolin is extracted from kaolinized granite by washing it out with powerful and remote 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.

Magnesium Aluminum Silicate

Magnesium Aluminum Silicate is obtained from silicate ores of the montmorillonite group. The ores are blended with water to produce a slurry, which is then processed to remove impurities and separate out the colloidal fractions. Refined colloidal fractions are dried to form a small flake and then is microatomized to form various powder grades (Palmieiri 1994).

Zeolite

Roskill Informations Services Ltd. (1988) reported natural Zeolites being recovered from deposits by selective opencast or strip mining processes. The raw material is then processed by crushing, drying, powdering, and screening. Synthetic Zeolite synthesis requires the following conditions: reactive starting materials; a high pH; a low-temperature hydrothermal state with concurrent low autogenous pressure at saturated water pressure; and a high degree of supersaturation of a large number of crystals.

Analytical Methods

Montmorillonite has been detected using far infrared spectra (Angino 1964). Bentonite and Kaolin are described by Angino (1964) using far infrared spectra and by Sadik (1971) using x-ray diffraction. Attapulgite has been detected with the use of transmission or scanning electron microscope (Zumwalde 1976), and by means of x-ray powder diffraction analysis (Keller 1979). The characterization of Hectorite was achieved through x-ray diffraction, infrared spectroscopy, and chemical analysis (Browne et al. 1980). Zeolites have been examined using scanning electron microscopy (Wright and Moatamed 1983; van Hoof and Roelofsen 1991) and x-ray diffraction (van Hoof et al. 1991). Magnetic angle spinning nuclear magnetic resonance (NMR) has confirmed the structural breakdown of Fuller's Earth (Drachman, Roch, and Smith, 1997).

IMPURITIES/COMPOSITION

Aluminum Silicate

Other minerals associated with natural Aluminum Silicates are anauxite, dickite, kaolinite, kochite, mullite, newtonite, pyrophyllite, takizolite, terierite, and ton (Budavari 1989).

Attapulgite

Attapulgite commonly is found with smectites, amorphous silica, chert, and other minerals (Bish and Guthrie 1993).

A typical composition is shown in Table 4 (Keller 1979).

Bentonite

The principle constituent is Montmorillonite. 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 (Informatics, Inc. 1974).

A typical composition is shown in Table 4 (Belmonte 1994).

Fuller's Earth

Principle deposits of Fuller's Earth include Montmorillonite, Bentonite, Attapulgite, and sepiolite (Gamble 1986).

TABLE 4

Mineral composition of individual samples of Magnesium Aluminum Silicate, Attapulgite, Bentonite, Hectorite, Kaolinite, and Montmorrillonite (Barr 1963)

	Silicate clays analyzed					
Mineral	Magnesium Aluminum Silicate (%)	Attaplugite (%)	Bentonite (%)	Hectorite (%)	Kaolinite (%)	Montmorillonite (%)
SiO_2	61.1	55.03	59.92	55.86	45.44	51.14
Al_2O_3	9.3	10.24	19.78	0.13	38.52	19.76
Fe_2O_3	—	3.53	—	0.03	0.80	0.83
FeO	0.9	—	2.96	—	—	—
MgO	13.7	10.49	1.53	25.03	0.08	3.22
CaO	2.7	—	0.64	Trace	0.08	1.62
K_2O	0.3	0.47	0.57	0.10	0.14	0.11
Na_2O	2.9	—	20.6	2.68	0.66	0.04
TiO_2	0.1	—	—	—	0.16	—
CO_2	1.8	—	—	—	—	—
LiO_2	—	—	—	1.05	—	—
F	—	—	—	5.96	—	—
MnO	—	—	—	—	—	Trace
ZnO	—	—	—	—	—	0.10
H_2O	7.2	19.86	Not reported	12.14	14.20	22.80
Reference	Palmieri 1994	Keller 1979	Belmonte 1994	Keller 1979	Keller 1979	Keller 1979

Hectorite

Principle impurities include calcite, dolomite, silica crystals, and grit (Barr 1963). A typical composition is shown in Table 4 (Keller 1979).

Kaolin

Quartz, mica, and feldspar are often found associated with the crude mineral and is often removed through screening and elutriation (Informatics, Inc. 1974).

Ferreira and Freitas (1976) surveyed Kaolin for any potentially pathogenic organisms and a mean viable count. *Pseudomonas aeruginosa*, *Salmonella typhosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Clostridium tetani* were absent. The mean viable count was 74×10^3/6 M. The bacteria present were mostly gram-positive aerobic spore-formers.

A typical composition is shown in Table 4 (Keller 1979).

Magnesium Aluminum Silicate

One trade-name group of products contain 1% to 6% by volume weight crystalline silica in the form of cristabalite; they also comment that a few grades may contain quartz as well (Kelse 1997).

A typical composition is shown in Table 4 (Palmeiri 1994).

Montmorillonite

A typical composition of Montmorillonite is shown in Table 4 (Keller 1979).

Zeolite

Valatina, Pylev, and Lemjasev (1994) analyzed the chemical compositions of five samples of Zeolite dusts taken from mines in Russia (Table 5). The benzo[a]pyrene content in the dusts of natural Zeolite tuffs (rock deposits) ranged from 0.0 to 3.6 μg/kg.

TABLE 5
Zeolite mine dust chemical analysis (Valatina, Pylev, and Lemjasev 1994)

Dust sample	1	2	3	4	5
Molar ratio of SiO_2/ Al_2O_3	9.0	8.3	9.8	7.4	9.4
Zeolite (%)	83	50.6	73	63	56
Silicon dioxide (%)	66.84	0	70.92	62.64	68.6
Aluminum oxide (%)	12.36	12.62	12.11	14.17	12.16
Iron (III) oxide (%)	0.92	4	1.03	2.65	0.2
Magnesium oxide (%)	1.53	1.34	0.53	1.19	0.93
Calcium oxide (%)	2.36	4.15	2.56	2.01	1.93
Sodium oxide (%)	2.65	0.15	0.62	1.75	2
Benzo[a]pyrene	2.5	3.6	0.1	1.3	0

USE

Cosmetic

According to the European Cosmetic Directive (EU reference no. 391 Annex II), Zirconium and its compounds are listed under substances that must not form part of the composition of cosmetic products, with the exception of complexes in Annex III, Part I. These complexes are aluminum zirconium chloride hydroxide complexes and the aluminum zirconium chloride hydroxide glycine products used in antiperspirants; and the zirconium lakes, salts, and pigments of coloring agents listed in reference 3 in Annex IV, Part I (Cosmetics Directive of the European Union 1995).

Aluminum Silicate, anhydrous, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Bentonite, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite are listed in the *Japanese Comprehensive Licensing Standards by Category* (CLS) (Rempe and Santucci 1998). Aluminum Silicate, anhydrous has no concentrations limits and is listed in all categories except eyeliner preparations and lip preparations. Calcium Silicate, is listed in all categories. Magnesium Aluminum Silicate, which is listed under Aluminum Magnesium Silicate, is listed in all categories. Magnesium Silicate is listed in all categories. Hectorite is listed in all categories except eyeliner preparations, lip preparations, and oral preparations. Montmorillonite is excluded from only eyeliner preparations. Pyrophyllite is listed in all groups except eyeliner, lip, oral, and bath preparations. Bentonite, Kaolin, and Zeolite are listed in all categories.

Information on use of ingredients in cosmetic formulations is available from the Food and Drug Administration (FDA) as part of a voluntary industry reporting program (FDA 1998). These data are presented in the first two columns of Table 6.

In addition, the Cosmetic, Toiletry, and Fragrance Association (CTFA) provides information from the industry directly to CIR on the current concentration of use (CTFA 1999a). In some cases a current concentration of use is provided even when there is no current use reported to FDA. It is presumed that an industry report of a current concentration of use means the ingredient is in use. These data are included in the third column of Table 6.

In those cases where there is a use reported to FDA, but there is no current concentration of use data available, the last column in Table 6 includes historical data from 1984 when FDA collected information on concentration as part of the voluntary reporting program described earlier (FDA 1984). If no historical data are available, no concentration is listed.

Aluminum Silicate

Aluminum Silicate functions as an abrasive, anticaking agent, bulking agent, and opacifying agent in cosmetics (Wenninger et al. 2000). In 1998 it was reported as an ingredient in 10 formulations in seven different categories (FDA 1998).

TABLE 6
Frequency of use and concentration of use as a function of product category

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
	Aluminum Silicate		
Mascara (167)	2	0.5	
Blushers (all types) (238)	1	—	—
Dentifrices (38)	—	37	
Shaving cream (139)	1	—	—
Cleansing (653)	2	2	
Paste masks (mud packs) (255)	1	—	1–5
Skin fresheners (184)	1	—	0.1–1
Other skin preparations (692)	2	3	
1998 total uses of Aluminum Silicate	10		
	Calcium Silicate		
Bath oils, tablets, and salts (124)	12	—	0.1–5
Bubble baths (200)	2	—	0.1–25
Other bath preparations (159)	2	—	0.1–25
Eye shadow (506)	11	1–8	
Powders (247)	35	2	
Blushers (all types) (238)	17	5–8	
Face powders (250)	40	0.3–10	
Foundations (287)	5	2–8	
Lipstick (790)	3	0.5	
Makeup bases (132)	1	0.5	
Rouges (12)	1	—	1–5
Other makeup preparations (135)	1	—	1–5
Other manicuring preparations (61)	1	—	1–5
Skin cleansing preparations (653)	1	8	
Men/s talcum (8)	—	8	
1998 total for Calcium Silicate	132		
	Magnesium Aluminum Silicate		
Other bath preparations (159)	1	—	—
Eye makeup remover (84)	20	—	0.1–25
Eye shadow (506)	4	1	
Eye lotion (18)	1	1	
Eye makeup remover (84)	2	—	0.1–25
Mascara (167)	33	0.4–5	
Eyeliner (514)	—	0.2–0.5	
Eyebrow pencil (91)	—	0.5	
Other eye makeup preparations (120)	16	1–5	
Cologne and toilet waters (656)	1	—	—
Other fragrance preparations (148)	1	—	>0–1
Hair conditioners (636)	1	—	0.1–1
Hair straighteners (63)	3	—	0.1–1
Hair dyes and colors (1572)	—	2	
Shampoos (noncoloring) (860)	3	1–2	
Other hair preparations (276)	3	—	—
Hair rinses (coloring) (33)	1	—	—
Foundations (287)	130	0.4–5	
Lipstick (790)	3	—	0.1–1
Makeup bases (132)	60	1–2	

(Continued on next page)

TABLE 6
Frequency of use and concentration of use as a function of product category *(Continued)*

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
Makeup fixatives (11)	3	2	
Other makeup preparations (135)	24	0.8	
Cuticle softeners (19)	1	—	—
Nail creams and lotions (17)	1	—	0.1–5
Dentifrices	—	0.7	
Bath soaps and detergents (385)	1	0.5–1	
Deodorants (underarm) (250)	5	0.5–1	
Other personal cleanliness products (291)	14	2	
Aftershave lotion (216)	9	—	1−>50
Other shaving preparations (60)	2	—	0.1–5
Skin cleansing preparations (653)	41	0.1–5	
Face and neck skin care preparations (263)	16	0.6–3	
Body and hand skin care preparations (796)	56	0.3–5	
Foot powders and sprays (35)	3	—	—
Moisturizers (769)	70	0.3–4	
Night creams, lotions, powders, and sprays (188)	11	0.3–2	
Paste masks (mud packs) (255)	34	3–5	
Other skin care preparations (692)	33	0.1	
Suntan gels, creams, and liquids (136)	6	2–5	
Indoor tanning preparations (62)	19	0.5–2	
1998 total for Magnesium Aluminum Silicate	632		
	Attapulgite		
Powders (fragrance) (247)	5	—	—
Body and hand skin care preparations (796)	—	8	
Paste masks (mud packs) (255)	5	8	
1998 total for Attapulgite	10		
	Bentonite		
Bath, oils, tablets, and salts (124)	—	5	
Eyeliner (514)	6	5	
Mascara (167)	1	0.8	
Other eye makeup preparations (120)	1	—	—
Hair conditioners (636)	1	—	—
Hair straighteners (63)	3	—	0.1–1
Foundations (287)	5	2–8	
Makeup bases (132)	3	1	
Cuticle softeners (19)	1	1	
Bath soaps and detergents (385)	1	0.5	
Other personal cleanliness products (291)	2	—	0.1–10
Skin cleansing preparations (653)	6	—	>0–10
Face and neck skin care preparations (excluding shaving) (263)	1	2–5	
Body and hand skin care preparations (excluding shaving) (796)	6	2–5	
Moisturizers (769)	2	3	
Night creams, lotions, powders, and sprays (188)	1	—	—
Paste masks (mud packs) (255)	44	12–80	
Skin fresheners (184)	1	—	—

(Continued on next page)

TABLE 6
Frequency of use and concentration of use as a function of product category *(Continued)*

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
Other skin preparations (692)	8	—	—
Suntan gels, creams, and liquids (136)	1	—	—
Other suntan preparations (38)	—	1	
1998 total for Bentonite	73		
	Fuller's Earth		
Paste masks (mud packs) (255)	2	—	—
Other skin preparations (692)	1	—	25–50
1998 total for Fuller's Earth	3		
	Hectorite		
Eyeliner (514)	3	—	—
Mascara (167)	1	0.7	
Shampoos (noncoloring) (860)	—	1	
Hair bleaches (113)	5	—	—
Foundations	—	15	
Other makeup preparations (135)	1	—	1–5
Basecoats and undercoats (manicuring) (48)	1	—	—
Nail polish and enamel (80)	1	—	—
Deodorants (underarm) (250)	1	0.7	
Other personal cleanliness products (291)	1	—	—
Paste masks (mud packs) (255)	2	0.4	
Skin cleansing preparations (653)	—	100	
Body and hand creams, lotions, powders, and sprays (796)	—	8	
Other skin preparations (692)	1	—	—
Paste masks (mud packs) (255)	—	8	
Other suntan preparations (38)	1	—	
1998 total for Hectorite	18		
	Sodium Magnesium Silicate		
Eyeliner	—	0.08	
Eye shadow (506)	11	0.08	
Mascara (167)	1	0.4	
Other eye makeup preparations (120)	1	—	—
Powders (fragrance) (247)	1	—	—
Tonics, dressings, and other hair-grooming aids (549)	1	—	—
Blushers (all types) (238)	2	—	—
Face powders (250)	3	0.4	
Foundations (287)	4	0.4	
Lipstick (790)	1	3	
Makeup bases (132)	—	0.1	
Other makeup preparations (135)	1	—	—
Dentifrices (38)	—	0.3	
Deodorants (underarm) (250)	—	0.5	
Skin cleansing preparations (653)	—	0.5	
Face and neck skin care preparations (excluding shaving) (263)	3	0.8–5	
Body and hand skin care preparations (excluding shaving) (796)	2	0.1	
Moisturizers (769)	1	1	

(Continued on next page)

TABLE 6
Frequency of use and concentration of use as a function of product category *(Continued)*

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
Paste masks (mud packs) (255)	1	5	
Skin fresheners (184)	—	5	
Other skin preparations (692)	1	—	1–5
1998 total for Sodium Magnesium Silicate	34		
	Kaolin		
Other bath preparations (159)	1		1–10
Eyebrow pencil (91)	5	15–17	
Eyeliner (514)	9	25–48	
Eye shadow (506)	171	3–29	
Mascara (167)	31	8–18	
Other eye makeup preparations (120)	15	20	
Powders (247)	40	5	
Hair conditioners (636)	5	4	
Tonics, dressings, and other hair-grooming aids (549)	—	15	
Other hair-coloring preparations (59)	1	5	
Blushers (all types) (238)	72	14–20	
Face powders (250)	58	30	
Foundations (287)	45	6–36	
Lipstick (790)	6	12–30	
Makeup bases (132)	24	7–25	
Rouges (12)	2	—	>0–50
Makeup fixatives (11)	3	—	1–5
Paste masks (mud packs) (255)	—	12–84	
Other makeup preparations (135)	20	10–24	
Bath soaps and detergents (385)	1	3	
Other manicuring preparations (61)	—	53–54	
Skin cleansing preparations (653)	—	0.01	
Face and neck skin care preparations (263)	—	3	
Moisturizers (769)	—	25	
Skin fresheners (184)	—	2	
Other skin care preparations (692)	—	3–100	
Suntan gels, creams, liquids (136)	—	25	
1998 total for Kaolin	509		

Attapulgite

Attapulgite functions as an abrasive, bulking agent, opacifying agent, and viscosity-increasing agent (Wenninger et al. 2000). The FDA reported in 1998 Attapulgite being used in 10 formulations (FDA 1998).

Bentonite

Bentonite functions as an absorbent, bulking agent, emulsion stabilizer, opacifying agent, suspending agent—nonsurfactant, and viscosity-increasing agent—aqueous in cosmetic formulations (Wenninger et al. 2000). In 1998, 94 formulations were reported (FDA 1998). Of the 94 formulations, 47% were reported within paste masks (mud packs) (FDA 1998).

Calcium Silicate

Calcium Silicate functions as an absorbent, bulking agent, and an opacifying agent in cosmetic formulations (Wenninger et al. 2000). The FDA reported 132 formulations containing Calcium Silicate in 1998, of which 30% of the formulations were face powders (FDA 1998).

Fuller's Earth

Fuller's Earth functions as an absorbent, anticaking agent, bulking agent, and opacifying agent (Wenninger et al. 2000). Fuller's Earth was reported in three formulations in 1998 (FDA 1998).

Hectorite

Hectorite functions as an absorbent, bulking agent, opacifying agent, suspending agent—nonsurfactant, and viscosity-increasing agent—aqueous (Wenninger et al. 2000). In 1998, Hectorite was reported in 18 formulations (FDA 1998). Rheox Inc. (1999a) reported Hectorite as being used in antiperspirants, suntan products, eye products, hair products, creams and lotions, lip products, facial masks, and nail products.

Kaolin

Kaolin functions as an abrasive, absorbent, anticaking agent, bulking agent, and opacifying agent in cosmetic formulations (Wenninger et al. 2000). Of the 509 formulations reported by FDA in 1998, 34% were eye shadows (FDA 1998).

Lithium Magnesium Silicate

Lithium Magnesium Silicate functions as a binder, bulking agent, and viscosity-increasing agent—aqueous in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Lithium Magnesium Sodium Silicate

Lithium Magnesium Sodium Silicate functions as a bulking agent and viscosity-increasing agent—aqueous (Wenninger et al. 2000). There were no current uses reported to FDA.

Magnesium Aluminum Silicate

Magnesium Aluminum Silicate functions as an absorbent, anticaking agent, opacifying agent, and viscosity-increasing agent—aqueous in cosmetics (Wenninger et al. 2000). It was reported that Magnesium Aluminum Silicate was used in 629 formulations in 1998 (FDA 1998). Of those 629 formulations, 21% were used in foundations.

Magnesium Aluminum Silicate (VEEGUM) was reported by Carlson (1977) to typically be used at a concentration of 1% to 2%, consistent with the data in Table 6. Another source reported Magnesium Aluminum Silicate used at concentrations of 10% to 50% for adsorbents, 0.5% to 2.5% for stabilizing agents, 1% to 10% for suspending agents, 2% to 10% for tablet and capsule disintegrants, 2% to 10% tablet binders, and 2% to 10% viscosity-increasing agents, again consistent with data in Table 6 (Palmieri 1994).

Additional historical data on concentration of use of this ingredient are available from a Toilet Good Association survey. Table 7 is a summary of that information (Toilet Goods Association 1969).

Magnesium Silicate

Magnesium Silicate functions as an absorbent, anticaking agent, bulking agent, opacifying agent, and viscosity-increasing agent—aqueous in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Magnesium Trisilicate

Magnesium Trisilicate functions as an abrasive, absorbent, anticaking agent, bulking agent, opacifying agent, and viscosity-increasing agent—aqueous in cosmetics (Wenninger et al. 2000).

TABLE 7
Magnesium Aluminum Silicate in cosmetic preparations (Toilet Goods Association 1969).

Product category	Use in product	Concentration (%)
Face cream/lotion (cleansing, hormone, night, acne, astringent)	Thickener, binder, emulsion stabilizer	2.1
Hand cream/lotion	Thickener, binder, emulsion stabilizer	1.3
Body cream/lotion (moisturizer, suntan preparations)	Thickener, binder, emulsion stabilizer, slip agent	1.6
Makeup (lotion, cream, medicated, matte, highlight)	Thickener, binder, emulsion stabilizer, pigment suspender	1.8
Rouge (cream, liquid, blusher, toner)	Thickener, binder, pigment suspender	1.8
Face mask	Thickener, binder	8.9
Powder aerosol	Anticaking	8.0
Powder compact/pressed	Oil absorption	1.0
Leg makeup	Thickener	3.9
Deodorant/antiperspirant	Thickener, emulsion stabilizer	1.8
Eye makeup (eyeshadow, mascara, eyeliner)	Thickener, emulsion stabilizer, pigment suspender	2.0
Depilatory	Thickener	2.0
Shave preparations	Thickener	0.5
Shampoo	Thickener	3.5
Cream sachet	Thickener, emuslion stabilizer	0.8

Montmorillonite

Montmorillonite functions as an abrasive, absorbent, emulsion stabilizer, opacifying agent, and viscosity-increasing agent—aqueous in cosmetics (Wenninger et al. 2000). There were no current uses reported to FDA.

Pyrophyllite

Pyrophyllite functions as an absorbent, colorant, and opacifying agent (Wenninger et al. 2000). There were no current uses reported to FDA.

Sodium Magnesium Silicate

Sodium Magnesium Silicate functions as binder and bulking agent (Wenninger et al. 2000). In 1998, Sodium Magnesium Silicate was reported in 34 formulations (FDA 1998).

Zeolite

Zeolite functions as an absorbent and deodorant agent in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Zirconium Silicate

Zirconium Silicate functions as an abrasive and opacifying agent in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Noncosmetic

Aluminum Silicate

Aluminum Silicate is approved, under the heading of indirect food additives, as a substance used as basic components of single or repeated use of the food contact surfaces cellophane (21 Code of Federal Regulations [CFR] 177.1200) and rubber (21 CFR 177.2600).

Attapulgite

Attapulgite is listed in the OTC Active Ingredient Status Report as proposed category I, as an antidiarrheal ingredient (FDA 1994). Attapulgite is listed by Gamble (1986) as being primarily used in absorbents, pesticides, oil and petroleum treatment, and as a filler in many products.

Bentonite

Bentonite is considered by FDA to be generally recognized as safe (GRAS) as a direct food additive (21 CFR 184.1155).

Bentonite is listed by Gamble (1986) as being 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. Another source reported Bentonite as being used as an adsorbent, emulsion stabilizer, and suspending agent (Belmonte 1994). Bentonite is categorized by the *National Formulary* as a suspending and/or viscosity-increasing agent (United States Pharmacopeial Convention, Inc. 1994).

Calcium Silicate

Calcium Silicate is listed in the OTC Active Ingredient Status Report as an external analgesic and skin protectant (FDA 1994). The *National Formulary* category is as a glident and/or anticaking agent (United States Pharmacopeial Convention, Inc. 1994).

The American Conference of Governmental Industrial Hygienists (ACGIH) TLV-TWA (threshold limit value–time weighted average) is 10 mg/m^3 for inhalable dust (ACGIH 1997).

Hectorite

Hectorite has two listings of category IISE in the OTC Active Ingredient Status Report (FDA 1994). It is listed as being used as an external analgesic and skin protectant. Barr (1957) stated that the Federal Drug Administration (sic) has given approval for the use of Hectorite in internally and externally applied products, as well as dentifrices, cosmetics, and externally approved pharmaceuticals.

Kaolin

According to FDA, Kaolin is considered GRAS as an indirect food additive (21 CFR 186.1256). Kaolin is listed as being used in antacids, anorectals (external and interrectal), antidiarrheals, skin protectants, and digestive aids (colloidal Kaolin) in the OTC Active Ingredient Status Report. The final rulings are as follows: antacids: category IIE; anorectals (both): category I; and digestive aid: category IISE. Proposed rulings are as follows: antidiarrheal: category IIIE; skin protectant diaper rash: category I; skin protectant poison ivy: category I; and skin protectant: category I. Category III is designated as the conditions for which the available data are insufficient to permit final classification at this time.

Gamble (1986) reports Kaolin's main use in the paper industry to fill and coat the surface of paper. Kaolin is also reported being used as a filler in rubber, paint extender, filler in plastics, ceramics manufacture, ink, adhesives, insecticides, medicines, food additives, bleaching, adsorbents, cement, fertilizers, crayons, pencils, detergents, porcelain enamels, paste, foundries, linoleum, floor tiles, and textiles.

The *National Formulary* classifies Kaolin as a tablet and/or capsule diluent (United States Pharmacopeial Convention, Inc. 1994).

The *Food Chemicals Codex* specifies limits of impurities for clay (Kaolin) as: acid-soluble substances <2%; Arsenic (as As) <3 ppm; Heavy Metals (as Pb) <40 ppm; Lead <10 ppm (National Academy of Science 1996).

Magnesium Aluminum Silicate

Magnesium Aluminum Silicate (MAS) is listed as being used in acne treatments and in antacids in the OTC Active Ingredient Status Report (FDA 1994). As an antacid, MAS is a category I listing, meaning it is generally recognized as safe and effective and is not misbranded. However, MAS is a category IISE listing as used for acne. MAS was listed as category IISE due to safety and/or effectiveness.

Other uses for Magnesium Aluminum Silicate have been reported as: adsorbent, suspending agents, tablet and capsule disintegrant, tablet binder, and viscosity-increasing agent (Palmieri 1994).

The *National Formulary* classifies Magnesium Aluminum Silicate as a suspending and/or viscosity-increasing agent (United States Pharmacopeial Convention, Inc. 1994).

VEEGUM, a tradename for Magnesium Aluminum Silicate, has been designated by the FDA as a raw material with the following number: FD-CRMCS no. R0010045 and has an individual Chemical Abstract Registry (CAS) number 12199-37-0.

Magnesium Silicate

Magnesium Silicate is classified as a glidant or anticaking agent by the *National Formulary* (United States Pharmacopeial Convention, Inc. 1994).

Magnesium Trisilicate

Magnesium Trisilicate is listed in the OTC Active Ingredient Status Report as being used as antacids, digestive aids, and overindulgence remedy (FDA 1994). In antacids, FDA has listed Magnesium Trisilicate as category I (generally recognized as safe and effective). FDA concluded that Magnesium Trisilicate use in digestive aids is category IISE (not generally recognized as safe and effective). FDA has proposed that Magnesium Trisilicate use in overindulgence remedies is category I.

Pyrophyllite

Pyrophyllite is listed under Code of Federal Regulations (21 CFR 73.1400) as a naturally occurring color additive and must conform to the following specifications: lead (as Pb) not more than 20 ppm; and arsenic (as As) not more than 3 ppm. Also Pyrophyllite may be used safely for coloring externally applied cosmetics, in amounts consistent with good manufacturing practice (21 CFR 73.2400).

Pyrophyllite is listed by Gamble (1986) as being used in refractories, rubber, ceramics, insecticides, plastics, paint, roofing, bleaching powder, textiles, cordage, and wall board.

Zeolite

Zeolites are reported by Gamble (1986) as being used in CO_2 recovery from natural gas, aromatic separates dimension stones, filler in paper, isolation of radioactive wastes, water aeration, dietary supplements for animals, neutralization of acidic soils, carriers for pesticides and fungicides, sorbents for oil spills, polishing agent in toothpastes, and petroleum solvents. International Agency for Research on Cancer (IARC) (1997) lists the three main uses of synthetic Zeolite as: detergents, catalysts, and adsorbents or desiccants.

Zirconium Silicate

Zirconium Silicate is reported by Kleber and Putt (1986) as being used in chewing gum and in a dental prophylaxis paste.

GENERAL BIOLOGY

Adsorption

The large volume of general data available on the adsorption of various chemicals, cells, etc., to these silicate clays is presented in Table 8. In addition, to this general information, specific reactions are described using specific silicate clays—these data are described below.

Hectorite

Bujdak and Rode (1996) reported that Hectorite-catalyzed glycine and diglycine oligomerizations were performed as drying/wetting cycles. Approximately 7% of glycine was converted to diglycine and diketopiperazine on Hectorite after 7 days. It may be noted that the Hectorite sample was altered by substituting Li(I) for Mg(II), which caused a greater effect on oligomerizations.

Porter et al. (1998) reported condensation reactions of the amino acid glycine on the surface of Cu(II)-exchanged Hectorite. Polymerization of gylcine oligomers was seen primarily at the edges or topmost layer. These reactions were facilitated by the availability of intergallery metal cations at the step edges or pores in the surface region.

Kaolin

Adenosine monophosphate molecules were adsorbed onto Kaolinite, modified with Mg^{2+} and irradiated with ultraviolet (UV) light. These synthesis products were tested for their bond types by enzymatic hydrolysis and analyzed by ion-exchange chromatography. Considerable portions of the products were phosphodiesterase hydrolyzed, which implies a 3′–5′, 2′–5′, or both, nature of the bonds (Strigunkova, Lavrentiev, and Ostroshchenko 1986).

Montmorillonite

Dougherty et al. (1985) incubated Montmorillonite saturated with magnesium chloride (10 mg) with 5×10^6 human neutrophils. Effects were determined by phase contrast microscopic examination and by the measurement of lactate dehydrogenase. Both untreated and clay treated with human albumin were used to stimulate neutrophil chemiluminescence. Montmorillonite was also incubated with human erythrocytes and the free hemoglobin was measured at 430 nm and the effect of clay on zymosan-activated serum was also investigated. Rapid neutrophil lysis was observed in cells exposed to untreated clay. After lysis, lactate dehydrogenase rapidly adsorbed to the surface of the clay. Clay pretreatment with human albumin blocked the enzyme surface adsorption and cell lysis. Neutrophil chemiluminescence was stimulated by untreated clay but not by clay pretreated with 5% albumin. Clay lysis of erythrocytes was incomplete as compared to neutrophil lysis. Zymosan-activated serum samples exposed to clay; complement activity as measured by neutrophil chemotaxis was suppressed in a dose-dependent manner.

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays

Compound adsorbed	Experimental design	Results	Reference
		Magnesium Aluminum Silicate	
Dicumarol	The drug dicumarol was given to dogs with 50% colloidal Magnesium Aluminum Silicate (MAS); the plasma level of dicumarol in dogs was measured	Significantly lower plasma levels and delayed appearance of dicumarol resulted from administration with 50% MAS; drug concentration at peak level was 16.7% (25.8% in controls) and peak plasma levels were seen at 12–24 h (8–12 h in controls)	Akers, Lach, and Fischer 1973
Streptomycin sulphate and neomycin sulphate	Adsorption studies were carried out in vitro in McIlvaine's Buffer and water	MAS had the greatest affinity for streptomycin sulphate in water (adsorption coefficient of $111 \cdot 10^{-3}$ for water and $33 \cdot 10^{-3}$) whereas the adsorption coefficient for MAS in water to neomycin sulphate was $34 \cdot 10^{-3}$	Ghazy, Kassem, and Shalaby 1984
Bromohexine HCL	MAS was mixed with bromohexine HCL to make tablets and were stored in polyethylene film for various times; the amount of bromohexine remaining in the tablet was determined	Bromohexine remaining in the tablets increased with increasing concentrations of MAS, indicating that MAS prevented the adsorption of bromohexine to polyethylene film; no bromohexine degradation was reported	Kukita et al. 1992
Tetracycline	In vitro and in vivo adsorption of tetracycline by VEEGUM was studied	The maximum serum concentration of tetracycline was decreased by 21%; the maximum adsorption in vitro occurred at pH 1.2, where the % adsorbed ranged from 91.5% to 97.2%	Healy et al. 1997
Trimethoprim	The concentration of trimethoprim in the blood was determined at 0, 15, and 30 min and 1, 2, 4, and 6 h	The mean decrease in the maximum blood concentration of trimethoprim was 49.94%	Babhair and Tariq 1983
Aminosidine sulphate, chloramphenicol, erythromycin, neomysin B sulphate, novobiocin sulphate, penicillin V, streptomycin sulphate, and tetracycline hydrochloride	Each antibiotic was added to 250 mg of magnesium trisilicate; the antibiotic activity was determined by cup-plate method using *Staphylococcus aureus*	Magnesium Trisilicate reduced the activity of all antibiotics except chloramphenicol	El-Nakeeb and Youssef 1968
Ampicillin and amoxycillin	In vitro adsorption and desorption studies were carried out at different pHs	Hydrated silica gel formed from decomposition of the antacid at pH 2.1 and Magnesium Trisilicate had no adsorptive effect on either antibiotic	Khali, Mortada, and El- Khawas 1984a
		Attapulgite	
Strychnine, quinine, and atropine	Adsorption isotherms for each of the drugs and the clay was determined using spectrophotometric or colorimetric methods	Attapulgite adsorbed strychnine better than atropine than quinine; an increase in the hydrogen ion concentration was found to have a slight decreasing effect on the adsorptive ability for strychnine	Evcim and Barr 1955

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays *(Continued)*

Compound adsorbed	Experimental design	Results	Reference
Strychnine and atropine	Activated attapulgite was added to both compounds and adsorption isotherms were calculated	Both compounds were adsorbed by Attapulgite; optimum adsorbent properties were calculated at pH 6.8 and 7.2	Barr and Arnista 1957
Agrobacterium radiobacter	The measurement of O_2 uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Attapulgite contained excess basic cations, which accounted for the initial high pH and the reduction on respiration elicited by the addition of buffer	Stotzky 1966
Vibrio cholerae and *Escherichia coli* enterotoxins	The toxins and Attapulgite were injected into the intestinal loop of rabbits	Attapulgite prevented the toxic effects caused by enterotoxins in the intestinal loop by adsorption; Attapulgite was effective when injected simultaneously with the toxin and before the toxin is injected	Drucker et al. 1977
Ampicillin and amoxycillin	In vitro adsorption and desorption studies were carried out at different pHs	Both drugs were adsorbed at pH 2.1; desorption experiments at pH values of 2.0 and 6.5 showed only partial release of the adsorbed antibiotics	Khali, Mortada, and El-Khawas 1984a
		Bentonite	
Escherichia coli, *Serratia marcescens*, and *Bacillus* species	Each organism was cultivated in broth portions with 3% and 10% Bentonite	All organisms were absorbed by Bentonite at each concentration; *Bacillus* species was almost completely absorbed at each concentration	Novakova 1977
Escherichia coli 0111 endotoxins (ETU 144, 150, and 153)	In vitro and in vivo endotoxin binding was studied	In vitro, Bentonite was an effective endotoxin binder and binding was pH dependent (lower pHs yielded better results); 75 mg completely eliminated endotoxemia. At pH 3.0, the ED_{50} was 20 mg	Ditter, Urbaschek, and Urbascek 1985
Zearalenone and nivalenol	20 or 50 g/kg of Bentonite was added to the feed of pigs contaminated with zearalenone and nivalenol and was ingested for 29 days	Bentonite was unsuccessful at overcoming the estrogenic or depressed performance effects caused by the mycotoxins	Williams, Blaney, and Peters 1994
Aflatoxins B_1, B_2, G_1, G_2, M_1	Various methods	2% Bentonite adsorbed 400 μg of B_1; 2% adsorbed 89% of M_1; 2.5% adsorbed 5 ppm of B_1 and G_1 and 0.5 to 5 ppm of B_2 and G_2; 10% adsorbed 70% B_1	Ramos, Fink-Gremmels, and Hernandez 1996
		Kaolin	
Strychnine and atropine	Kaolin was added to both compounds and adsorption isotherms were calculated	Both compounds were adsorbed by Kaolin	Barr and Arnista 1957

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays *(Continued)*

Compound adsorbed	Experimental design	Results	Reference
Aminosidine sulphate, chloramphenicol, erythromycin, neomysin B sulphate, novobiocin sulphate, penicillin V, streptomycin sulphate, and tetracycline hydrochloride	Each antibiotic was added to 250 mg of Kaolin; the antibiotic activity was determined by cup-plate method using *Staphylococcus aureus*	Kaolin adsorbed significant amounts of aminosidine, neomysin, streptomycin, and tetracycline; Kaolin had no effect on antibiotic activity	El-Nakeeb and Youssef 1968
Agrobacterium radiobacter	The measurement of O_2 uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Kaolin did not maintain the pH therefore the bacteria could not maintain respiration even with an optimal pH for growth	Stotzky 1966
Bacillus subtilis, *Bacillus megaterium*, *Aerobacter aerogenes*, *Escherichia intermedia*, *Pseudomonas aeruginosa* and *P. aeroginosa* C-II, *Flavobacterium* species, *Proteus vulgaris*	The measurement of O_2 uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Kaolin in unadjusted pH systems reduced respiration of the bacteria below that of cultures without clay; but in adjusted systems some stimulation of respiration with the addition of Kaolin was apparent	Stotzky and Rem 1966
Mycelial homogenates of 27 species of fungi	Fungal mycelium and Kaolinite were cultured together and the O_2 uptake and pH were recorded	Kaolinite concentrations <4% generally did not effect respiration; respiration was only markedly inhibited at concentrations >40%	Stozky and Rem 1967
Crystal violet	2 g of Kaolin was added to 100 ml of a crystal violet solution	Adsorption was examined over a pH range of 2.5–9.5; adsorption increased with increasing pH	Armstrong and Clarke 1971
Staphylococcus aureus	Suspension of the organism, Kaolinite, and NaCl were studied	Increasing electrolyte concentration was accompanied by increased edge-to-face Kaolinite flocculation and organism-Kaolin aggregates	Steel and Anderson 1972
Escherichia coli	*E. coli* was cultivated in broth portions with 3% and 10% Kaolinite	*E. coli* was absorbed by Kaolin at both concentrations; the greatest adsorption occurred at 10% Kaolin at all phases of bacterial growth	Novakova 1977
^{125}I-labeled *Pseudomonas aeruginosa* toxin	The in vitro adsorption of the toxin by Kaolin was investigated over a range of pHs	The maximum adsorption occurred at pHs below 4.1; minimal values occurred at pH 4.1, 7.4, and 8	Said, Shibal, and Abdullah 1980
Acetohexamide, tolazamide, and tolbutamide	In vitro (pH 7.4) and in vivo (rats) adsorption studies were carried out	All 3 drugs bound and acetohexamide had the greatest binding; the hypoglycemic activity of the 3 drugs were suppressed and blood glucose concentrations were increased; desorption of the drugs from Kaolin ranged from 1.8% to 24.5%	Said and Al-Shora 1980

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays *(Continued)*

Compound adsorbed	Experimental design	Results	Reference
Coliphages T1 and T7 of *Escherichia coli*	1 ml suspensions of the coliphages were added to various concentrations of Kaolin	Adsorption of both coliphages by Kaolin were approximately the same 99%	Schiffenbauer and Stotzky 1982
Trimethoprim	The concentration of trimethoprim in the blood was determined at 0, 15, and 30 min and 1, 2, 4, and 6 h in the presence of Kaolin-Pectin	The mean decrease in the maximum blood concentration of trimethoprim was 29.42%	Babhair and Tariq 1983
Cationic surfactants: distearyl dimethyl ammonium chloride (74%); lauryl dimethylbenzyl ammonium chloride (50%)	A Kaolinite solution with added copper ions was added to surfactants and the metal ion uptake was recorded	Cationic surfactant result: the equilibrium between the metal ions and the organic cations was not effected	Beveridge and Pickering 1983
Anionic surfactants: sodium alkylbenzene aulphonate (80%); Monoethanolamine lauryl sulphate (34%); lauryl alcohol polyethylene condensate (28%)		Anionic surfactants: increased metal uptake by the clay was observed	
Nonionic surfactants: alcohol ethoylates; tridecaml ethoxylate (90%); cetystearyl alcohol ethoxylates; stearic acid ethoxylate; cocnut monoethanolamide ethoxylate; octadecylamine ethoxylate; castor oil ethoxylate; nonyl phenol ethoxylates; dinonyl pheno ethoxylate; polypropylene glycol ethoxylates		Nonionic surfactants: many surfactants had no effect and some caused enhanced loss of the metal ions from solution	
Escherichia coli 0111 endotoxins (ETU 144, 150, and 153)	In vitro and in vivo endotoxin binding to Kaolin	In vitro Kaolin was an effective endotoxin binder and binding was pH dependent (lower pHs yielded better results); 300 mg of Kaolin eliminated endotoxemia, at pH 7.4, the ED_{50} was 900 mg	Ditter, Urbaschek, and Urbascek 1983
Reovirus type 3	Chymotrypsin, ovalbumin, and lysozyme were added to Kaolinite and reovirus type 3	Chymotrypsin and ovalbumin reduced the adsorption of reovirus but lysozyme did not	Lipson and Stotzky 1984
Ampicillin and amoxycillin	4 g of Kaolin was ingested and 2 h later, 500 mg of the drugs were administered. This protocol was repeated 2 h later and urine (human) samples were collected	All volunteers showed reduced drug bioavailability following treatment; after 8 h, the reduced bioavailability for ampicillin ranged from 51.2 to 76.3 and 63.6 to 80.6 for amoxycillin	Khali, Mortada, and El-Khawas 1984b

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays *(Continued)*

Compound adsorbed	Experimental design	Results	Reference
Ampicillin and amoxycillin	In vitro adsorption and desorption studies to Kaolin (light, natural, and fine) were carried out at different pHs	The 3 types of Kaolin adsorbed only ampicillin and adsorption decreased as the pH increased; only partial release of the antibiotics was seen at pH 2.0 and 6.5	Khali, Mortada, and El-Khawas 1984a
Reovirus type 3 and coliphage T1	Competitive adsorption studies were carried out with Kaolin in estuarine water and distilled water	Reovirus type 3 and coliphage T1 did not share common adsorption sites on Kaolin and the coliphage did not interfere with the reovirus adsorption in estuarine water; the reovirus had no apparent effect on the adsorption of the phage in estuarine water	Lipson and Stotzky 1985
LT toxins of *Vibrio cholerae* and *Escherichia coli*, the ST toxin of ETEC, and the verotoxin of EHEC	Not specified	Kaolin inactivated the LT toxin and adsorption was a result of hydrogen bonding; it was ineffective against the verotoxin when the pH was alkaline; Kaolin was only slightly effective against the ST toxin	Brouillard and Rateau 1989
		Montmorillonite	
Agrobacterium radiobacter	The measurement of O_2 uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Montmorillonite spurred bacterial respiration by maintaining the initial pH; when the pH was adjusted to 7.0 respiration was its highest and similar to the buffered systems	Stotzky 1966
Bacillus subtilis, *Bacillus megaterium*, *Aerobacter aerogenes*, *Escherichia intermedia*, *Pseudomonas aeruginosa* and *P. aeroginosa* C-II, *Flavobacterium* species, *Proteus vulgaris*	The measurement of O_2 uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Montmorillonite increased the respiration of all species regardless of pH and characteristics of the bacteria primarily by maintaining the pH of the systems favorable for growth	Stotzky and Rem 1966
Mycelial homogenates of 27 species of fungi	Fungal mycelium and Montmorillonite were cultured together and the O_2 uptake and pH were recorded	Montmorillonite concentrations <4% generally did not effect respiration; respiration was markedly inhibited at concentrations of 4% and above	Stozky and Rem 1967
Cationic drugs: chlorpheniramine maleate, amphetamine sulfate, and propoxyphene hydrochloride; Anionic drugs: not specified Nonionic drugs: xanthines, theophylline, and caffeine	Dissolution and dialysis were carried out in vitro	All the cationic drugs and certain nonionic drugs bound tenaciously; the anionic drugs and nonionic drugs that exist as nonionics bound very weakly and rapidly pass into solution	McGinity and Lach 1976

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays *(Continued)*

Compound adsorbed	Experimental design	Results	Reference
Carbon tetrachloride, ethylene dibromide, trichlorethylene	10–1000 ppb/water of the three compounds were exposed to aluminum-saturated Montmorillonite and calcium-saturated Montmorillonite	Aluminum-saturated Montmorillonite absorbed 17% of trichloroethylene and 6% of the other cmpds; calcium-saturated Montmorillonite did not absorb carbon tetrachloride or trichloroethylene	Rogers and MacFarlane 1981
Coliphages T1 and T7 of *Escherichia coli*	1 ml suspensions of the coliphages were added to various concentrations of Montmorillonite	Adsorption of T1 coliphages by Montmorillonite was 84% and T7 was 96%	Schiffenbauer and Stotzky 1982
Cationic surfactants: distearyl dimethyl ammonium chloride (74%); lauryl dimethylbenzyl ammonium chloride (50%)	A Montmorillonite solution with added copper ions was added to surfactants and the metal ion uptake was recorded	Cationic surfactant result: metal ion uptake was reduced by competing surface sites	Beveridge and Pickering 1983
Anionic surfactants: sodium alkylbenzene aulphonate (80%); monoethanolamine lauryl sulphate (34%); lauryl alcohol polyethylene condensate (28%);		Anionic surfactants: increased metal uptake by the clay was observed	
Nonionic surfactants: alcohol ethoylates; tridecaml ethoxylate (90%); cetystearyl alcohol ethoxylates; stearic acid ethoxylate; coconut monoethanolamide ethoxylate; octadecylamine ethoxylate; castor oil ethoxylate; nonyl phenol ethoxylates; dinonyl pheno ethoxylate; polypropylene glycol ethoxylates		Nonionic surfactants: surfactants reduced the amount of metal ion adsorbed by the clay	
Reovirus type 3	Chymotrypsin, ovalbumin, and lyso-zyme were added to Montmorillonite and reovirus type 3	Chymotrypsin, ovalbumin, and lysozyme reduced the adsorption of reovirus	Lipson and Stotzky 1984
Poliovirus-1 (Lsc 2ab strain)	500, 15, 3 mg/L of Sodium Montmorillonite and the virus were suspended in seawater and the adsorption, desorption, and virus survival were studied	99.9% of the virus was absorbed in less than 30 min; 500 mg/L of Na-Montmorillonite significantly increased the survival duration of of the virus and desorption tests showed elution of 76%	Gantzer, Quignon, and Schwartzbrod 1994
Reovirus type 3 and coliphage T1	Competitive adsorption studies were carried out with Montmorillonite in estuarine water and distilled water	Reovirus type 3 and coliphage T1 did not share common adsorption sites on Kaolin and the coliphage did not interfere with the reovirus adsorption in estuarine water or distilled water; the reovirus suppressed the adsorption of the coliphage in estuarine water	Lipson and Stotzky 1985

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays *(Continued)*

Compound adsorbed	Experimental design	Results	Reference
		Pyrophyllite	
Agrobacterium radiobacter	The measurement of O_2 uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Pyrophyllite did not maintain a favorable pH for sustained respiration in either buffered or nonbuffered systems	Stotzky 1966
		Zeolite	
Zearalenone	5% of a synthetic anion-exchange zeolite and a cation-exchange zeolite and 250 μg/g of zearalenone were added to the feed of rats	The anion-exchange zeolite was completely effective and the cation-exchange zeolite was not	Smith 1980
Aflatoxin B1	Two samples of natural Zeolites in different liquids were incubated with B_1	The average aflatoxin retention rate was 605; effectiveness was lower in media containing nitrogen compounds	Dvora'k 1989

Bujdak and Rode (1996) reported peptide formation on the surface of three Montmorillonite samples. The Montmorillonite-catalyzed reaction produced diglycine and diketopiperazine from glycine.

Ferris et al. (1996) studied the catalytic properties of Na^+-Montmorillonite by adding daily ImpA to a decanucleotide ($[^{32}P]$-dA$(pdA)_8$pA, where Im = imidazole; pA = adenosine-5′-phosphate; pdA = 3′-deoxyadenosine-5′-phosphate; ^{32}P = radioactively labeled phosphate group). Polyadenylates were formed after two additions of ImpA, with the main products being monomers ranging from 11 to 14. Polynucleotides, with more than 50 monomers, were formed after 14 additions. The principle oligomeric products contained 20 to 40 monomers.

Ertem and Ferris (1998) reported Montmorillonite-catalyzed ImpA and ImpA-A5′ reactions. Oligomer yields decreased significantly when the addition of alkylammonium or aluminum poly oxo cations blocked the interlayer surfaces of the Montmorillonite particles.

Absorption, Distribution, Metabolism, and Excretion

Magnesium Trisilicate

Page, Heffner, and Frey (1941) measured the urinary excretion of silica in five men given 5 g of synthetic Magnesium Trisilicate orally for 4 consecutive days. Urine samples were collected for 24 h on the second day after the end of administration and analyzed for silica content. The mean 24-h excretion of all subjects was 16.2 mg of SiO_2. On the second, third, and fourth days after administration, the mean excretion rose to 172, 178, and 162 mg SiO_2. A total of 20 mg of Magnesium Trisilicate was taken and contained 9.2 g of SiO_2. An approximation of 5.2% SiO_2 excretion was estimated.

Benke and Osborn (1979) conducted a study in which groups of four to six male Sprague-Dawley Cox rats were fasted for 17 to 18 h and then were administered Magnesium Trisilicate orally in doses of 40, 200, or 1000 mg/kg of their body weight. Control animals received 10 ml of quartz-distilled water. All suspensions contained <0.5 ppm of silicon and aluminum. Urine samples were collected over an 8-h period, and the remaining urine in the bladder was collected afterwards. The concentrations of silicon was measured by induction-coupled radiofrequency (RF) plasma optical emission spectrometry. Silicon excretion was most rapid in the first 24 h after dosing. The control values were subtracted from the final values and the following number resulted. The urinary silicon excretion at 40, 200, and 1000 mg/kg Magnesium Trisilicate was 16.8%, 5.1%, and 1.5%, respectively.

Dobbie and Smith (1982) reported a 24-h urinary excretion study in which Si was determined by atomic absorption spectroscopy in one male and one female participant. A normal diet was given to the participants and four urine collections were made. A single dose of Magnesium Trisilicate was ingested at the beginning of the second 24-h collection. Magnesium Trisilicate doses given were as follows: 2, 5, and 10 g to the male subject and 2.5, 5, and 7.5, and 10 g in the female subject. The amount of Si excreted at the 5-g dose was greater than any other dose in the male subject and was greater than the 2.5- and 7.5-g doses in the female subject. The value of Si excretion for the male and female subjects were 3.63 and 3.31 mmol/day, respectively. Maximum excretion occurred in the first 24 h after ingestion.

The oral bioavailability of silicon and aluminum in Magnesium Trisilicate was studied by Cefali et al. (1995). Twelve female beagle dogs were administered a single 20-mg/kg dose of Magnesium Trisilicate and their blood was sampled at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing. The plasma

samples were assayed for silicon and aluminum by graphite furnace atomic adsorption. No dogs displayed emesis, but four had soft stool. The area under the curve (AUC, mg · h/L), concentration maximum (C_{max}, mg/L), and time maximum (T_{max}, h) for silicon absorption was 8.8, 0.75, and 6.9, respectively. The AUC (mg · h/L), C_{max} (mg/L), and T_{max} (h) for aluminum absorption was 315, 24, and 5.7, respectively. There was no statistically significant absorption of aluminum from the aluminum containing compounds.

Montmorillonite

Retention of monodisperse and polydisperse Montmorillonite particles inhaled by dogs, rats, and mice was studied by Snipes, Boecker, and McClellan (1983a). Cations normally present in Montmorillonite were exchanged with ^{134}Cs. Polydisperse and monodisperse ^{134}Cs-labeled Montmorillonite suspensions were administered to groups of 40 rats and mice and to 120 beagle dogs by a multiport nose-only inhalation exposure system. Aerosol concentrations ranged from 10^{-3} to 10^{-1} mg of fused Montmorillonite per liter 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. Rats and mice were counted on exposure days 2, 4, 8, 16, 32, 64, 128, 256, 365, 512, 730, and 850 and the dogs were also counted on the same schedule, but also at 4, 5, 7, and 9 years after inhalation exposure. Excreta collections were made for animals from each exposure group. Five rats and five mice from each group were killed 4 h after exposure. The remaining rats and mice were killed at various times after exposure. Two dogs were scheduled for termination at times ranging from 4 h to 9 years. All animals were necropsied and tissues from lungs, lung-associated lymph nodes (LALNs), gastrointestinal tract, spleen, kidneys, abdominal lymph nodes, blood, skeleton, muscle, and skin were prepared for analysis of ^{134}Cs exposure. Results of the counts were converted into disintegrations per minute.

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 per gram 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 deposit 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 LALNs 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-day half-time in the lymph nodes and 6900-day half-time clearance in the gastrointestinal tract. The transport rate of the particles in the dog was 0.0002 day^{-1} of the lung burden. The long-term biological clearance half-term day was 690 days for rats and 490 days for mice. The lymph node accumulation process was modeled by a short-term process that became negligible after a few days (Snipes, Boecker, and McClellan 1983a).

Snipes, Muggenburg, and Bice (1983b) instilled radio-labeled (^{134}Cs) fused Montmorillonite particles into specific lung lobes or injected intraperitoneally into 32 beagle dogs. Necropsy was performed at 34, 182, and 365 days 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 of ^{134}Cs for 29 dogs and from 42 to 64 μCi of ^{134}Cs for lung burdens for the other three dogs. Effective translocation half-time of lung instillations was 390 days. The accumulation rate of ^{134}Cs-labeled particles in the lymph nodes was 0.03% per day. Individual lung lobes cleared particles to one or two lymph nodes, and specific lymph nodes accumulated particles from one to three lung lobes. Lymph nodes that collected particles from the lung included the left mediastinal node, left tracheobronchial lymph node (TBLN), right TBLN, left middle TBLN, and right middle TBLN. The destination for translocated particles were primarily the nodes proximate to the tracheal bifurcation. Particles injected into the peritoneal cavity were translocated mainly to mesenteric lymph nodes and left sternal and right sternal lymph nodes. A small percentage of particles went to the left TBLN.

Zeolite

The oral bioavailability of silicon and aluminum in Zeolite A was studied by Cefali et al. (1995). Twelve female beagle dogs were administered a single 20-mg/kg dose of Zeolite A and blood was sampled at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing. The plasma samples were assayed for silicon and aluminum by graphite furnace atomic adsorption. No dogs displayed emesis but four had soft stool. The AUC (mg · h/L), C_{max} (mg/L), and T_{max} (h) for silicon absorption was 9.5, 1.07, 7.9, respectively. The AUC (mg · h/L), C_{max} (mg/L), and T_{max} (h) for aluminum absorption was 342, 29, and 3.5, respectively. The AUC and C_{max} values were elevated after the addition of the silicon containing compounds compared to the baseline and the AUC was significantly elevated. There was no statistically significant absorption of aluminum from the other aluminum-containing compounds.

In a study by Cefali et al. (1996), the bioavailability of silicon and aluminum in Zeolite A administered in either a capsule, an oral suspension, or an oral solution relative to an intravenous bolus infusion administered over a 1- to 1.5-min period was investigated. Twelve beagle dogs were given single doses of Zeolite A and their plasma samples, drawn at 0 and 36 h, were analyzed for silicon and aluminum concentrations by graphite furnace

atomic absorption. The plasma aluminum AUC values from the oral capsule and suspension were not statistically different from those during the control period. However, the aluminum AUC of the oral solution was statistically greater than the AUC of the corresponding control period. The extent of absorption of aluminum form the oral dosage forms was less than 0.1% relative to the intravenous infusion.

In Vitro Assays

Aluminum Silicate

Nadeau et al. (1987) tested Fiberfrax, an aluminum silicate, in several in vitro assays for red blood cell (RBC) hemolysis, lactate dehydrogenase activity (LDH), β-galactosidase (β-GAL) activity, lactic acid production, cellular ATP activity, and the cellular DNA contents. The mean length and diameter of this sample were determined to be 8.3 μm and 0.2 μm, respectively. Approximately 60% of this Fiberfrax sample was nonfibrous.

For the hemolysis assay, RBCs from rats were isolated and exposed to 100, 250, 500, 750, or 1000 μg/ml of fibers for 1 h. The percentage of release of hemoglobin was compared with that of a fully lysed sample. The target cells for the other experiments were obtained by bronchoalveolar lavage from black hooded rats. Each of the experiments tested both fresh cell monolayers and 1-day-old monolayers. Fiber samples were added to the cultures at two doses, 33.3 μg/ml and 166.7 μg/ml. LDH activity was based on the formation rate of NADH at 340 nm. The β-GAL activity was based on the measurement of *p*-nitrophenyl release. The amount of metabolite released from PAMs (pulmonary alveolar macrophages) into the medium was the measurement of lactic acid production. PAMs were treated with 1 ml of dimethyl sulfoxide to release the nucleotides and the ATP was measured later by a bioluminesence assay.

Fiberfrax particles produced no hemolytic activity at any concentration except 1000 μg/ml. Even at 1000 μg/ml, the particles had very weak hemolytic properties with only 2.0% hemolysis. In fresh PAM monolayers, Fiberfrax was very cytotoxic at 166.7 μg/ml. The extracellular releases of LDH and β-GAL were approximately 60% to 70% and 40% to 50%, respectively. A low cell viability was confirmed by an 80% decrease in ATP cell contents. Even at the lower dose, 33.3 μg/ml, a significant cytotoxic effect resulted, as judged by enzyme releases and ATP cell contents. Again in the day-old cultures, Fiberfrax was highly cytotoxic to PAM. LDH and β-GAL activities were as great and ATP cell contents were significantly decreased. At the lower dose, a moderate cytotoxic effect was observed. Decreases in lactic acid production were more pronounced at 166.7 μg/ml. No significant effect on total DNA cell content was noted in either the fresh or day-old cultures (Nadeau et al. 1987).

Attapulgite

Colony formation of human embryo intestinal cells (I-470) was examined by Reiss, Millette, and Williams (1980). At a dose of 0.001 to 1 mg/ml of Attapulgite with fibers <2 μm, colony formation was not modified. Colony formation was inhibited by 35% and 43% at doses of 2.5 and 5.0 mg/ml, respectively.

Oscarson, Van Scoyoc, and Ahlrichs (1981) added Attapulgite to a culture of bovine RBCs to study the extent of hemolysis. Saline was added to cultures as a control and in a separate experiment, the polymer poly-2-vinylpyridine-*N*-oxide was also added to study its inhibiting effects. No other details were given. The concentration of Attapulgite that caused 50% hemolysis in 1 ml of a 3% solution of RBCs was determined as 0.06 mg Attapulgite/ml of silicate-erythrocyte-buffer suspension. A concentration of 0.2 and 1.0 μm/ml of polymer caused 20% and 3% hemolysis, respectively. This was somewhat less hemolysis than without the polymer.

Chamberlain et al. (1982) tested two samples, one with short fibers and one with long fibers, of Attapulgite for their cytotoxicity in three cell lines: mouse peritoneal macrophages, human type II alveolar tumor (A549) cells, and Chinese hamster V79-4 lung cells. Attapulgite samples of 50, 100, and 150 $\mu g/ml^{-1}$ were added to mouse peritoneal macrophages for 18 h. The medium and cell lysates were assayed for LDH activity. The control received no dust sample. In the second experiment Attapulgite, 100 $\mu g/ml^{-1}$ and 200 $\mu g/ml^{-1}$, were added to A549 cultures and incubated for 5 days. The diameters of the cells were assessed for giant cell formation. The control treatment received no dust. In the last experiment, the survival of V79-4 cells in the presence of a series of concentrations of each dust was determined. Specific concentrations were not given. The cells and dust samples were incubated for 6 days and counted after the incubation. The controls received no dust.

The mouse macrophages released 57.7% LDH from interaction with 150 $\mu g/ml^{-1}$ of short fiber Attapulgite and was considered cytotoxic. However, the short fiber sample was considered inert to the A549 cells and V79-4 cells. The long fiber Attapulgite was cytotoxic to all three cell types. It was noted by investigators that mouse peritoneal macrophages are sensitive to both fibrogenic and carcinogenic dusts; whereas nonmacrophage cell lines such as V79-4 and A549 cells are insensitive to fibrogenic dusts but sensitive to the fiber morphology of carcinogenic dusts (Chamberlain et al. 1982).

Gormley and Addison (1983) investigated the cytotoxic effect of Attapulgite with a particle size of 2.6 μm. Clay suspensions, 20 and 80 μg/ml, were added to P388D1, a macrophage-type cell line for 48 h. Three sets of controls were included: a positive control, 20 μg of quartz DQ_{12}/ml; and two negative controls, 80 μg of TiO_2/ml, and an undusted set of cultures. The following assessments were made: cell viability; the activity of LDH; the activity of *p*-nitrophenyl-*N*-acetyl-β-D-glucosamide; L-(+)-Lactic acid production; and total cellular protein concentrations. Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) $\pm$ the standard deviation. The 20-μg/ml dose of Attapulgite produced a 65.8% $\pm$ 9.2% viability and the 80 μg/ml dose produced a 30.9% $\pm$ 17.4% viability. Cellular LDH activities fell with decreasing cell viability, whereas the percentage of LDH in the medium increased.

Similar results were seen with glucosamidase. Also, the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded.

The induction of squamous metaplasia in tracheal organ cultures was investigated by Woodworth, Mossman, and Craighead (1983). Suspensions of Attapulgite at concentrations of 1, 4, and 16 mg/ml were added to the mucosal surface of the tracheal explants for 1 h. After experimental treatments, extracts were transplanted to another surface more suitable for cell attachment. Mucocillary differentiation was maintained for 4 weeks and the explants were examined at 2, 4, and 6 weeks after exposure to Attapulgite. The extent of squamous metaplasia was evaluated by SEM (scanning electron microscope). The explants were labeled with [^{3}H]-thymidine and the labeling index was scored. Four weeks after exposure to Attapulgite, the explants underwent both proliferative and metaplastic alteration. Attapulgite induced an increase in metaplasia at low doses (1.0 and 4.0 mg/ml), but the increase was not statistically significant. The labeling index was also increased slightly but statistically significant. SEM was used to determine the association of fibers with metaplastic lesions. Most fibers aggregated at the margins of the explant, although small numbers of individual fibers were distributed along the mucosal surface. These fibers either rested on nonciliated cells or protruded into the mucosal surface. They were often encompassed by accumulations of epithelial cells. Metaplastic foci tended to be small. Many foci associated with the lesions but some were located at sites where no lesions could be seen.

The binding capacity, in vitro cytotoxicity, and percentage of hemolysis were investigated in a study by Harvey, Page, and Dumas (1984). Binding assays were carried out using the known carcinogens benzo(α)pyrene (B(α)P), nitrosonornicotine (NNN), and *N*-acetyl-2-aminoflurene (NAAF) and 2 mg/ml of Attapulgite. A 2% suspension of sheep erythrocytes were added to 30 mg of Attapulgite and incubated for 50 min. Cytotoxicity was measured using 1000 μg of Attapulgite and macrophage-like P399D1 cells and using the Trypan blue dye exclusion method. Hemolysis was calculated by measuring the optical density at 540 nm. All experiments included the positive control UICC chrysotile A and the negative control titanium dioxide. Chrysotile binds significantly more to all three carcinogens than the other fibers ($p < .005$) except Attapulgite. Attapulgite and chrysotile had very comparable binding capacities. Again Attapulgite and chrysotile had the greatest hemolysis and cytotoxicity compared to the negative control. On a scale of 1 to 5, 5 being the greatest, Attapulgite scored a 3.72 and 4.26 in hemolysis and cytotoxicity, respectively.

The cellular interactions between Attapulgite and rat hepatocytes were examined in a study by Denizeau et al. (1985a). Primary cultures of rat hepatocytes were exposed to 10 μg/ml of Attapulgite fibers for 20 h. Ultrastructural analysis was performed by transmission electron microscopy. Fiber length was not indicated in this study. Fibers are phagocytized by the cells and numerous phagolysosomes are distributed throughout the cytoplasm. The phagolysosomes also appear in the vicinity of charged vacuoles. Invaginations of the plasma membrane engulfing fibers and formation of vacuoles are identifiable. Deeper in the cytoplasm vacuoles with various shapes show the presence of fibers.

Beck and Bignon (1985) incubated leukemic mouse cells with two samples of 10, 50, or 100 μg/ml of Attapulgite. Viable cell counts were taken at 0, 24, 48, and 72 h. A positive control consisting of UICC amosite and untreated negative controls were also used in this experiment. The majority of fibers in the Attapulgite samples were <1.0 μm. No evidence of cytotoxicity was measured over the 72-h period. The results from the Attapulgite samples were indistinguishable from the untreated controls.

The cytotoxic effects of Attapulgite on rabbit alveolar macrophages and rat pleural mesothelial cells were investigated by Jaurand et al. (1987). Attapulgite samples with a mean fiber length of 0.77 μm were added at concentrations 4 and 8 μg/cm^2 to rabbit alveolar macrophage cultures for 4 and 20 h; control cultures received medium with no fibers. Enzyme release, activity of cytoplasmic LDH and lysosomal β-GAL was tested. The presence of LDH activity in cultures was the gauge of cytotoxicity and the presence of β-GAL was the gauge of cell stimulation. Attapulgite at both concentrations was cytotoxic at 20 h. β-GAL release percentages for Attapulgite and quartz after 20 h were almost identical.

Again Attapulgite was added at concentrations of 1, 2, 4, and 10 μg/cm^2 to rat pleural mesothelial cells. The cell number was determined daily with the use of a Nachet NS 1002 image analyzer. Attapulgite was not cytotoxic except at 10 μg/cm^2. At the lower doses, cell number increases were comparable to that of the controls (Jaurand et al. 1987).

Nadeau et al. (1987) tested Attapulgite for its effects on cells in several in vitro assays for RBC hemolysis, LDH activity, β-GAL activity, lactic acid production, cellular ATP activity, and the cellular DNA contents. The mean length and diameter of this sample were determined to be 0.8 μm and 0.1 μm, respectively. The same study was conducted on Aluminum Silicate and all protocol and procedures are explained under that section. Attapulgite particles produced no hemolysis except at 1000 μg/ml. Even at 1000 μg/ml, the particles showed very weak hemolytic properties with only 2.0% hemolysis. Analysis with the fresh PAM monolayers revealed Attapulgite to be very cytotoxic at 166.7 μg/ml. The extracellular releases of LDH and β-GAL were approximately 60% to 70% and 40% to 50%, respectively. A low cell viability was confirmed by an 80% decrease in ATP cell contents. Even at the lower dose, 33.3 μg/ml, a significant cytotoxic effect resulted, as judged by enzyme releases and ATP cell contents. Again in the day old cultures, Attapulgite was highly cytotoxic to PAM. LDH and β-GAL activities were very large and ATP cell contents were significantly decreased. At the lower dose, a moderate cytotoxic effect was observed. Decreases in lactic acid production were more pronounced at 166.7 μg/ml. No significant effect on total DNA cell content was noted in either the fresh or day-old cultures.

Garcia, Dodson, and Callahan (1989) investigated the effects of Attapulgite on cultures of human umbilical vein and bovine artery endothelial cell monolayers. Chrysotile asbestos was also studied as a positive control. Rapid phagocytosis of Attapulgite and chrysotile particulates was evident in endothelial cell monolayers. Attapulgite was markedly toxic according to a gradient of time-dependent and concentration-dependent endothelial cell injury measured by specific ^{51}Cr release. Chrysotile was much less toxic. Responses of bovine pulmonary artery and human vein endothelial cells to fiber phagocytosis and fiber-induced injury were similar. Fiber-mediated stimulation in human umbilical cell monolayers of the arachidonate metabolite prostacyclin paralleled endothelial injury. Attapulgite was stimulatory in this experiment, whereas chrysotile was only weakly cytotoxic. Superoxide dismutase and catalase produced significant protection against fiber-mediated endothelial cell injury. Chelation by deferoxamine of elemental Fe in the fiber preparations was also protective.

Perderiset et al. (1989) reported the hemolytic activity of Attapulgite on human red blood cells at five concentrations (0.05, 0.1, 0.2, 0.4, and 0.5 mg/ml). Additional studies tested the hemolytic activity of dipalmitoyl phosphatidylcholine (DPPC) and bovine serum albumin (BSA)-treated Attapulgite (2 mg/ml). The mean fiber length was <2 μm. The percentage of hemolysis was determined by measuring the absorbance of the supernatant at 540 nm. At 0.5 mg/ml, Attapulgite caused 82% hemolysis. The maximum amount of BSA adsorbed was 70 ± 10 μg/mg of Attapulgite, and the maximum occurred at an initial concentration of 200 μg/ml. For DPPC, the maximum amount of BSA adsorbed was 210 ± 14 μg/mg of Attapulgite, and the maximum occurred at an initial concentration of 250 to 300 μg/ml. Both compounds reduced the hemolytic effect of Attapulgite due to adsorption on the particle's surface.

Nolen, Langer, and Herson (1991) tested nine different samples of Attapulgite for their membrane-lysing activity using human RBCs. The HC_{50} (concentration of particulate in μg/ml required to lyse 50% of the erythrocytes in a suspension containing 1.8×10^8 cells/ml) was determined quantitatively. Three samples of Chrysolite were used as positive controls. No other details of the experiment were given. The fiber characteristics were determined by light microscopy and x-ray diffraction and the HC_{50} values are presented in Table 9.

Attapulgite's cytotoxicity was investigated in rat pleural mesothelial cells (RPMCs) by Yegles et al. (1995). A suspension of 0.5 mg/ml of Attapulgite was added to RPMCs, and a 3,(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) viability test and anaphase/telophase abnormalities test were performed. The clay sample had no fibers measuring greater than 4 μm. Cytotoxicity was expressed as the concentration that provides 75% of cell viability compared to untreated controls (IC_{75}). Attapulgite was only poorly toxic with an IC_{75} of >100 $\mu g/cm^3$. Untreated controls averaged about 3.4% of abnormal anaphases; no significant anaphase abnormalities were seen with Attapulgite as well.

Bentonite

The hemolysis of human erythrocytes and methylene blue adsorption by two Bentonite samples were investigated by M'anyai et al. (1969). A white Bentonite sample consisted of 50% illite, 25% quartz, and 25% Montmorillonite; the yellow Bentonite sample consisted of predominately Montmorillonite. The data in Table 10 show that the hemolytic effect varied as a function of both of the amount of clay (mg) and the surface area (m^2).

TABLE 9
Fiber characteristics of nine Attapulgite samples tested for their membranolytic activity using human red blood cells (Nolen, Langer, and Herson 1991)

		Fiber length (μm)				
Sample	Fiber character	<1.0	1.1–5.0	5.1–10.0	>10.0	HC_{50}* (μg/ml)
1	Fibrous	71.5	26.3	1.7	0.5	400
2	Fibrous	92.7	7.1	—	—	Inactive
3	Nonfibrous	90.2	9.3	0.3	0.3	746
4	Fibrous	78.0	21.3	0.7	0.2	211
5	Fibrous	75.1	22.4	2.0	0.6	369
6	Nonfibrous	91.1	8.7	0.1	0.1	76
7	Nonfibrous	83.4	16.6	—	—	83
8	Nonfibrous	83.1	16.8	—	—	109
9	Fibrous	59.4	37.5	2.6	0.6	51
Chrysolite 1	Fibrous	77.2	20.5	1.8	0.5	41
Chrysolite 2	Fibrous	84.9	13.6	0.6	0.4	82
Chrysolite 3	Fibrous	88.8	10.6	0.4	0.2	59

*The HC_{50} is the concentration of silicate clay (in μg/ml) required to lyse 50% of the erythrocytes in a 1.8×10^8cells/ml suspension.

TABLE 10
Hemolysis and methylene blue adsorption results (M'anyai et al. 1969)

Mineral	Sample description	50% hemolysis in 1 ml of a 2% erythrocyte suspension as function of: Amount of clay (mg)	50% hemolysis in 1 ml of a 2% erythrocyte suspension as function of: Surface area of clay (m^2)	Amount of methylene blue adsorbed by 1 m^2 clay surface (mg)
Bentonite	White	1.66	0.039	3.59
Bentonite	Yellow	1.0	0.135	2.13
Montmorillonite	Ca-substituted	5.0	0.50	1.46
Montmorillonite	$^{+}$Quartz	0.8	0.02	—
Kaolin		2.0	0.06	1.09
Kaolin	Fat	1.5	0.07	1.60
Kaolin	White	4.0	0.06	0.12
Kaolin	Pink	5.0	0.115	0.19

Beck and Bignon (1985) dosed peritoneal macrophages with two samples of Bentonite and the triphenyltetrazolium chloride (TTC) reduction, LDH activity, and methylene blue adsorption were used to assess cytotoxicity. One sample of Bentonite contained 3% SiO_2 and the other 34%. Bentonite inhibited TTC reduction similar to the fibrogenic dusts such as quartz. However, the extracellular LDH activity was not increased and methylene blue adsorption was very high.

Hatch et al. (1985) examined the cytotoxicity of Bentonite to rabbit alveolar macrophages. The alveolar macrophages were incubated with 1.0 mg/ml of Kaolin for 20 h at 37°C. Control cultures received 1.0 mg/ml of TiO_2. The viability percentage of the macrophages and the ATP content of the cells as index of cytotoxicity were determined. Bentonite caused a large reduction in both the viability and ATP levels. The viability index and ATP levels were presented as percentage reductions and were 64.7% and 92.0%, respectively. Controls figures were 18.3% and 0.7%, respectively.

TTC reduction, LDH activity, and methylene blue adsorption were measured as an index of cytotoxicity in a study by Adamis et al. (1986). Bentonite was added to peritoneal macrophages obtained from rats. No specific dose of Bentonite or other details were stated. TTC reduction was much greater and proved Bentonite to be cytotoxic. Extracellular LDH was almost half for Bentonite compared to control values. Methylene blue adsorption was significantly higher for Bentonite.

Murphy, Roberts, and Horrocks (1993a) investigated the cytotoxicity of Bentonite to human umbilical vein endothelial (HUVE) cells, undifferentiated N1E-115 neuroblastoma cells, and ROC-1 oligodendrogial cells. Indices of cytotoxicity used in this study were morphological examination, LDH activity, and fatty acid release. A suspension of Bentonite (1 to 2 μm in fiber length) was added to the cultures at concentrations of 0.1, 0.03, and 0.01 mg/ml and incubated for 1, 6, and 24 h. Following incubations, the cells were examined morphologically. The medium and cells were extracted for free fatty acid quantitation. LDH activities were assayed after 24 h of incubation at a Bentonite concentration of 0.10 mg/ml.

Bentonite did not lyse ROC-1 oligodendrogial and the neuroblastoma cells and did not cause a dose-dependent increase in fatty acids at 24 h. No significant increases in LDH activity were detected utilizing any of these cell lines. However, Bentonite caused a dose-dependent increase in fatty acid concentrations only after 24 h of incubation. A 4.5-fold increase in fatty acid concentrations over control values was calculated. Increases over control activities of LDH were 141% with Bentonite. Within 1 h, Bentonite associated with the plasma membrane of HUVE cells and the morphology was drastically changed after treatment (no details given). Cell lysis was also apparent with treatment. After trypan blue staining, 94% of HUVE cells were nonviable with Bentonite treatment (Murphy, Roberts, and Horrocks 1993a).

In a separate study by Murphy et al. (1993b), the cytotoxicity of Bentonite was examined in two cell lines: primary murine spinal cord neurons and differentiated N1E-115 neuroblastoma cells. A clay suspension with a concentration of 0.1 mg/ml was added to the cultures. The neuronal cells were incubated for 1 h with Bentonite. Photomicrographs were taken at 5, 15, and 60 min following treatment. For the N1E-115 cells, incubation lasted 18 h and photomicrographs were taken at 5 and 15 min and 3, 6, and 18 h after the treatment. Morphological changes were observed using a phase contrast microscope. Within 5 min, clay particles were observed on the neuronal cell bodies. Cell bodies appeared granular within 15 min. The cells were completely lysed after 60 min and there was no evidence of any remaining cell bodies or processes. Cell membrane contact was apparent after 5 min in N1E-115 cultures. No morphological changes were apparent at this point. At 18 h, the cells were covered with

clay but cellular processes remained intact. N1E-115 cell lysis did not occur and no cytotoxicity was recorded as a result of Bentonite treatment.

Calcium Silicate

Hunt, Pooley, and Richards (1981) tested three samples of Calcium Silicate (A, B, and C) for biological reactivity in three in vitro test systems. Table 11 presents the differences in SiO_2 and Al_2O_3 percentages between the three samples.

In the first test system, 50, 100, 150, and 200 mg of the three samples of Calcium Silicate, UICC chrysotile (positive control), and titanium dioxide (negative control) were added to rabbit erythrocytes. The cultures were incubated for 50 min. The percentage of hemolysis was calculated. Rabbit erythrocytes were also incubated with 10, 30, and 50 mg heated, crushed samples of Calcium Silicate to calculate the percentage of hemoglobin binding. In the second study, rabbit alveolar macrophages were incubated with 5 mg of the Calcium Silicate samples for time intervals up to 60 min. The results were expressed as total viable cells. In the third study, sonicated Calcium Silicate samples (100 to 2000 μg) were added to rabbit lung fibroblasts. On days 7, 10, 17, and 24 after treatment, the cultures were analyzed for cellular DNA, protein, other cellular material, and hydroxyproline. Cytological studies on the same cells were carried out using dust concentrations of 50 to 400 μg and staining the cultures to visualize reticulin fibers.

In order to obtain 20% hemolysis, 0.4 mg of chrysotile, 2.8 mg of A, 25.0 mg of B, and 15.0 mg of C are required. Titanium dioxide did not produce 20% hemolysis at any concentration. Sonication of all samples enhanced hemolysis and a "respirable" preparation of A had the same hemolytic activity as chrysotile. Sample B binds more hemoglobin than A or C but not more than chrysotile. Samples B and C had enhanced hemolytic activity when heated above 300°C. Heating had no effect on sample A. All samples produced similar macrophage mortality and at concentrations of 5 mg, only 60% of the cells were surviving at 60 min. Chrysotile at 5 mg resulted in a 20% viability. Samples A and B produced greater DNA and protein concentrations at day 7. However, sample A induced greater protein concentrations at day 24 with normal hydroxyproline levels. Sample B at day 24 had decreased concentrations of protein and hydroxyproline with an increase in mineral concentration. Sample A produced few changes in fibroblast morphology and reticulin deposits.

TABLE 11
Aluminum and Silicon content in Calcium Silicate samples used in biological reactivity study (Hunt, Pooley, and Richards 1981)

Calcium Silicate sample	SiO_2 %	Al_2O_3 %
A	57.3	2.6
B	52.3	4.4
C	53.7	1.0

TABLE 12
Sample characterisitcs of five Calcium Silicates tested for hemolytic activity in vitro (Skaug and Gyseth 1983)

Sample	Chemical formula	SiO_2 %	Fibrous character
CaSi A, natural wollastonite	$CaSiO_3$	—	+++
CaSi B, natural wollastonite	$CaSiO_3$	2	+
CaSi C, synthetic wollastonite	$CaSiO_3$	9	–
CaSi D, synthetic tobermorite	$Ca_5Si_6O_{17} \cdot 2.5\ H_2O$	10	–
CaSi E, synthetic tobermorite	$Ca_5Si_6O_{17} \cdot 2.5\ H_2O$ $Ca_6Si_6O_{17}(OH)_2$	2	+

Sample B produced sparse and irregular deposition of reticulin (Hunt, Pooley, and Richards 1981).

Skaug, Davies, and Glyseth (1984) tested five Calcium Silicate dust samples for hemolytic activity in vitro. Electron microscopy and x-ray diffractions techniques were used to characterize the Calcium Silicates and the results are presented in Table 12. The Calcium Silicate samples A to E, chrysotile B (positive control), and titanium dioxide were added to RBCs at concentrations of 0, 5, and 10 mg/ml. The effect of sonication of the dust samples and the addition of 30 mM $CaCl_2$, EDTA, and EGTA were also investigated. Sample E produced the greatest hemolysis at nearly 40%. The hemolytic activity of the synthetic Calcium Silicate samples were greater. In all experiments, greater dust concentrations increased hemolysis. Sonication increased the hemolytic activity of the synthetic samples but had no effect on the natural samples. The 30 mM $CaCl_2$ increased the hemolysis of samples D and E, but not C. EDTA did not decrease hemolysis for samples D and C, and EGTA did not inhibit hemolysis of samples B, C, D, and E.

Five samples of Calcium Silicate also were used to test cytotoxic effects on mouse peritoneal macrophages in vitro. Calcium Silicate concentrations of 0, 20, 40, and 60 $\mu g/cm^3$ were added to mouse peritoneal macrophages for 18 h. The medium and cell lysates were assayed for LDH and β-glucuronidase (β-GLUC). The positive-control dust utilized was DQ12 quartz standard and the negative-control dust was magnetite. Characterization of the five samples were carried out by means of x-ray diffraction and scanning electron microscopy. The results of the mineral characterization are presented in Table 13. The samples A, B, C, and D had little effect on LDH release but sample E, the fibrous tobermorite, was clearly cytotoxic. Samples A and B caused release of large levels of β-GLUC. Sample E also caused the release of significant amounts of β-GLUC due to its cytotoxicity. Samples C and D caused the release of amounts comparable to the negative controls (Skaug, Davies, and Glyseth 1984).

TABLE 13
Mineral characterization of five samples of Calcium Silicate used to test cytotoxic effects on mouse peritoneal macrophages in vitro (Skaug, Davies, and Glyseth 1984)

Sample	Description	Chemical formula	% SiO_2 added	Presence of fibers
A	US wollastonite	$CaSiO_3$	—	+
B	Natural wollastonite	$CaSiO_3$	2	+
C	Synthetic wollastonite	$CaSiO_3$	9	−
D	Synthetic tobermorite	$Ca_5Si_6O_{17} \cdot 2.5\ H_2O$	10	−
E	Synthetic tobermorite and xonotlite	$Ca_5Si_6O_{17} \cdot 2.5\ H_2O$ $Ca_6Si_6O_{17}(OH)_2$	2	+

Hectorite

In a study by Gormley and Addison (1983) mentioned earlier, the cytotoxic effects of Hectorite were investigated. The Hectorite sample had a particle size of 2.8 μm. The procedures are detailed in the study under the Attapulgite heading. Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) $\pm$ the standard deviation. The 20-μg/ml dose of Hectorite produced an 83.4% $\pm$ 10.9% viability and the 80 μg/ml dose produced a 56.4% $\pm$ 13.3% viability. Cellular LDH activities decreased with decreasing cell viability while the activity of LDH in the medium increased. Similar results were seen with glucosaminidase. Also, the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded.

Banin and Meiri (1990) reported that they added Hectorite to murine neuroblastoma cells at a concentration range of 70 to 1000 μg/ml, although details were not provided. They concluded that clear morphological signs of cell deterioration were evident and, at the concentrations listed, an acute toxic effect was seen.

Kaolin

Results from a study by M'anyai et al. (1969) on the hemolysis and methylene blue adsorption by Kaolin are presented in Table 10.

Kaolin was heated to temperatures of 290°C, 350°C, 500°C, 650°C, 800°C, and 950°C and changes in the internal structure and surface properties were investigated and compared to alterations in hemolytic activity in vitro. The measurement of methylene blue adsorption and investigation of the crystal structure by x-ray diffraction were made. In addition, Kaolin was added to human erythrocytes and the amount of lysed hemoglobin release was determined following an 1-h incubation. Complete dehydration of Kaolin resulted in the formation of metakaolinite between the temperatures 500°C to 650°C. The formation of metakaolinite resulted in complete loss of hemolytic activity. Heating to higher temperatures, 800°C and 950°C, resulted in the formation of γ-Al_2O_3 (mullite) or SiO_2 (cristobalite), which led to greater intensification of hemolytic activity. The extent of hemolysis depended on the crystal structure and hydration of the surface (M'anyai et al. 1970).

Oscarson et al. (1981) added Kaolin to a culture of bovine RBCs to study the extent of hemolysis. Saline was added to cultures as a control and in a separate experiment, the polymer poly-2-vinylpyridine-*N*-oxide was also added to study its inhibiting effects. No other details were given. The concentration of Kaolin that caused 50% hemolysis in 1 ml of a 3% solution of RBCs was determined as 0.6 mg Kaolin/ml of silicate-erythrocyte-buffer suspension. A concentration of 0.2 and 1.0 μM/ml of polymer caused 50% and 20% hemolysis, respectively. This was somewhat less hemolysis than without the polymer.

Mossman and Craighead (1982) adsorbed 3-Methylcholanthrene (3MC) onto heat-sterilized preparations of Kaolin (4, 8, and 16 mg dust/ml medium). The tracheas of female golden Syrian hamsters were excised, and prepared for organ cultures and exposed to 3MC/Kaolin preparations. After 4 weeks in vitro, the organ cultures were examined morphologically or implanted subcutaneously into syngeneic weanling female hamsters. The hamsters were palpated for tumors at 3-week intervals and any masses >5 mm in diameter were excised. Animals with no tumors were killed at 105 to 110 weeks of age and the tracheal implants were removed. The tracheal organ cultures and tumors were fixed for microscopic examination. Explants exposed to Kaolin had differentiated mucociliary epithelium for periods of several weeks. In vitro the columnar mucosal cells acquired a cuboidal configuration and the foci of the epithelial hyperplasia appeared at sites where microscopically evident accumulations of particles were deposited on the tracheal epithelium. No keratinizing squamous metaplasia was evident. Neoplasms developed in the tracheal implants exposed to 3MC-coated Kaolin. Tumor development was dosage dependent. No sarcomas developed only carcinomas. In the highest Kaolin/3MC-treated group, 28% of the animals developed tumors. Tumors failed to develop in tissues treated with Kaolin alone.

The comparative effects of Kaolinite (Kaolinite is the raw mineral that comprises Kaolin) on cellular and artificial membranes were examined using three test systems: tracheal epithelial cells, sheep erythrocytes (RBCs), and preparations of phospholipid-cholesterol vesicles in a study by Woodworth, Mossman, and Craighead (1982). Kaolinite doses of 0.003, 0.01, 0.03, and 0.1 mg/ml were added to tracheal epithelial cells for 24 h. Control cultures received no particulate. The ^{51}Cr release

was determined by liquid scintillation. Spontaneous release was determined from the control cultures. The second experiment, a hemolytic assay, combined RBC and Kaolinite doses of 0.1, 0.5, 1.0, 5.0, and 20.0 mg/ml were added at 37°C for 1 h. The optical density was determined at 540 nm. One milliliter of the preparation of liposomes (11.5 μg lipids) was added to 1 ml of a Kaolinite suspension. After 1 h, the optical density of the mixture was measured at 380 nm. The percentage of CrO_4^{2-} release was calculated. Control cultures received no particulate.

Kaolinite induced release of ^{51}Cr by tracheal epithelium was almost 50% at the highest dose. The cells phagocytized the particles, as demonstrated by SEM and phase-contrast microscopy. This process was most evident after 24 h. Cells containing intracellular particles demonstrated retraction of lamellopoidal extensions, surface blebbing, and a change in morphology from flattened to round.

A dose-dependent relationship between mineral concentration and hemolysis was demonstrated. Hemolysis was rapid. Approximately 50% of the RBCs were hemolyzed within 10 min. SEM revealed remnants of RBCs in cultures with complete hemolysis.

CrO_4^{2-} release at 10 mg/ml of Kaolinite was ~35% after 1 h. A dose-dependent relationship between particle concentration and CrO_4^{2-} release was again demonstrated (Woodworth, Mossman, and Craighead 1982).

In a study by Gormley and Addison (1983) described earlier, the cytotoxic effects of two Kaolins (K-1 and K-2) were investigated. K-1 had a particle size of 3.2 μm, and K-2 had a particle size of 3.9 μm. The procedures are detailed in the study Gormley and Addison (1983) under the Attapulgite heading.

Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) $\pm$ the standard deviation. The 20-μg/ml dose of Kaolin (K-1) resulted in a 101.4% $\pm$ 6.7% viability and the 80-μg/ml dose produced a 69.5% $\pm$ 6.5% viability. With a 20-μg/ml dose of Kaolin (K-2), viability was 93.6% $\pm$ 4.5%, with the 80 μg/ml dose, it was 60.0% $\pm$ 4.1%. It may be noted that K-1 has a finer particle size but a smaller surface area as compared to K-2. Cellular LDH activities decreased with decreasing cell viability, whereas the percentage of LDH in the medium increased. Similar results were seen with glucosaminidase. Also the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded (Gormely and Addison 1983).

The cytotoxicity of Kaolinite toward mouse peritoneal macrophages was examined in a study by Davies et al. (1984). This three-part study investigated whether or not respirable china clay (Kaolinite) was cytotoxic toward macrophages in vitro, the components responsible for the toxicity, and the factors responsible for the components toxicity. The assessment of toxicity was indicated by the activity of LDH assayed from the medium and cell lysates.

China clay dusts (60 μg/culture) from 12 separate drying plants were added to mouse peritoneal macrophage cultures and incubated for 18 h. The medium and cell lysates were collected and assayed for LDH activity. All 12 cultures had changes that indicated dust cytotoxicity. Between 19.5% and 60.0% LDH was released from the cultures. Four other dust samples, three of quartz (5,10,15, 20 μg/culture) and one of magnetite, were also assayed. The cytotoxicity of quartz indicated a dose-dependent relationship and was quite toxic. The magnetite dust had little effect on LDH release.

Mineral composition of the dusts was determined using x-ray diffraction analysis. A summary of the dust samples' composition was as follows: Kaolinite (84% to 96%), mica (3% to 6%), quartz (1%), and feldspar (0% to 7%). Due to the possibility of other dust cytotoxicity, the biological effects of the ancillary minerals and Kaolin was studied. Two high-purity Kaolins were tested in the same method as above and were clearly cytotoxic toward the macrophages. By x-ray diffraction, these two Kaolins were both 98% pure Kaolin. The feldspar sample had lower activity than titanium dioxide, a material considered nonfibrogenic and is used as a control dust in cell studies. The mica dust samples were cytotoxic but much lower than that of the Kaolin. By mineral analysis, it was found that mica dusts had 34% Kaolinite. Quartz was ruled out as the cytotoxic agent due to the very low concentrations (1%) in the initial experiment.

In a separate experiment, Kaolin pretreated with poly-2-vinyl pyridine-*N*-oxide (PVPNO) (0.45 μg/mg), was added to mouse peritoneal macrophages. (Note: PVPNO has been demonstrated to reduce the cytotoxicity of Kaolin [Davies and Preece 1983]). Electron micrographs were taken of the macrophages with and without the pretreated Kaolin for analysis of the factors causing the toxicity. The ultrastructural alterations and number of particles within the cells appeared to be similar in both the treated and nontreated cultures. It was concluded that PVPNO has no effect on the inhibition of the uptake of Kaolin. Dust particles were found adjacent to cell surfaces and in membrane-bound intracytoplasmic vesicles. However, no particles penetrated or were seen penetrating the nucleus and no lysed cells were seen.

In the last set of experiments, the physical structure of Kaolin and how it relates to dust toxicity was studied. Four components of Kaolin's structure were examined: gibbsite or mica-like surfaces, positively charged edges, negative charged particles, and an amorphous 'gel' coating on kaolinite. Transmission electron micrographs of gibbsite or mica-like surfaces indicated low toxicity and were ruled out as a possible marked toxic factor. A colloidal gold decoration technique was to study the positively charged edges of Kaolinite. Gold binds to the positively charged particles of Kaolinite and treatment of polyacrylic acid abolishes the gold decoration. In this study, mouse peritoneal macrophages were incubated with polyacrylic treated Kaolin (120 μg/culture). Only a small drop in the cytotoxicity of Kaolin was observed. The electrophoretic mobility of negatively charged Kaolin particles was also studied. Increased amounts of ammonium chloride produced a significant decrease in electrophoretic mobility. It is important to note that the greater concentrations did not produce negatively charged Kaolin particles. These same aluminum-treated Kaolins were added to mouse

peritoneal macrophages (120 μg/culture) and the cytotoxicity changed very little based on the amount of LDH activity released. The last experiment examined the effect of the amorphous 'gel' coating of Kaolin and its cytotoxicity. Plasma-ashing and the same LDH assay were performed on the samples. The first group, Kaolin (40 mg/cm^3), was plasma-ashed after 24 h and no effect was observed. Plasma-ashing after 72 h did reduce cytotoxicity. The second group of Kaolin dusts were mixed with formalin-fixed lung tissue and then immediately plasma-ashed. The cytotoxicity was not reduced. The last groups included Kaolin recovered from air-dried lungs of Fischer rats exposed to china clay dust (10 mg/m^3) for 40 h/week for 1 year, left for 1 year, then ashed to a constant weight. Inhalation of these dusts was significantly less toxic. Reductions in cytotoxicity was probably due to alterations in the surface coating of Kaolin (Davies et al. 1984).

Beck and Bignon (1985) dosed peritoneal macrophages with a sample of Kaolin and the TTC reduction, LDH activity, and methylene blue adsorption were used to assess cytotoxicity. The sample contained 30% SiO_2. The results from this study classified Kaolin as an inert dust and nontoxic. Methylene blue adsorption was slight.

Gormley, Kowolik, and Cullen (1985) used luminol-dependent chemiluminescence (CL) to assess the in vitro production of reactive oxygen species by human neutrophils and monocytes after exposure to Kaolinite. Either opsonized or nonopsonized Kaolinite dust was added to either neutrophil or monocyte suspensions and luminol. The suspensions were assayed for CL and measured in millivolt. Concentrations of dust ranged from the maximum of 3 mg/ml downwards. A control suspension of zymosan (2 mg/ml) was also assayed for CL production. Neutrophils challenged with opsonized dust had relatively low dose-dependent CL production compared to controls. However, when neutrophils challenged with nonopsonized dust, CL production peaked at 67%. Again dose-dependent responses were obtained when monocytes were tested. However, monocytes had a greater CL response in the presence of opsonized dust. These results were the reverse of the earlier neutrophil responses as a very low monocyte CL production was obtained with nonopsonized dust.

In a study by Wallace et al. (1985), the cytotoxicity of native and surface-modified Kaolin and the effect of pulmonary surfactant were studied. Cell membrane damage and cytotoxicity were measured by the release of alveolar macrophage cytoplasmic enzyme LDH, the lysosomal enzymes β-n-acetylglucosaminidase (β-NAG) and β-GLUC, and sheep blood cell hemolysis. Dipalmitoyl lecithin (DPL) emulsions made from synthetic L-α-lecithin β,γ-dipalmitoyl were added to Kaolin to produce a concentration of 7.5 mg dust/ml. Controls of saline and Kaolin without DPL were also utilized. For the hemolysis assays, the mixtures were resuspended in phosphate-buffered saline (PBS) at a concentration of 2.0 mg dust/ml PBS.

Fresh sheep blood erythrocytes were mixed with dust suspensions in concentrations of 0.1 to 1.0 mg/ml. Untreated Kaolin and DPL-treated Kaolin erythrocytes were incubated for 1 h at 37°C. Negative controls were made with erythrocytes in PBS and positive controls were made by lysing erythrocytes. All samples were read at 540 nm using a spectrophotometer and the percentage of lysis was calculated. The lecithin treated Kaolin suppressed erythrocyte activity to near "background levels." The hemolysis value for the maximum nontreated Kaolin concentration (1 mg/ml) was 42%, whereas the hemolysis value for the lecithin-treated Kaolin at the same concentration was 2%. Adsorption isotherm data estimated that 0.1 mg Lecithin/mg Kaolin would provide full surface coverage and suppress the hemolytic capacity to 97% lower than the native Kaolin.

In the second experiment of the same study, alveolar macrophage enzyme release studies were carried out using macrophages from Sprague-Dawley rats. Untreated Kaolin and DPL-Kaolin samples at a concentration of 1 mg/ml were mixed with macrophages and incubated for 2 h at 37°C. The results were similar as in the above experiment. The nontreated Kaolin caused release of enzymes: 570% LDH, 600% β-GLUC, and 570% β-NAG of the control values. The treated Kaolin did not cause the release of these enzymes. These results imply that Kaolin damages erythrocytes and macrophages through cell membrane–dust surface interactions and that pulmonary surfactants can absorb the mineral surfaces for a short time (Wallace et al. 1985).

Mossman and Be'gin (1989) conducted a study in which Kaolin samples were coated with the enzymes L-alpha-dipalmitoyl glycerophosphorylcholine (DGPL) and phospholipase A_2 (PLA_2) and the hemolytic potential of both coated and noncoated samples were studied in vitro. The samples were incubated with sheep erythrocytes and the optical density of the supernatant at 540 nm was determined to measure hemoglobin release. With increasing amounts of DGPL, neutralization of the hemolytic potential occurred at 75 to 85 mg DGPL/g of Kaolin. The residual adsorbed value was 83.0 mg DGPL/g Kaolin. The digestive removal of DGPL by Kaolin was measured at the applied specific activity of 0.96 units PLA_2 per molecule DGPL on Kaolin. Most of the produced lysolecithin remains adsorbed at 2 h.

Banin and Meiri (1990) added Kaolinite to murine neuroblastoma cells at concentrations of 100 to 1000 μg/ml. Within minutes, the Kaolinite increased the increasing permeability of the membranes, depolarized resting potential, and the maintaining action potentials in response to stimulation were lost. Within 30 min, the cells had alterations of morphological deterioration. Microvilli retracted, the surface assumed an unruffled, smooth appearance, and large holes developed in the plasma membrane.

Murphy, Roberts, and Horrocks (1993a) investigated the cytotoxicity of Kaolinite using three cell lines: HUVE cells, undifferentiated N1E-115 neuroblastoma cells, and ROC-1 oligodendrogial cells. Indices of cytotoxicity used in this study were morphological examination, LDH activity, and fatty acid release. Exact experimental details are provided in the Bentonite section under the same heading.

Kaolinite did not lyse ROC-1 oligodendroglia and the neuroblastoma cells and did not cause a dose-dependent increase in fatty acids at 24 h. No significant increases in LDH activity were detected utilizing either of these cell lines. However, Kaolinite increased fatty acid concentrations after 24 h of incubation in a dose-dependent fashion. A 1.7-fold increase in fatty acid concentrations over control values was calculated. Increases over control activities of LDH were 146% with Kaolinite. Within 1 h, Kaolinite associated with the plasma membrane of HUVE cells and the morphology was drastically changed after treatment (no details given). Cell lysis was also apparent. After trypan blue staining, 90% of HUVE cells were nonviable with Kaolinite treatment (Murphy, Roberts, and Horrocks 1993a).

Kaolinite dust was tested for potential human leukocyte elastase (HLE)-inhibiting effects (Oberson et al. 1996). HLE inhibition was evaluated by incubating 15 nM HLE for 1 h in the presence of 5 μg of Kaolinite. Suc$(Ala)_3$pNA was then added for 30 min. Activity was measured at 410 nM. The 5 μg Kaolinite abolished (90% inhibition) the activity of 0.45 μg HLE.

Montmorillonite

Results from a study by M'anyai et al. (1969) on the hemolysis and methylene blue adsorption by Montmorillonite are presented in Table 10.

Oscarson, Van Scoyoc, and Ahlrichs (1981) added Montmorillonite to a culture of bovine RBCs to study the extent of hemolysis. Saline was added to cultures as a control and in a separate experiment, the polymer, poly-2-vinylpyridine-*N*-oxide, was also added to study its inhibiting effects. No other details were given. The concentration of Montmorillonite that caused 50% hemolysis in 1 ml of a 3% solution of RBCs was determined as 0.006 mg Montmorillonite/ml of silicate-erythrocyte-buffer suspension. A concentration of 0.2 and 1.0 μM/ml of polymer reduced hemolysis to 23% and 0%, respectively.

The comparative effects of Montmorillonite on cellular and artificial membranes were examined using three test systems—tracheal epithelial cells, sheep erythrocytes (RBCs), and preparations of phospholipid-cholesterol vesicles—in a study by Woodworth, Mossman, and Craighead (1982). Montmorillonite doses of 0.003, 0.01, 0.03, and 0.1 mg/ml were added to tracheal epithelial cells for 24 h. Control cultures received no particulate. The ^{51}Cr release was determined by liquid scintillation. Spontaneous release was determined from the control cultures. A second experiment, a hemolytic assay, combined RBC and Montmorillonite doses of 0.1, 0.5, 1.0, 5.0, and 20.0 mg/ml at 37°C for 1 h. The optical density was determined at 540 nm. Control cultures received no particulate. One milliliter of the preparation of liposomes (11.5 μg lipids) was added to 1 ml of a Montmorillonite suspension. After 1 h, the optical density of the mixture was measured at 380 nm. The percentage of CrO_4^{2-} release was calculated. Control cultures received no particulate.

Montmorillonite induced release of ^{51}Cr by tracheal epithelium was almost 60% at the highest dose. The cells phagocytized the particles, as demonstrated by SEM and phase-contrast microscopy. This process was most evident at after 24 h. Cells containing intracellular particles demonstrated retraction of lamellopoidal extensions, surface blebbing, and a changed morphology from flattened to round.

A dose-dependent relationship between mineral concentration and hemolysis was demonstrated. Hemolysis was rapid. Approximately 50% of the RBCs were hemolyzed within 10 min. SEM revealed remnants of RBCs in cultures exhibiting complete hemolysis.

CrO_4^{2-} release at 10 mg/ml of Montmorillonite was ~40% after 1 h. A dose-dependent relationship between particle concentration and CrO_4^{2-} release was again demonstrated (Woodworth, Mossman, and Craighead 1982).

In the Gormley and Addison study (1983) described earlier, the cytotoxic effects of three samples of Montmorillonite (CaM-1, CaM-2, and NaM) were investigated. CaM-1 and -2 have calcium substitutions in their lattices whereas NaM has sodium substitutions. Particle sizes ranged from 2.0 to 3.1 μm. The procedures are detailed under the Attapulgite heading. Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) ± the standard deviation. The 20-μg/ml dose of CaM-1 with particle size of 3.1 μm produced a 79.1% ± 19.2% viability and the 80-μg/ml dose produced a 51.9% ± 15.6% viability; CaM-2 with a particle size of 2.5 μm produced viabilities of 21.2% ± 3.5% (20 μg/ml) and 13.1% ± 2.2% (80 μg/ml); and NaM with a particle size of 2.0 μm produced viabilites of 47.3% ± 7.4% (20 μg/ml) and 37.2% ± 4.6% (80 μg/ml). The sample CaM-1 had the largest surface area, whereas sample NaM, had the smallest. Sample CaM-2 had the lowest viability percentage despite the median particle size and surface area. Investigators attributed the marked toxicity of sample CaM-2 due to the presence of ~1% of quartz and 10% cristobalite in the sample. Sample NaM, which also exhibited a greater toxicity, contained ~5% quartz and ~2% calcite. Cellular LDH levels fell with decreasing cell viability whereas the percentage of LDH in the medium increased. Similar results were seen with glucosaminidase. Also, the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded.

Gormley, Kowolik, and Cullen (1985) used luminol-dependent CL to assess the in vitro production of reactive oxygen species by human neutrophils and monocytes on exposure to Montmorillonite. Either opsonized or nonopsonized Montmorillonite (containing a calcium as its exchange ion) dust was added to either neutrophil or monocyte suspensions and luminol. The suspensions were assayed for CL and measured in millivolt. Concentrations of dust ranged from the maximum of 3 mg/ml downwards. A control suspension of zymosan (2 mg/ml) was also assayed for CL production. Neutrophils challenged with opsonized dust resulted in relatively low dose-dependent CL production compared to controls. However, when neutrophils were challenged with nonopsonized dust, a marked response of CL peak production at 114% was elicited. Again dose-dependent responses were obtained when monocytes were tested. However,

monocytes elicited a slightly higher response in the presence of opsonized dust. These results proved to be the reversal of the earlier neutrophil responses. A very low monocyte CL production was obtained with nonopsonized dust.

Banin and Meiri (1990) reported a study in which Montmorillonite was added to murine neuroblastoma cells at a concentration range of 100 to 1000 μg/ml, but no details were given. The authors concluded that clear morphological signs of cell deterioration were evident and, at the concentrations listed, an acute toxic effect was seen.

Murphy, Roberts, and Horrocks (1993a) investigated the cytotoxicity of Montmorillonite to three cell lines: HUVE cells, undifferentiated N1E-115 neuroblastoma cells, and ROC-1 oligodendrogial cells. Indices of cytotoxicity used in this study were morphological examination, LDH activity, and fatty acid release. Exact experimental details are provided in the Bentonite section under the same heading.

Montmorillonite did not lyse ROC-1 oligodendroglia and the neuroblastoma cells and did not cause a dose-dependent increase in fatty acids at 24 h. No significant increases in LDH activity were detected utilizing either of these cell lines. However, Montmorillonite caused a dose-dependent increase in fatty acid levels only after 24 h of incubation. A 10-fold increase in FA levels over control values was calculated. Increases over control activities of LDH were 154%. Within 1 h, Montmorillonite associated with the plasma membrane of HUVE cells and the morphology was drastically changed after treatment (no details given). Cell lysis was also apparent with treatment. After trypan blue staining, 99% of HUVE cells were nonviable with Montmorillonite treatment (Murphy, Roberts, and Horrocks 1993a).

In a study by Murphy et al. (1993b), the cytotoxicity of Montmorillonite was examined in two cell lines: primary murine spinal cord neurons and differentiated N1E-115 neuroblastoma cells. A clay suspension with a concentration of 0.1 mg/ml was added to the cultures. The neuronal cells were incubated for 1 h with Montmorillonite. Photomicrographs were taken at 5, 15, and 60 min following treatment. For the N1E-115 cells, incubation lasted 18 h and photomicrographs were taken at 5 and 15 min and 3, 6, and 18 h after the treatment. Morphological changes were observed using a phase-contrast microscope. Within 5 min, clay particles were observed on the neuronal cell bodies. Cell bodies appeared granular within 15 min. The cells were completely lysed after 60 min and there was no evidence of any remaining cell bodies or processes. Cell membrane contact was apparent after 5 min in N1E-115 cultures. No morphological changes were apparent at this point. At 18 h, the cells were covered with clay but cellular processes remained intact. N1E-115 cell lysis did not occur and no cytotoxicity was recorded.

Montmorillonite dust was tested for potential HLE-inhibiting effects (Oberson et al. 1996). HLE inhibition was evaluated by incubating 15 nM HLE for 1 h in the presence of 5 μg of Montmorillonite. Suc(Ala)$_3$pNA was then added for 30 min. Activity was measured at 410 nM. The 5 μg Montmorillonite (98% inhibition) abolished the activity of 0.45 μg HLE.

Pyrophyllite

The cytotoxicity of Pyrophyllite dust on rat alveolar macrophages was investigated in a study by Zhang, Zhang, and Song (1997). Cytotoxicity was measured by the potassium content of the macrophages and the levels of LDH. Alveolar macrophages were isolated from bronchi alveolar lavages of male Wistar rats. These animals were divided into six groups based on the dust concentrations. The groups were as follows: quartz (75.72 μg/ml) dust group; Pyrophyllite mine (PM) dust group A, 200 μg/ml (75.72 μg/ml SiO_2 and 30.42 μg/ml Al_2O_3); PM dust group B, 200 μg/ml (75.72 μg/ml SiO_2 and 30.42 μg/ml Al_2O_3); Pyrophyllite carving mills (PCM) dust group A, 200 μg/ml (31.68 μg/ml SiO_2 and 40.58 μg/ml Al_2O_3); PCM dust group B, 200 μg/ml (31.68 μg/ml SiO_2 and 40.58 μg/ml Al_2O_3); normal control of saline. Both PM group B and PCM group B were imitated groups of the natural dusts from the mines used to study the toxicity of SiO_2 and Al_2O_3. They did not include the metals Fe, Cu, Ni, and Zn as did both samples A. The cell cultures were incubated at 37°C for 16 and 22 h.

The LDH activity of quartz was greater than all other groups except PM group A incubated at 22 h. When compared to the saline controls, all exposed groups had significantly lower increases in LDH activity. Both the LDH activities of the PM dust groups were greater than those of the PCM dust groups ($p < .5$). However, no differences between the PM groups A and B or between the PCM groups A and B were detected. The K^+ content of the saline controls was greater than all exposed groups. The quartz group had the lowest concentrations of K^+ followed by the PM dust groups and then the PCM dust groups. Again, no differences between either A or B groups was observed. It was concluded that Pyrophyllite dust exposure is cytotoxic to alveolar macrophages and people working in a PM have greater risk of respiratory problems than people working on PCMs.

Mineralogical analysis of the dust samples taken from the mines was performed using an atomic absorption spectrophotometer. The SiO_2 content was 37.9% higher in the PM group than in the PCM group 15.8%. Al_2O_3 concentrations were lower in the PM dust groups (15.2%) than in the PCM dust groups (20.3%). Toxicity due to metals in the samples A was ruled out. The samples B did not include the metals and had similar LDH activity as the samples A (Zhang, Zhang, and Song 1997).

Zeolite (Zeolite A)

Zeolite A at concentrations of 0.1 to 100 μg/ml was incubated for 48 h with normal human osteoblast-like cells. An induction of a dose-dependent increase in DNA synthesis and the proportion of cells in mitosis occurred. This mitogenic action was dependent on cell seeding density. Alkaline phosphatase activity and osteocalcin release were also increased but no significant effect on collagen production per cell occurred. Zeolite treatment increased the steady-state mRNA levels of transforming growth factor β (Keeting et al. 1992).

Zeolite (Clinoptilolite)

Total degradation of rat peritoneal macrophages incubated with Clinoptilolite dust particles occurred during 15- and 30-min time periods at concentrations of 1.0 and 0.5 mg/ml, respectively. Dust particles measured <5 μm. Thirty-eight percent of macrophages and 57.5% of RBCs were killed within 30 min at a Zeolite concentration of 0.25 mg/ml. Dose-dependent CL was observed in the first 10 to 20 s when luminol was added to the cultures. Catalase (30% to 50%) decreased the cytotoxic effects of Zeolite, whereas ethanol, sodium azide, and mannitol had no effect (Korkina et al. 1984).

Zeolite (Mordenite)

Syrian hamster and rat alveolar macrophages were exposed to nontoxic concentrations of Mordenite and the reduction of cytochrome *c* in the presence and absence of superoxide dismutase, and the amount of O_2 released were indicators of cytotoxicity. Other fibrous particles were used as positive controls. Mordenite as compared to the positive controls was less active at comparable concentrations (Hansen and Mossman 1987).

Zeolite (Nonfibrous Japanese Zeolite)

Japanese Nonfibrous Zeolite was incubated with two cell lines, Chinese hamster V79-4 and A579 at concentrations ranging from 5 to 100 μg/ml. Two samples of erionite and a sample of UICC crocidolite, a positive control, were also tested. Concentrations that inhibited plating were estimated using the LD_{50}. Compared to the positive control and the erionite samples, the Zeolite had a much greater LD_{50} value and was nontoxic in the A549 assay (Brown et al. 1980).

ANIMAL TOXICOLOGY

Acute Oral

Calcium Silicate

Calcium Silicate FDA compound 71-41 was suspended in 0.85% saline and administered to 10 male rats by intubation. Each animal that received a dose of 5000 mg/kg died within 24 h. Doses of 100, 500, 1000, 2000, 3000, and 4000 mg/kg were selected to determine the acute LD_{50} using the Litchfield-Wilcoxson method. Groups of 5 male rats were administered the doses and were killed for necropsy. The LD_{50} was determined as 3400 mg/kg; at the highest dose, necropsy findings included bloody gastric mucosa with distension, hydrothorax, and congested lungs. In a second LD_{50} assessment, Calcium Silicate was prepared as 24.1% (w/v) suspension and administered orally to a group of 10 male rats at a single dose of 5000 mg/kg. No signs of toxicity or abnormal behavior were observed within a 7-day period. No deaths occurred. All animals were killed and on necropsy no gross findings were observed. The acute oral LD_{50} was considered to be greater than 5000 mg/kg (Litton Bionetics, Inc. 1974).

Hectorite

Five male and five female Sprague-Dawley rats were administered a single dose of 5 g/kg of the test article by gavage. The animals were observed the day of dosing and 15 days after for gross and visible toxic or pharmacological effect. No such effects were seen and none of the animals died. All animals were killed for necropsy. No findings were reported. The acute oral LD_{50} was >5.0 g/kg of body weight (FDRL Inc. 1980b).

Kaolin

A report by the Federation of American Societies for Experimental Biology (1977) included an acute oral study in which 120 rats were fed doses of Kaolin ranging from 100 to 210 g/kg. Fourteen rats were controls. Kaolin was inert and nonstatic except for the danger of bowel obstruction resulting in perforation. The clinical signs were listlessness, anorexia, oliguria, hypothermia, and dyspnea. These were a pathological reaction from overdistension 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.

McClurg, Beck, and Powers (1980) fed a group of 10 male Sprague-Dawley rats a control diet plus 0.5 ml Kaolin 20%–pectin 1%. The control diet was then fed for 48 h and 72 h later stool samples were collected. The samples were analyzed for volume, sodium, potassium, and fat content. The results were 103% increase in sodium; 184% increase in potassium; fat excretion remained at baseline.

Magnesium Aluminum Silicate

Suspensions of 1 ml of Magnesium Aluminum Silicate at doses of 100–2000, 5000,10000, 20000, and 50000 mg/kg were administered to a series of 37 mice. At the greatest dose, the mortality rate was 33%. The LD_{50} was considered to be $>50{,}000$ mg/kg (Munch 1944).

Zirconium Silicate

In a study conducted by Stookey et al. (1967), the LD_{50} of Zirconium Silicate was determined. Oral intubations of a 60% aqueous slurry of Zirconium Silicate containing 1% carboxymethylcellulose to prevent settling was given to 80 albino mice. Doses ranged from 70 to 200 gm/kg body weight. A dosage of 200 g of Zirconium Silicate per kilogram body weight was not sufficient to create a 50% mortality rate in mice. Dosages greater than 200 g were not tested due to the limitations of the mouse gastrointestinal tract. A 37.5% mortality rate was recorded for the dosage of 200 g/kg of body weight.

Short-Term Oral

Bentonite

Carson and Smith (1982) fed Bentonite at concentrations 0%, 2.5%, 7.5%, or 10% to male weanling rats to determine the most effective level to overcome the effects of T-2 toxicosis.

Increasing the concentration of Bentonite resulted in significant increases in body weight and feed consumption. The most effective concentration tested was 10%. Bentonite had no effect on the activity of nonspecific hepatic esterase.

The role of Bentonite in the prevention of T-2 toxicosis in rats was further investigated by Carson and Smith (1983). Groups of 10 male Wistar rats were fed diets containing 5% Bentonite for 2 weeks and the feed consumption and growth were recorded. Each diet was administered with or without 3 μg T-2 toxin/g of feed for 2 weeks. Bentonite reduced the decreases in final body weight and feed consumption as compared to controls. The livers from this test group were excised and assayed for nonspecific esterase (E.C.3.1.1.1). Five percent Bentonite had no significant effect on the activity of this enzyme. In a second experiment, Bentonite was supplemented in the control diet at 2.5%, 5.0%, 7.5%, and 10%. Bentonite at 2.5% greatly increased feed consumption and final body weights and feeding. Ten percent Bentonite overcame the toxicosis completely. In a third study, rats were fed 0%, 5%, 7.5%, or 10% Bentonite for 2 weeks and then dosed with [^{3}H] T-2 toxin. The urine and feces were collected at 21 h and tissues were excised for determination of residual ^{3}H. Feeding Bentonite had little effect on the fraction of the dose excreted in the urine. Feeding 5%, 7.5%, and 10% Bentonite resulted in significant increases in the fecal excretion of ^{3}H when compared to controls. Bentonite had no effect on residual ^{3}H in the liver or kidneys but all concentrations reduced residual ^{3}H in muscle. Rats fed 5% Bentonite had more ^{3}H in the digesta in the small intestine and in the wall of the intestinal tissue when compared to controls. Intestinal transit time was reduced as well.

Bartko et al. (1983) fed a group of five sheep a diet containing 0.15 g/kg body weight of Zeolite for 3 months. Other sheep received no additions to their normal diet. At the end of the study, no difference in health effects was found between the two groups. The health effects included general behavior, total and acute acidity, content of volatile fatty acids in rumen contents, hematological values, content of microelements, transaminase activity, and acid-base homeostasis in the blood.

Magnesium Aluminum Silicate

Munch (1945) gave groups of 10 mice daily doses of either 5 or 10 g/kg of body weight orally for 10 days. Two days separated the first five doses from the second five doses. No signs were observed in any mouse at any time when administered 5 g/kg. The animals were killed and no pathological changes were seen at necropsy. No tissue was taken for further examination. One mouse died after five doses of 10 g/kg and one mouse died after nine doses of 10 g/kg. Neither mouse had lesions at postmortem examination.

This same author administered VEEGUM orally to 10 rabbits for a total of 10 doses. The first four animals were given 5 g/kg of body weight; the fifth animal was a control. The second four animals were given 10 g/kg of body weight; the fifth was also a control. No changes in body weight, no signs at toxicity, and no deaths were recorded. All animals were killed and at necropsy no lesions were seen in the stomach, liver, kidneys, or other viscera. No tissue was taken for microscopic examination (Munch 1945).

Zeolite (Clinoptilolite)

In a 148-day feed-lot experiment reported by McCollum and Galyean (1983), 48 cross-bred steers were fed a 70% sorghum diet with Clinoptilolite substituted at 0%, 1.25%, and 2.5% of the diet dry matter. No differences were found among treatments in average daily weight gain, feed intake or feed efficiency.

Pond, Yen, and Crouse (1989) fed 32 castrated male pigs various diets of calcium, iron, and Clinoptilolite to study tissue storage of major and trace elements with the addition of Clinoptilolite. At day 84, all pigs were killed and analyzed. Dietary concentrations of calcium, iron, and Clinoptilolite had no effect on daily weight gain, daily feed intake, or the ratio of weight gain:feed intake of growing pigs.

Zeolite (Clinoptilolite and Sodium Zeolite A)

Weanling Landrace $\times$ Yorkshire pigs were fed diets containing 3% Clinoptilolite with or without 150 ppm cadmium chloride or 3% Sodium Zeolite A with or without 150 ppm cadmium chloride for 31 days. Pigs fed cadmium and Zeolites did not have decreased hematocrit and hemoglobin values similar to those of pigs fed diets without the Zeolites. Hepatic cadmium concentration was significantly reduced in animals fed with Clinoptilolite. Hepatic iron was not affected significantly by either Zeolite; hepatic iron and zinc were decreased by dietary cadmium. Hepatic zinc was increased by Sodium Zeolite A (Pond and Yen 1983b).

Zeolite A

Various diets containing no Zeolite, 0.3% Zeolite A, or 0.5% Clinoptilolite were fed to cross-bred pigs for 6 weeks. The average daily weight gain, average daily feed intake, and feed:weight gain ratio were unaffected by supplementation of either Zeolite. Energy utilization was improved by feeding diets containing either Zeolite (Shurson et al. 1984).

Subchronic Oral

Magnesium Aluminum Silicate

The Food and Drug Research Laboratories (FDRL 1958a) carried out a 90-day feeding study using 220 weanling albino rats divided into five groups. The largest dose group consisted of 10 male and 10 female rats; control animals totaled 25 rats of each sex. A commercial ration was supplemented with 2%, 5%, 10%, and 20% VEEGUM. Control diets were unmodified. Body weight and feed intake were recorded daily and the efficiency of feed utilization (EFU; gram gained per 100 g) was calculated. Hematological examinations were made at 6 and 12 weeks on half of the test group. Blood sugar and nonprotein nitrogen determinations and urine analyses were also completed. Four rats in the 20% group, four rats in the 10% group, and control group

were placed on a modified program to estimate the balance between the intake of dietary ash and the ash excreted. Rats fed the 20% diet were examined at 8 weeks and rats fed the 10% diet at 12 weeks. All animals were killed at the end of the 90-day period. Liver, kidneys, spleen, heart, and adrenal glands weights were determined. Microscopic examination of the liver, kidneys, spleen, and portions of the gastrointestinal tract of four rats of each sex and control, 10%, and 20% groups were carried out.

The average body weights and net gains were not adversely affected by the ingestion of VEEGUM up to 10% in the diet. Growth was diminished slightly but with statistical significance ($p = .05$) when 20% VEEGUM was fed to both sexes. With EFU corrections, only the 20% dose significantly lowered the observed EFU value. One male rat of the 2% group died and one of each sex of the 10% group died. These rats had fibrinous exudates in the thorax, hemorrhagic lungs, and evidence of respiratory infection at necropsy. Gross findings for the rest of the animals revealed no significant abnormalities other than in the lungs. The incidences of pulmonary lesions did not differ among controls and test animals. Organ weights fell within normal limits. Hematological observations were within normal limits, including the rats of the 20% group. Blood sugar and nonprotein nitrogen values were also within normal limits. Females of the 20% group had slightly increased values compared to controls but still were in the normal range. Silicon content of the spleens of control animals were about the same as in the 2% group. However, in the 5% and 10% groups, the silicon content was slightly increased. Microscopic examination disclosed no abnormalities in the liver, kidneys, and gastrointestinal tract. Ash data indicated that 81% of VEEGUM of the 20% group was excreted and 73% of the 10% group was excreted (FDRL 1958a).

FDRL (1958b) fed two groups of four mongrel dogs, two female and two male for each group, a basal diet and a diet supplemented with 10% VEEGUM for 90 days. At 6 and 12 weeks, complete blood counts were made and blood sugar and nonprotein nitrogen were determined. Urine specimens were examined at 12 weeks for acidity, sugar, albumin, and microscopic elements in the sediment. At the end of 90 days, all dogs were killed for necropsy. Silicon content of the spleen was also determined. Body weight did not change despite a depression of appetite with the addition of VEEGUM. No abnormalities were seen upon hematological examination at the 6- or 12-week periods. Two of the test animals had slightly increased blood sugar at the end of the testing period. All other values for sugar and nonprotein nitrogen levels were normal. No difference in organ weight was seen. Silicon concentration of the spleens of the test animals were slightly elevated compared to controls (143 versus 103 mg/spleen). No microscopic lesions were compound induced.

CTFA (1999b) reported that in feeding tests with dogs and rats ingesting large amounts of VEEGUM (10% of ration) for 90 days, all responses were negative and VEEGUM was considered nontoxic.

Magnesium Trisilicate

Page, Heffner, and Frey (1941) gave six white rats daily doses of 0.6 g of Magnesium Trisilicate for 6 months. A litter was born and divided into two groups, a control and a treated group. The treated group received Magnesium Trisilicate doses from the time of weaning that corresponded to a daily dose of 3 or 4 pounds for a healthy human. This litter was also mated. Tissues from the animals of the first and second generation were examined microscopically. No evidence of tissue changes were recorded.

Dobbie and Smith (1982) gave six male guinea pigs a suspension in tap water of 250 mg/L Magnesium Trisilicate over a 4-month period for 5 days each week. Atomic absorption spectroscopy established that the soluble Si in the suspension was 267 μmol/L. Normal tap water was given to six control animals 7 days a week and 2 days a week to the test guinea pigs. At 4 months, all animals were killed for necropsy. The kidneys were processed for microscopic examination. All six animals had renal lesions that involved the distal nephron. Lesions of the distal tubule were dilation or cystic change. Some tubules were plugged with proteinaceous material. The interstitium of the kidneys was expanded by chronic inflammatory cells and excess collagen fibers. No lesions were seen in control animals.

Chronic Oral

Zeolite (Synthetic Zeolite A)

Groups of 50 male and female Wistar rats were fed 1, 10, 100, or 1000 mg/kg of Synthetic Zeolite A in their diets for up to 104 weeks. Clinical signs, mortality, and gross and microscopic lesions were recorded. No differences in body weight gain or clinical parameters were observed between control and treated animals. Based on feed intake, the Zeolite intake of the 10-, 100-, and 1000-mg/kg groups was 0.62, 6.1, and 58.5 mg/kg body weight/day for males and 0.65, 6.53, and 62.2 mg/kg body weight/day for females, respectively. No significant treatment-related lesions were observed in any of the organs examined and there was no effect on the types or incidence of any neoplastic changes seen (Gloxhuber et al. 1983).

Acute Parenteral

Aluminum Silicate

Musk et al. (1988) exposed Syrian golden hamsters to saline suspensions of Aluminum Silicate at 3.75 and 0.75 mg/100 g body weight by intratracheal instillation and sacrificed the animals at day 1. Their lungs were lavaged and the lavage fluid was characterized using cellular and biochemical indicators (lactic dehydrogenase, albumin, macrophages, polymorphs, and RBCs) of pulmonary damage. Either dose did not alter the biological parameters tested in comparison to those animals only exposed to saline.

Lemaire et al. (1989) gave Fiberfrax, an aluminum silicate, by intratracheal instillation at doses of 1, 5, and 10 mg to groups of

five rats. The details of this experiment are explained by Lemaire et al. (1989) under the Attapulgite heading in this section. The average length of Fiberfrax fibers were 8.3 μm and <50% were under 5 μm. The significant inflammatory response was mainly numerous lymphocytes and epithelioid giant cells. The lesions were located predominantly around the terminal bronchioles. Areas of early fibrosis were seen in the lesions. Every test animal developed type C lesions, described above. A dose-dependent reaction was suggested due to more extensive lesions seen in animals dosed with 10 mg. The bronchoalveolar lavage fluid had macrophages as the predominant cells followed by neutrophils and then by lymphocytes.

Pigott and Ishmael (1992) studied the effects of intrapleural injections of Aluminum Silicate in rats. A single intrapleural injection of 20 mg of four Aluminum Silicate samples (Saffil, aged Saffil, aluminosilicates A and B) and chrysotile A asbestos was administered to dose and control groups consisting of 24 rats of each sex. The control group received only a saline injection. The predominant length of the fibers in each sample were Saffil, 10 to 20 μm; aged Saffil, 20 to 40 μm; aluminosilicate A, 20 to 40 μm; and aluminosilicate B, 0 to 10 μm. Each rat was allowed to live out its lifespan or until it appeared distressed until 85% mortality was reached. All animals, were then killed and organs were taken for microscopic examination. Reactions to both forms of Saffil were very similar. In almost all animals, a minimal focal chronic pleurisy/fibrosis was minimal with adhesion formation. Pericardial adhesions and mesothelial proliferation with some Saffil fibers were seen. The reactions to both aluminosilicate samples were very similar. Minimal to moderate focal chronic pleurisy/fibrosis was often associated with mesothelial proliferation. Aluminosilicate B caused three malignant mesotheliomas, one pleural and two peritoneal. A benign testicular mesothelioma was seen in one rat dosed with Saffil, two dosed with aged Saffil, and four dosed with aluminosilicate A. Incidences of tumors are presented in Table 14.

Attapulgite

Pott et al. (1987) injected three samples of 25 mg of Attapulgite dust intraperitoneally into 40 Wistar rats. 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%.

Stanton et al. (1981) reported that 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 one of two Attapulgite samples. 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 years. 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 UICC crocidolite, 14/29 developed pleural mesotheliomas.

Be'gin et al. (1987) delivered Attapulgite with a mean fiber length of 0.8 μm and diameter of 0.02 μm to the lungs of sheep by bronchioscopic cannulation. The tracheal lobe of 16 sheep was subjected to a single exposure of 100 mg of Attapulgite in 100 ml of saline. A bronchoalveolar lavage (BAL) was conducted at 2, 12, 24, 40, and 60 days, and necropsy was conducted on day 60. Total BAL cells, macrophages, and neutrophils, fibronectin content, and LDH and 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.

Jaurand et al. (1987) injected samples (20 mg/ml of 0.9% NaCl) of Attapulgite fibers with the median length of 0.77 μm into the pleural cavities of 36 2-month-old Sprague-Dawley rats. 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.

Wagner, Griffiths, and Munday (1987) injected 20 male and 20 female, SPF Fischer rats intrapleurally with single injections of Attapulgite. Three samples of Attapulgite named after the location of their discovery (Lebrija, Torrejon, and Leichester) were utilized in this study. No concentrations were provided.

TABLE 14

Tumors in rats treated with intrapleural injections of four Aluminum Silicate samples (Pigott and Ishmael 1992)

Tumor	Control	Chry. Asbestos	Saffil	Saffil aged	Alumosil. A	Alumosil. B
Total no. of animals	62	81	71	68	57	67
No. of benign	44	55	57	56	46	49
No. of malignant	17	26	16	14	10	19
Malignant mesothelioma	0	7	0	0	0	3

TABLE 15
Toxic reactions to intrapleural injections of Attapulgite (Wagner, Griffiths, and Munday 1987)

Dust	Mesothelioma	Nonmesothelioma
Lebrija Attapulgite	2	38
Torrejon Attapulgite	14	26
Leichester Attapulgite	30	2
Crocidolite	34	6
Kaolin	0	40
Saline	1	39

However, fiber length information was provided. Lebija Attapulgite had fiber lengths of ≤ 2 μm. Torrejon Attapulgite contained at the most 0.54% of fibers ≥ 6 μm. Leichester Attapulgite contained about 19% of fibers ≥ 6 μm. 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. Two controls were used in this experiment; Kaolin and saline. One positive-control crocidolite was also used. The results from this experiment are summarized in Table 15.

Lebrija Attapulgite dust extracted from the lung had fibers ≤ 2 μm. Material examined from Torrejon Attapulgite was fibrous and have fiber length up to 8 μm. Leichester Attapulgite fibers from extracted lungs were up to 25 μm. The investigators considered these fibers to be tumorigenic. Kaolin was a nonfibrous dust and crocidolite was fibrous. The authors concluded that exposure to Torrejon, and Leichester Attapulgite should be avoided (Wagner, Griffiths, and Munday 1987).

Lemaire et al. (1989) reported a study in which groups of five rats received single intratracheal instillations of Attapulgite at 1, 5, and 10 mg. One month after treatment, BAL and microscopic examination of the lungs were performed. The average length of the fibers were 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.

In a study by Renier et al. (1989), intrapleural injections of 20 mg of different Attapulgite fiber samples in 1 ml of saline were given to 2-month-old Sprague-Dawley rats. 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. Attapulgite in the present experiment was not carcinogenic (Renier et al. 1989).

Lemaire (1991) reported a study in which groups of five animals received doses of 1, 5, or 10 mg of Attapulgite by transtracheal injection to examine alveolar macrophage (AM) production of interleukin-1 (IL-1) and macrophages-derived growth factor (MDGF) from fibroblasts. Saline and UICC chrysotile B asbestos were used as controls. At 1 month, Attapulgite produced granulomas and the UICC chrysotile B produced fibrosis. At 8 months, 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 UICC chrysotile B. Enhanced production of IL-1 was seen in all treated groups. MDGF production was only seen in animals with lung fibrosis.

Coffin, Cook, and Creason (1992) injected a single dose of 0.5, 2, 4, 8, 16, or 32 mg of Attapulgite intrapleurally into six groups of 25 Fischer 344 rats. 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.

Bentonite

Sykes et al. (1982) investigated the effects of Bentonite dust administered by intratracheal instillation in rats. A 0.5-mg dose of Bentonite with a mean size of 0.3 μm was instilled intratracheally. Control animals were injected with sterile saline and TiO_2 (a nontoxic dust). Animals were killed at 1, 2, 6, 24, and 48 h; and 4 and 7 days after instillation. Bronchopulmonary lavage (BPL) was carried out and AMs and polymorphonuclear (PMN) leukocytes were recovered. The activity of LDH and protein content of the lavage fluid were also determined. In a second experiment, after instillation of 5 mg of Bentonite, the animals were killed at 1, 7, 49, and 100 days. In addition to the above, peroxidase and lysozyme activity were measured.

In the first experiment, a rapid influx of PMN leukocytes was detected at 6 h. PMN leukocyte response peaked at $\sim 19 \times 10^6$ cells after instillation and started declining more slowly up to 4 days. At 7 days, the PMN leukocyte numbers were 2.5×10^6. The greatest increase in the numbers of AMs recovered occurred at 4 and 7 days. 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 days. LDH activity at 24 h was maintained at 40 mU cm^{-3} and then increased (73 mU cm^{-3}) with the influx of PMN 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, large number of PMN leukocytes were recovered at day 1. However the severity of the response did not differ significantly from the 0.5 mg dose. By 7 days,

the numbers had decreased and was similar to control values. A significant decrease in the number of AMs compared to controls was observed at 24 h after instillation. This decrease was followed by a sharp increase that exceeded control values by 7 days. Total number estimates were similar to those of the first experiment. LDH activity and protein concentration from Bentonite and TiO_2 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 days, but returned to control values at 49 and 100 days (Sykes et al. 1982).

Marek and Blaha (1985) gave subplantar injections of 0.05 ml of a 5% solution of Bentonite to male Wistar rats. The rats either received both hind paw injections 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. The injection was of Kaolin. 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.

Tatrai et al. (1983) administered a single dose of 40 mg of Bentonite suspended in 1 ml of physiological saline containing 40,000 IU of crystalline penicillin intratracheally to male CFY rats. The Bentonite's 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 days after exposure. Body and lung weight 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 days, 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 the 24th 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 the 48th and 72nd h. Exposure after 90 days, included dust storage foci filled with 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 increase but distribution remained the same. After 90 days, the value was the same as seen at 72 h. (Tatrai et al. 1983).

Hatch et al. (1985) assessed the ability of Bentonite to increase susceptibility to bacterial pneumonia. Bentonite was injected intratracheally into mice at concentrations of 1, 10, and 100 μg. 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-day period. Control animals were exposed to TiO_2, a nontoxic dust. 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 (Hatch et al. 1985).

In a study by Tatrai et al. (1985), male CFY rats were given a single dose of 60 mg of Bentonite, in 1 ml of physiological saline containing 40,000 IU crystalline penicillin, by the intratracheal route. 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, the 2nd and 4th week, and the 3rd, 6th, and 12th month. The acid phosphatase activity and the progression of fibrosis was determined. The lungs were processed for microscopic examination and fibrosis determined by Belt and King's classification. The results from this experiment are presented in Table 16. Acid phosphatase activity was increased at 72 h and had returned to normal by the first month.

Bentonite dust was administered intratracheally as a single 60-mg dose to Sprague-Dawley rats in a study by Adamis et al. (1986). The animals were killed 3, 6, and 12 months after exposure. The right lung was studied microscopically and the lipids, phospholipids, and hydroxyproline 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 months, 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

TABLE 16
Toxic effect of intratracheal instillation of Bentonite (Tatrai et al. 1985)

	Time after instillation		
End point	72 hours	1st month	12th month
Acid phosphatase activity	72	—	—
Fibrosis	N/A	Loose reticulin fibrils, no collagen	Loose reticulin fibrils, no collagen

difference was detected at 6 and 12 months. Hydroxyproline content of treated rats (mg/g lung wet weight) was very similar to controls at 3, 6, and 12 months (Adamis et al. 1986).

Calcium Silicate

Bolton et al. (1986) injected three Calcium Silicate samples into the peritoneal cavity of three groups of 36 rats. Each rat was given a single injection of 25 mg of dust and allowed to live out their life span. At necropsy, little dust or dust-related fibrosis was visible in the peritoneal cavity. No mesotheliomas developed in any of the animals.

Richards, Tetley, and Hunt (1981) compared the biological reactivity of three samples of Calcium Silicate (A, B, and C) in vivo to that of chrysotile and titanium dioxide. Titanium dioxide and saline were considered negative controls, while chrysotile was considered a positive control. Groups of 32 female, MRC hooded rats were instilled intratracheally with 0.25, 0.50, 1.0, or 5.0 mg of Calcium Silicate. At weeks 1 and 4 after instillation, the control and treated rats were killed. The lungs were lavaged and the reactivity of the minerals to free cell populations, lavaged lung tissue, and pulmonary surfactant was conducted. All mineral doses of 5 mg induced an increase in the number of free cells at week 1. Only sample B increased in cell numbers at lower doses. At the end of 1 week, sample B was considered more reactive than either sample A or C, but chrysotile was considered more reactive than sample B. At 4 weeks, the effects seen from samples A and B are almost completely reversed and were comparable to that of titanium dioxide. Sample B at 4 weeks produced a greater or a comparable activity to chrysotile. No mineralogical analysis of the Calcium Silicate samples was provided.

Kaolin

Zaidi et al. (1981) investigated the effect of *Candida albicans* in modifying the fibrogenisis caused by Kaolin. Five groups of guinea pigs were injected intratracheally with *C. albicans* (500 μg); Talc dust (75 mg); Talc and *C. albicans*; Kaolin (75 mg); or Kaolin and *C. albicans*. Two animals from each group were killed at 1, 7, 15, 30, 60, 90, 120, and 180 days after injection. The lungs were collected for bacteriological and microscopic examination. The combined effect of Kaolin and the organism incited an acute inflammatory reaction similar to Kaolin dust alone at day 1. However, Kaolin and the organism produced thick reticulin and collagenous fibrosis, unlike Kaolin alone. Talc produced only a thin reticulin fibrosis not enhanced by the presence of the organism. The enhanced fibrogenicity was attributed to the adjuvant activity of Kaolin with the polysaccharide glucan component of *C. albicans*.

Edwards et al. (1984) gave 12 fetal lambs and six fetal monkeys subarachnoid injections of Kaolin. A sterile suspension of 2% Kaolin in saline was injected into the cisterna magna. Fetal lambs received 1 to 3 ml of Kaolin and fetal rhesus monkeys received 0.5 to 1.0 ml. 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 140 to 145 days for the sheep and 160 to 165 days for 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 one monkey underwent ventriculoamniotic shunting at 120 days after gestation.

Ventricular dilatation was apparent at 1 week following Kaolin injections. The cerebral mantle was markedly thinned, with relative preservation of the cortex and severe attenuation of the white matter. The average cortical thickness of the cingulate gyrus in the Kaolin-injected sheep was 716 μ compared to 1225 μ in control animals. The corpus callosum was an average of 125 μ in thickness in the sheep compared to 475 μ in control animals. Microscopic examination of the cortical neurons were 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 five 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 (Edwards et al. 1984).

Hatch et al. (1985) assessed the ability of Kaolin to increase susceptibility to bacterial pneumonia. Kaolin was injected intratracheally into mice at a dose of 100 μg. 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-day period. Control animals were exposed to TiO_2, a nontoxic dust. 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.

Wagner, Griffiths, and Munday (1987) used Kaolin as a negative control in a previous intrapleural injection study. The protocol and results are cited under Attapulgite in this section.

Fugiyoshi, Hayashi, and Oh-ishi (1989) reported a study in which Kaolin, a known activator of factor XII, was injected intraperitoneally into mice at 2.5 mg/mouse to study the Kaolin-induced writhing response. The writhing responses were observed in the 10 min after treatment and the mean number of responses was 9.2. Sixty minutes after the Kaolin injection, captopril (20 μg/mouse) was injected and the writhing response was observed again for 10 min after injection. Captopril is an antihypertensive and vasodilator. A second study was conducted by administering bromelain (10 mg/kg intravenously) followed by the injection of Kaolin 30 min later. Bromelain is a standardized complex of proteases from the pineapple plant purported to have primarily antiedema, antiinflammatory, and coagulation-inhibiting effects. The response was not reproduced.

Montmorillonite

Heat-treated Montmorillonite in doses of 5, 15, and 45 mg was given to groups of four Sprague-Dawley rats by intratracheal

instillation. Following a 3-month 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 AMs. 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 (Schreider, Culbertson, and Raabe 1985).

Zeolite

A single intratracheal administration of 50 mg of Zeolite dust was given to male rats and observations were made at 1 and 3 days, and 1 and 3 months after injection. Time-dependent increases in phagocytosis were observed. Morphological changes in the lungs was described as exogenous fibrous alveolitis (Kruglikov, Velichkovsky, and Garmash 1990).

Zeolite (Clinoptilolite)

Kruglikov et al. (1992) reported a study in which a single intratracheal instillation of 50 mg of Clinoptilolite was made to male rats. On days 1, 3 to 5, and 18 after injection, lung tissues were examined histopathologically. On the first day, the smallest Zeolite particles were phagocytized by neutrophils, whereas larger particles were phagocytized by macrophages. About a fourth of macrophages had phagocytized more than six dust particles per cell and <2% of macrophages were degenerated. At 3 to 5 days, no more particles were seen in neutrophils and their numbers had decreased. However, the percentage of macrophages containing more than six dust particles in the cytoplasm increased to 90%. Only 7% of macrophages degenerated. On day 18, the pattern of phagocytosis was similar to that at days 3 to 5, but 4% of macrophages were degenerated.

Tatrai and Ungv'ary (1993) instilled single intratracheal doses of 30 and 60 mg of Clinoptilolite particles to groups of 50 male and female (equal numbers) Wistar rats. The particles were <5 μm and were suspended in 40,000 IU crystalline penicillin. Controls received only saline instillations. All survivors were killed at the end of the study. Examination for gross and microscopic lesions were conducted. None of the treated groups had a significant increase in the incidence of any specific neoplasms compared to the controls. No positive trend was noted in the occurrence of neoplasms. Neoplasms seen within both control and treated animals were similar in the anatomical sites in which they were found and their histological feature.

Zeolite (Mordenite)

Suzuki (1982) gave two groups, one of 18 and one of 5 male Swiss albino mice, a single injection of 10 or 30 mg Zeolite intraperitoneally. The control animals were untreated. Ten months after exposure, no neoplastic changes were observed in the treated animals. Nearly all (98%) of the sample particles were <5 μm.

Suzuki and Kohyama (1984) administered a single injection of 10 mg of Mordenite to a group of 50 male BALB/c mice. The control animals received saline injections. The Mordenite sample was comprised of 94% of particles <3 μm. No peritoneal tumors were observed in any of the control animals. Mild peritoneal fibrosis was seen in treated mice, but no peritoneal or any other organ neoplasms were observed between 7 to 23 months.

Tatrai, Wojn'arovits, and Ungv'ary (1991) made intratracheal instillations of 60 mg of Mordenite to groups of 10 rats. The animals were killed at 1 week, and 1, 3, 6, and 12 months after exposure. Lesions in the lungs were observed. Nonspecific confluent bronchopneumonia was observed at 1 week after exposure and sequestration of macrophages at 1 month after exposure. Mild fibrosis was observed at later times. After 12 months, the aluminum:silicon ratio in macrophages was similar to the ratio in natural Zeolites.

Tatrai et al. (1992) reported the changes in cervical and hilar lymph nodes in the test animals treated in the above study as seen by electron microscopy and light microscopy. By the end of the first year, dust storing macrophage foci developed in the lymph nodes with minimal fibrosis. Also 3/10 of the rats had atypical hyperplasia. Electron microscopy showed the dust stored in macrophages without structural changes. However, dispersive x-ray microanalysis of the intracellularly stored dust revealed the ratio of the two main elements, aluminum and silicon, changed with respect to aluminum as compared to the original Zeolite sample.

Zeolite (Nonfibrous Japanese Zeolite)

A single intrapleural injection of 20 mg of Nonfibrous Japanese Zeolite was administered to two groups of 20 male and 20 female Fischer 344 rats. Control rats received saline injections alone. Mean survival time for control animals was 720 days and 715 days for treated animals. One pleural mesothelioma was found in the control group and one pleural and one peritoneal mesothelioma was found in the treated group (Wagner et al. 1985).

Zeolite (Synthetic Zeolite 4A)

A single intraperitoneal injection of 10 mg of Synthetic Zeolite 4A was given to groups of 50 male BALB/c mice. The average particle length of the sample was 2.24 μm. Treated animals were observed for 7 to 23 months after exposure and no mesothelioma were observed (Suzuki and Kohyama 1984).

Zeolite (Synthetic Zeolite MS4A and MS5A)

Maltoni and Minardi (1988) reported a study in which groups of 20 male and 20 female Sprague-Dawley rats received a single intraperitoneal injection of 25 mg of Zeolite MS4A (sodium aluminum silicate) or MS5A (calcium aluminum silicate) or water

only (control). Observations were made for the animal's entire life span and microscopic examination was performed. One peritoneal mesothelioma in an Zeolite MS4A-exposed rat was found at 141 weeks after treatment.

These same authors administered single intrapleural injections and single subcutaneous injections of 25 mg of Zeolite MS4A and MS5A or water to separate groups of 20 male and 20 female Sprague-Dawley rats. No difference in incidences of tumors was found among control and treated animals (Maltoni and Minardi 1988).

Zirconium Silicate

In a study by Harding (1948), a 3-ml dose of a 10% suspension of Zircon in milk and saline was injected intraperitoneally into three cavies (guinea piglike rodent). The animals were killed nearly a year later. At microscopic examination, a dry opaque material was embedded in the peritoneum of the abdominal wall over the small intestine, and in the omentum. Growth was not affected.

The accumulation of Zirconium Silicate in tissue was reported by Stookey et al. (1967). In one study, six young adult male rats were anesthetized and were given subcutaneous injections into their back. Half of the rats were injected with saline to serve as controls and the other half were injected with 0.3 ml of an aqueous 50% slurry of Zirconium Silicate. Three weeks after the injections, the animals were killed. Tissue surrounding the injection site was excised and prepared for microscopic examination. Zirconium Silicate deposits were observed as discrete nodules with a narrow surrounding connective tissue wall in the deep connective tissues of the back. Saline controls had no lesions and in some cases, healing was complete.

In another study in this report, eight young adult female rats were divided into four equal groups according to body weight and their tissues were subjected to microscopic examination following saline and Zirconium Silicate or sodium zirconium lactate injections. Group 1, the control group, was given a single injection of 0.05 ml of isotonic saline in four different areas: subcutaneous injections in the right buccal mandibular mucosa; periosteal injections in the left buccal mandibular periosteum; intramuscular injections on the ventral side of the left thigh; subcutaneous injections in a shaved area on the back located about 1 inch behind the shoulders of the midline. Group 2 was similarly injected with 0.05 ml of a 20% slurry of Zirconium Silicate. Groups 3 and 4 were injected with 0.05 ml of a 20% solution of sodium zirconium lactate and a 20% slurry of flour of pumice. All animals were killed 1 week after the injections and tissue samples for histological sections were taken at each injection site. An identical study with the same experimental procedures as the above study used adult male guinea pigs. In each species, saline injections produced no effect, Zirconium Silicate caused minimal toxicity, and sodium zirconium lactate plus pumice was toxic. The results from these two studies are listed in Table 17.

The results pertain to both the rat and guinea pig studies. Zirconium Silicate deposits were described as well circumscribed masses of particulate material surrounded by a narrow zone of new connective tissue. Nonspecific muscle damage, without necrosis due to the presence of the particulate matter and the volume of injected material, was localized to the immediate vicinity of the injection site. Macrophages along a border of a mass of Zirconium Silicate had reflective material within their cytoplasm. Dispersed particles were phagocytized by macrophages, with little or no associated inflammatory response. No evidence of bone resorption was found adjacent to periosteal deposits.

In another study by these authors, skin and muscle tissue samples were taken for microscopic examination. Eight adult rats were anesthetized and a deep incision was made on the ventral side of the left rear leg. The incision was made in the quadratus femoris muscle. The animals were exposed to 50 mg of pumice flour, silica dioxide, and Zirconium Silicate, respectively. Insertion of the appropriate substance was made into the muscle

TABLE 17
Toxic reactions to injected Zirconium Silicate (Stookey et al. 1967)

Animal species	Agent injected	Concentration (%)	Degree* of tissue reaction			
			Oral mucosa	Subcutaneous tissues	Periosteal tissue	Intramuscular tissue
Rat	Saline		0	0	0	0
Rat	Zirconium Silicate	20	+	+	0	+
Rat	Sodium zirconium lactate and pumice	45 and 20	+++	+++	+++	+++
Guinea pig	Saline		0	0	0	0
Guinea pig	Zirconium Silicate	20	+	+	+	+
Guinea pig	Sodium zirconium lactate and pumice	45 and 20	+++	+++	+++	+++

*0 = reaction absent.
+ = mild inflammatory reaction of little consequence.
++ = mild reaction with granulomatous response.
+++ = destructive granulomatous reaction.

TABLE 18
Toxic reactions to implantation of Zirconium Silicate in muscle tissue (Stookey et al. 1967)

Agent embedded in muscle	Amount (mg)	Degree of tissue reaction*	
		Subcutaneous tissue	Intramuscular tissue
Pumice	50.0	+	+
Silica dioxide	50.0	++	+++
Zirconium Silicate	50.0	+	+
Control		0	0

*0 = reaction absent.
+ = mild inflammatory reaction of little consequence.
++ = mild reaction with granulomatous response.
+++ = destructive granulomatous reaction.

incision and into the skin 1 cm lateral to the muscle incision. Control animals had the same muscle incision, but no foreign material was inserted. One animal from each group was sacrificed 10 days following surgery. The remaining animals were sacrificed 30 days from the incision. All tissue was fixed and prepared for microscopic examination. Table 18 presents the data from this experiment.

Adjacent tissues were free of inflammation or evidence of injury at 10 and 30 days. Deposits of Zirconium Silicate were identified and were surrounded by a narrow zone of new connective tissue. No necrosis was identified (Stookey et al. 1967).

Short-Term Parenteral

Attapulgite

Pott et al. (1987) conducted a study in which three samples of Attapulgite labeled Georgia, Lebrija, and Morimoiron were injected intraperitoneally to study their carcinogenic effects in rats. Each sample was injected one time each week for 9 weeks at 60 mg per injection. The number of female Wistar rats for each of the samples (Georgia, Lebrija, and Morimoiron) was 112, 115, and 114, respectively. Fiber analysis was made of each of the samples Morimoiron, Georgia, and Lebrija. The <50% fiber length was 0.7, 0.5, and 0.8 μm, respectively, and a <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 years after treatment for necropsy. At necropsy, neoplasms or organs with suspected neoplasm tissue were fixed for microscopic examination. These three samples were noncarcinogenic. The results are presented in Table 19.

In another experiment by the same investigators, a fourth sample of Attapulgite from Caceres was tested. Intraperitoneal injections of 2, 4, and 4 mg were administered consecutively for 3 weeks. 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. The results were considered moderate in relation to the dose. The Caceres Attapulgite sample results are also presented in Table 19 (Pott et al. 1987).

Kaolin

Toxicity of some of the minerals present in coal-mine dust was examined by Martin, Daniel, and Le Bouffant (1975). Five hundred female SPF Sprague-Dawley rats were divided into groups each with 10 animals. The rats were exposed over a period of 3 months to 50-mg/rat 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 bronchiolovascular lymphoid sheaths. No information regarding nonexposed lungs was presented. The opinion of the investigators was that exposure to

TABLE 19
Carcinogenic effect of intraperitoneal injection of Attapulgite from four sources (Pott et al. 1987)

Attapulgite sample source	No. of rats	% of rats with tumors	Lifespan (weeks) after treatment of					
			All rats				Rat with tumors	
			Time to death for <20% of all rats	Time to death for <50% of all rats	Time to death for <80% of all rats	All rats dead by this time	Time to death of first rat with tumor	Average time to death of rats with tumors
Mormoiron	114	3.5	92	116	138	164	47	92
Lebrija	115	3.5	95	116	134	164	98	114
Georgia	112	3.6	89	108	129	163	75	100
Caceres	30	40.0	94	109	132	142	74	116

Kaolin results in "pulmonary toxicity" and possesses "fibrogenic capacity" (Martin, Daniel, and Le Bouffant 1975).

Magnesium Silicate

An emulsion of Magnesium Silicate, 500 mg in 1 ml of saline, was injected subcutaneously into groups of 10 female Wistar rats once daily at 2, 4, 6, 13, or 20 days. As controls, 12 nontreatment rats were killed on the first experimental day and 12 rats were injected with 1 ml of saline once daily for 20 days. The trabecular bone, sinusoids, and hematopoietic cells were processed for microscopic examination. No significant change in the volume percentage of hematopoietic cells, sinusoids, or trabecular bone was present in the day-2 treatment group. After 4 days of treatment, the volume percentage of hematopoietic cells increased rapidly, sinusoids decreased rapidly, and trabecular bone decreased gradually. The volume percentage of hematopoietic cells was about 2.6 times normal, and that of sinusoids and trabecular bone was about 30% and 60% of normal, respectively, after 20 days of treatment. The tibia metaphyses had the following changes after 4, 6, 13, and 20 days of treatment; sinusoids were compressed by the markedly proliferated myelocytic element and severely narrowed the distance between the sinusoidal wall and the surface of trabecular bone was markedly increased. Atrophy of the thin trabecular bone was seen but no significant changes in osteocytes, osteoblasts, or osteoclasts were seen (Shibayama, Nishioto, and Nakata 1993).

Zeolite (Clinoptilolite)

Three intrapleural injections of 20 mg of Clinoptilolite were given in monthly increments to a group of 44 male and 49 female rats. Control animals received only saline injections. The Zeolite sample was described as having the formula: (Na,K) $Ca[Al_6Si_{30}O_{72}] \cdot 20H_2O$, with Cu, Pb, Zn, Ni, Co, Mo, Mn, Ti, Sr, Ba, and Hg contamination. Particle size measurements were recorded as follows: <3 μm, 6.5%; 5 μm, 5.9%; 10 μm, 5.9%; 10–30 μm, 20.6%; 30–100 μm, 35.1%; 100–500 μm, 26.1%. Pulmonary lymphosarcomas, pleural and abdominal lymphosarcomas, and lymphatic leukemias were observed in 47/93 treated animals and 5/45 saline-treated animals. No mesothelioma or pulmonary neoplasms were observed in the controls. Mesothelioma and bronchial carcinoma were detected in 2/93 and 1/93 treated animals, respectively (Pylev et al. 1986).

Zeolite (Phillipsite)

Three intrapleural injections of 20 mg of Phillipsite given in monthly increments were administered to a group of 44 male and 49 female rats. Control animals received only saline injections. The Zeolite sample was described as having the formula: $(Na_{1.38}K_{0.53}Ca_{0.87}Mg_{0.25})(Si_{11.93}Al_{4.03}O_{32}) \cdot 9H_2O$. Particle size measurements were recorded as follows: <5 μm, 14.5%; 10–30 μm, 32.8%; 50–70 μm, 16%; ≥ 100 μm, 36.7%. Neoplasms were found in 41/101 Zeolite-treated rats (50 tumors). Tumor types included 1 pleural mesothelioma, 2 pulmonary adenocarcinoma, 29 hemoblastosis, 7 mammary gland neoplasms, and 11 neoplasms found at other sites. In control animals, 16 neoplasms (pulmonary, pleural, and abdominal lymphosarcomas, lymphocytic leukemias, and mammary gland neoplasms) were identified in 14/52 rats (Pylev et al. 1986).

Zirconium Silicate

Harding (1948) reported results when an adult rabbit received intravenously four doses over 1 week of a 5-ml suspension of a 10% solution of Zircon. The animal was killed 33 weeks later. At microscopic examination revealed small clumps of crystals were close to the portal tracts of the liver. The clumps were in the Kupfer cells. Fibrosis was detected. Small clumps of crystals were also observed in the spleen and alveolar walls and spaces of the lungs.

In another study in this report, six young rats were injected intratracheally with 1 ml of a 10% solution of Zircon. Three rats were killed after 7 and 9 months. The lungs were radiographed and sectioned for microscopic examination. Much of the material was found free within the alveoli and lymph vessels of the lungs. A small amount was found within phagocytic cells. Swollen histiocytes were seen in a few alveoli. Fibrosis was not evident (Harding 1948).

Inhalation

Attapulgite

Wagner, Griffiths, and Munday (1987) exposed 40 (20 male and 20 female) SPF Fischer rats to Attapulgite dust in an inhalation chamber. The rats were exposed to two samples of Attapulgite (named by the region in which they were mined, Lebrija and Leichester) at a concentration of 10 mg/m^3 for 6 h/day for 5 day/week until they were killed. At 3, 6, and 12 months, four animals were killed. All 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. Mineralogical analysis, examination of ashed lung sections and examination of macerated lung tissue, were also performed. Kaolin, the negative-control dust, and Chrocidolite UICC, the positive-control dust, were also administered at a dose of 10 mg/m^3.

At microscopic examination, one peritoneal mesothelioma, one adenocarcinoma, and three bronchoalveolar hyperplasia were found in rats treated with Lebrija Attapulgite. Thirty-five rats had no proliferative changes. In rats treated with Leichester Attapulgite, proliferative lesions observed included two mesothelioma, one peritoneal mesothelioma, one malignant alveolar neoplasm, two benign alveolar neoplasms, and eight bronchoalveolar hyperplasias. Twenty-seven rats had no proliferative lesions. Rats exposed to the negative-control Kaolin had two bronchoalveolar tumors. Rats in the positive-control Crocidolite group had one adenocarcinoma and three bronchoalveolar tumors. The mean fibrosis grades of each treatment group are presented in Table 20.

TABLE 20
Toxicity of inhaled Attapulgite dust (Wagner, Griffiths, and Munday 1987)

Dust source	Total no. of rats	Mean fibrosis grade as function of time after exposure			
		3 months	6 months	12 months	24 months
Lebrija Attapulgite	40	3.1	2.6	3.2	3.2
Leichester Attapulgite	40	3.0	3.1	4.0	—
Kaolin	40	2.8	2.75	2.4	2.1
Crocidolite UICC	40	4.1	3.3	3.1	3.8

The classification of proliferative lesions and neoplasms corresponding to the mean fibrosis grades are as follows: (1) bronchoalveolar hyperplasia—no malignant proliferation of the epithelia; (2) benign alveolar neoplasm; (3) malignant alveolar neoplasm; (4) adenocarcinoma; (5) squamous carcinoma; (6) adenosquamous carcinoma; and (7) mesothelioma.

The Lebrija Attapulgite dust extracted from the animal lungs did not have short fibers and the presence of granular material and long fibers. The Leichester Attapulgite dust also had the presence of long fibers. Kaolin is a nonfibrous dust. UICC Crocidolite is a fibrous dust but lengths were not published in this study (Wagner, Griffiths, and Munday 1987).

Calcium Silicate

Bolton et al. (1986) exposed white male Wistar rats to clouds of Calcium Silicate dust at a concentration of 10 mg/m^3 for 7 h/day, 5 days/week, for a total of 224 days over an elapsed period of 12 calendar months. A total of four inhalation chambers were used with 48 animals/chamber. One chamber was reserved for control animals receiving only filtered air. The remaining three chambers were used to test three samples (A, B, and C) of Calcium Silicate. Twelve rats were killed from each of the chambers at the end of the dusting period. The final surviving animals were killed at the end of 19 months after exposure. At necropsy, tissue samples and one lung were taken from all major organs for microscopic examination. The other lung was taken for lung-dust analysis. The lung was dried and prepared for infrared analysis. Blood samples were taken 5 days prior to the start of the exposure and 3 days after the exposure.

All Calcium Silicate–treated groups had dust-containing macrophages scattered throughout the alveolar regions of the lung at the end of the exposure period. Occasional fibers were seen in animals with exposure to the Calcium Silicate 3. The frequency of dust-containing macrophages declined at the end of the dust exposure. Fewer dust-containing cells were in animals exposed to samples C than A or B. The number of animals with interstitial fibrosis for samples A, B, C, and controls were three, five, five, and five, respectively. In all cases, the alveolar septa were thickened with abnormal deposits of reticulin and in old animals with collagen. Although most cells were relatively flat in some areas, some cells were cuboidal and had the appearance of adenomatosis. Peribronchiolar fibrotic areas were close to the respiratory bronchioles and small granulomatous nodules with macrophages and fibroblasts were seen in rats exposed to sample A. Mediastinal lymph nodes from all treated animals showed no particulate material at the end of exposure. Small primary neoplastic lesions were found in two animals exposed to sample B. One lesion was described as a small squamous cell carcinoma and the other as an adenoma. No pathological changes were observed in all other organs. All examined blood parameters were within normal ranges for both animals studied before and after exposure (Bolton et al. 1986).

Kaolin

Kaolin was used as a negative control in a previous inhalation study. The protocol and results are cited under Attapulgite in this section (Wagner, Griffiths, and Munday 1987).

Zeolite (Synthetic Zeolite A)

A group of 15 male and 15 female Wistar rats were exposed to 20 mg/m^3 of Synthetic Zeolite A for 5 h/day, three times a week for 22 months. The Zeolite was characterized by $(Na_{12}(Al)_2)(SiO_2)_{12}\cdot 27H_2O$ and consisted of particles ranging from 0.5 to 10 μm. Thirty untreated males were the control group. Histopathological examinations of the trachea and the lung were completed. Moderate to extensive respiratory disease was seen in treated and control groups. No neoplasms were observed in any group (Gloxhuber et al. 1983).

In another study by Gloxhuber et al. (1983), a chronic inhalation study of Zeolite A batch F 325 dust was conducted. Groups of 15 male and 15 female hamsters and 15 male and 15 female rats were exposed for 5-h periods three times a week for 12 months for hamsters and 22 months for rats. Control animals were exposed to untreated air. The trachea and lungs of the animals were examined microscopically. Microscopic examination was limited to the trachea and lungs of 10 treated hamsters and 8 controls and to 10 treated rats and 5 controls due to deaths caused by a specific infection. Both species had moderate signs of respiratory disease in the treated and controls. In Zeolite-exposed hamsters, macrophages with accumulations of foreign material were found, mainly in alveoli. No other lesions of inflammation or connective tissue reactions were seen. Rat lungs had grey-white deposits in macrophages of the alveoli and the peribronchiolar lymph nodes near the hilus. Isolated

clay deposits were found in the mediastinal lymph nodes but no reactions were seen about the deposits.

Zeolite (Synthetic Nonfibrous Zeolite)

Groups of 20 male and 20 female Fischer 344 rats were exposed in inhalation chambers to a mean respirable dust concentration of 0 or 10 mg/m^3 of a Synthetic Nonfibrous Zeolite. Exposures were for 7 h/day, five days/week for 12 months. All animals were observed for their life span. Three males and three females per group were killed at 3, 6, 12, and 24 months after exposure. Erionite and UICC crocidolite were used as positive controls. The mean survival time for animals exposed to the Zeolite was 797 days, 504 days for animals exposed to erionite, 718 days for animals exposed to UICC crocidolite, and 738 days for untreated animals. One pleural mesothelioma and one pulmonary adenocarcinoma were seen in Zeolite-exposed rats. No neoplasms were found in controls; 27 mesotheliomas were found in erionite-treated rats and 1 squamous-cell carcinoma of the lungs was found in UICC crocidolite-treated rats (Wagner et al. 1985).

Dermal Irritation

Hectorite

A primary irritation study patterned after the Draize method was conducted using six white rabbits. 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 and the test subject was nonirritating to the skin of rabbits (FDRL Inc. 1980a).

Magnesium Aluminum Silicate

VEEGUM (2 g) was applied daily to the external ears of four rabbits for 10 days. These applications were made to both abraded and intact skin. The abraded skin healed completely within 4 to 6 days after application. No gross effects were noted in any of the animals. No tissue was taken for microscopic examination (Munch 1944).

VEEGUM was applied to the closely clipped intact and abraded abdominal skin of two groups of four rabbits each. A nonabsorbent paper binder was place onto the treated area. The dose was 3.4 g/kg of body weight. After 24 h, the binder was removed and any residual test material was removed by washing. Dermal irritation was recorded at 24 h and once daily after application for 7 days. All the animals were killed and necropsy was performed. No deaths and no systemic toxicity occurred from percutaneous absorption. The acute dermal LD_{50} was >3.5 g/kg of body weight. Dermal irritation generally consisted of moderate erythema and slight edema. The edema completely subsided within an additional 24 h, and erythema completely subsided in all animals between days 2 and 4. No major necropsy findings were reported (Hazelton Laboratories, Inc. 1968).

Eight male white rabbits were used in a primary skin irritation test with a solution of 4% MAS; 0.3 ml of the test substance was applied to the intact and abraded skin of the backs of four rabbits. The test substance was applied under occlusive patches for 24 h. The plaster was removed 24 h after application and the skin reactions were evaluated at 24 and 72 h. The primary irritation index was 0.1, suggesting that Magnesium Aluminum Silicate is a weak primary skin irritant (CTFA 1970a).

Three male guinea pigs were used in a cumulative skin irritation test with a solution of 4% MAS (in deionized water). The test substance (0.05) was applied to the flank of the animals once daily for 3 consecutive days. Skin reactions were evaluated at 24 h after each application. The cumulative irritation index was 0.0 and MAS had no cumulative skin irritation under the test conditions (CTFA 1970a).

Sodium Magnesium Silicate

CTFA (1970b) reported a study in which eight male, white rabbits were used in a primary skin irritation test with a solution of 4% Sodium Magnesium Silicate (in deionized water). The test substance (0.3 ml) was applied to the intact and the abraded skin on the backs of four rabbits. The test substance was applied under occlusive patches for 24 h. The plaster was removed 24 h after application and the skin reactions were evaluated at 24 and 72 h. The primary irritation index was 0.0, suggesting that Sodium Magnesium Silicate has no primary skin irritation under these test conditions.

CTFA (1970b) reported that three male guinea pigs were used in a cumulative skin irritation test with a solution of 4% Sodium Magnesium Silicate (in deionized water). The test substance (0.05 ml) was applied the flank of the animals once daily for 3 consecutive days. Skin reactions were evaluated at 24 h after each application. The cumulative irritation index was 0.0 and Sodium Magnesium Silicate had no cumulative skin irritation under the test conditions.

Ocular and Mucosal Irritation

Bentonite

Preparations of Prophypaste, Bentonite, tragacanth, trypsin, and sterile water were injected either intralamellarly or directly into the anterior chamber of six adult New Zealand rabbits at concentrations ranging from 1 to 5 mg/ml. No significant reactions were recorded with sterile water, Prophypaste, tragacanth, or combinations of tragacanth and Bentonite. Bentonite caused severe iritis after injection into the anterior chamber, but no corneal or retrocorneal reaction was noted grossly or microscopically. In five of the eyes where Bentonite was injected intralamellarly, widespread corneal infiltrates and retrocorneal membranes were observed within 2 to 5 days. The sixth eye had no reaction, only 0.1 ml of 0.25 mg/ml was injected. Anterior chamber taps of the eyes showed viscous mucopurulent material. Microscopic sections showed pseodoeosinophils, retrocorneal membranes,

and fibrovascular membranes in the anterior segment. Polarized light revealed highly birefringent particles were found at the injections sites, but not in the retrocorneal masses (Austin and Doughman 1980).

Hectorite

A primary eye irritation study using nine New Zealand white rabbits was carried out according to the Wolcott Procedure. A 0.1-ml liquid or semisolid (100 mg of the solid) sample was instilled into the one eye of each rabbit. Six of the nine animals' eyes were not rinsed and the eyes of three of the animals 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 days. 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 (FDRL Inc. 1981).

Magnesium Aluminum Silicate

Hazelton Laboratories, Inc. (1968) made a single application of 100 mg of VEEGUM or 0.1 ml of a 50% weight/volume to rabbit eyes. An aqueous suspension was made into the conjunctival sac of the left eye of each of six (undiluted) and three (50% suspension) rabbits. Three eyes (undiluted) were washed for 4 s after application and the remaining six eyes were not irrigated but held closed for 1 s. Control rabbits were not treated. Observations were made at 1, 4, 24, 48, and 72 h and at 4 and 7 days following application. Irritation was graded according to the Draize system. On day 7, the eyes were treated with 2% sodium fluorescein strain to provide evidence of corneal damage. Irritation generally consisted of moderate conjunctival hyperemia in all eyes and slight iritis in five of the eyes (one in the nonirrigated, undiluted group and two in each of the other groups). In the nonirrigated eye treated with the dry material, the iritis persisted until 72 h, whereas it was only present at the 1- and 4-h observations in the other eyes. The irritation gradually subsided completely in all within 2 to 4 days. The sodium fluorescein test was negative for corneal damage.

CTFA (1970a) reported that three male, white rabbits were used in an eye irritation test using a 4% solution of MAS. The test substance (0.01 ml) was instilled into the conjunctival sac of one eye of the animals without irrigation. Acute reactions were evaluated at 1 and 4 h, and 1, 2, 3, 6, and 7 days after application according to the Draize scoring system. The average irritation score at the time of maximum score (1 h) for the cornea, iris, and conjunctivae was 0, 0, and 6.7, respectively. The average total score was 6.7 suggesting that MAS produced minimal eye irritation under these test conditions.

Sodium Magnesium Silicate

Three male, white rabbits were used in an eye irritation test using a 4% solution of Sodium Magnesium Silicate (in deionized water). The test substance, 0.1 ml, was instilled into one eye of the animals without irrigation. Eye reactions were evaluated at 1 and 4 h, and 1, 2, 3, 6, and 7 days after application according to the Draize scoring system. The average irritation score at the time of maximum score (1 h) for the cornea, iris, and conjunctivae was 0, 0, and 6.0, respectively. The average total score was 6.0, suggesting that Sodium Magnesium Silicate had minimal eye irritation under these test conditions (CTFA 1970b).

Zeolite (Zeolite A)

In an acute ocular study, rats tolerated a single dose of 10 g of Zeolite A without any adverse reaction (Gloxhuber et al. 1983).

Zirconium Silicate

Gingival tissue was histologically examined in a study conducted by Stookey et al. (1967). Six weanling albino rats were given an oral prophylaxis using a paste containing 75% Zirconium Silicate and 25% distilled water. The animals were anesthetized and given a routine prophylaxis for 30 s per mandibular hemijaw. Three of the animals were killed 1 h following treatment. The other three animals were killed 24 h following treatment. Gingival tissue of the buccal surface of the mandibular molar areas were removed for microscopic examination.

No unusual tissue response was observed in either group. At 1 h, scattered particles of Zirconium Silicate were noted on the surface of the gingiva. Occasional particles could be identified in the superficial epithelium. Only an occasional mild local inflammatory response was noted in the subepithelial tissue. It was presumed to be secondary to the prophylaxis procedure (Stookey et al. 1967).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Calcium Silicate

FDRL Inc. (1973) conducted a study in which adult, Dutch-belted female rabbits were artificially inseminated and received oral intubations of Calcium Silicate at doses of 250, 500, 750, 1000, 1250, 1500, and 1600 mg/kg on days 6 through 18 after insemination. On day 29, cesarean section was performed and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. Body weights of live pups were recorded. The urogenital tracts of the animals were examined in detail. All fetuses underwent detailed gross examination. Calcium Silicate administered at 1600 mg/kg to pregnant rabbits for 13 consecutive days had no clear discernible effect on nidation or on maternal or fetal survival. Skeletal or soft tissue abnormalities did not differ from the number occurring in control groups.

Kaolin

Groups of 12 Sprague-Dawley female rats were fed three diets: control diet, 20% Kaolin diet, or iron-supplemented 20% Kaolin diet. The diets were fed for 37 to 86 days, 69 to 85 days, and 96 to 117 days 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 RBC 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 RBC levels (Patterson and Staszak 1977).

Magnesium Aluminum Silicate

According to Sakai and Moriguchi (1975), "MAS has neither teratogenic nor had adverse effects on the mouse fetus." MAS was administered at doses of 600, 3000, and 6000 mg/kg/day orally to pregnant mice (ICR-JCL) for 6 days on the 7th to 12th day of gestation. No significant differences between MAS-administered and control groups were observed in body weight gain, gross lesions, implantations, resorbed or dead fetuses, or growth inhibition of live fetuses. Incidences of skeletal anomalies were significantly greater in MAS-exposed fetuses, but none resulted in skeletal malformation. Development, external differentiation, body weight gain, and behavior were normal in all offspring.

Zeolite (Type A)

Type A Zeolite containing 15.8% sodium 19.0% silicon, and 20.1% aluminum was tested for its teratogenic potential by Nolen and Dickerman (1983). Sprague-Dawley rats and New Zealand rabbits were utilized under the standard FDA Segment II protocol. Zeolite A in distilled water was given to rats by gavage at concentrations of 74 or 1600 mg/kg of body weight on days 6 to 15. Rabbits were given doses of 74, 345, and 1600 mg/kg of Zeolite A by oral gavage on days 6 to 18. Vehicle controls were included but no details were provided. Type A Zeolite produced no adverse effects on the dam, embryo, or fetus in either the rats or rabbits at any dose.

Zeolite (Clinoptilolite)

Pond and Yen (1983a) investigated whether Clinoptilolite offers protection against the toxic effect of long-term cadmium ingestion by examining the effects of long-term ingestion of Clinoptilolite on reproduction and on the postnatal development of the progeny. Four groups of female Sprague-Dawley rats were fed the following diets: control; control and Clinoptilolite; control plus cadmium; and control plus cadmium and Clinoptilolite. At 13 weeks, male rats were placed with the females for mating. The female reproductive performance was unaffected by any of the various diets. The supplemental level of Clinoptilolite resulted in reduced body weight during gestation; body weight at parturition and postpartum was similar for rats of all diet groups.

GENOTOXICITY

Attapulgite

DNA damage caused by Attapulgite was evaluated through the measurement of unscheduled DNA synthesis (UDS) in a study conducted by Denizeau et al. (1985b). Hepatocytes taken from male Sprague-Dawley rats were prepared according to the collagenase perfusion technique. Attapulgite fibers were added at concentrations of 1 and 10 μg/ml to the primary cultures 2 h after the cells were seeded. 2-Acetylaminofluorene (AAF), a known UDS-inducing agent of rat hepatocytes, was added to the cultures at 0.05 and 0.25 μg/ml for each concentration of Attapulgite. Therefore, Attapulgite was used alone in this UDS assay system or in combination with AAF. The cultures were incubated for 20 h. Labeled thymidine was added to final concentration of 4 μCi/ml. The amount of thymidine in the DNA was evaluated by liquid-scintillation counting. Cytotoxicity was also measured in this study by measuring LDH activity using a spectrophotometer.

A significant increase in [^{3}H]-thymidine incorporation took place with the addition of AAF (0.05 and 0.25 μg/ml). However, at both Attapulgite concentrations, no significant increase in DNA-specific activity was observed. No alteration occurred in the UDS (induced by AAF) by secondary agents when both the fibers and AAF were applied. No statistically significant fiber effect of AAF-fiber interaction was recorded. Extracellular LDH activity was observed after 20-h incubations of Attapulgite at 1 and 10 μg/ml applied to the cells. No significant differences were found between the LDH activity in the treated samples versus the controls (Denizeau et al. 1985b).

Beck and Bignon (1985) tested Attapulgite and UICC chrysotile asbestos B for UDS in primary hepatocyte cultures. Attapulgite fibers (96%) averaged 0.8 μm in length. Cells were also exposed to AAF alone and mixed with fibers. Within 20 h, both types of fibers were found in various cell structures, i.e., plasma membrane invaginations, cytoplasmic vacuoles, and phagolysosome-like components. Chrysotile B and Attapulgite did not induce a significant UDS response or modulate the response to AAF.

The UDS and cellular growth was studied utilizing rat pleural mesothelial cells (RPMCs) in a study conducted by Renier et al. (1989). RPMCs were cultured to confluence on glass coverslips in multiwell plates. Concentrations 2, 4, and 10 μg/cm^2 of Attapulgite and [^{3}H]-thymidine were added to cultures for 20 h. UDS was not modified at concentrations of 2 and 4 μg/cm^2 of Attapulgite. However, in one experiment, 10 μg/cm^2 produced a significant increase in UDS. Cellular growth was measured by counting in situ with an inverted phase-contrast microscope after 24 h of treatment of 1, 2, 4, and 10 μg/cm^2 of Attapulgite. Results were similar to that of the UDS. Attapulgite was considered noncytotoxic at concentrations of 1, 2, and 4 μg/cm^2. However, at 10 μg/cm^2, cell growth was inhibited. No specific details were given.

Adachi et al. (1992) studied the effect of asbestos fibers on DNA by measuring the yield of 8-hydroxy-2′-deoxyguanosine (8-OH-dGuo). 8-OH-dGuo is an OH adduct at the 8-position of a guanine base thought to induce an AT-to-GC transversion in DNA which may lead to a point mutation. For comparison purposes, Attapulgite was also studied. Results for

Attapulgite were not different from controls (Adachi et al. 1992).

Calcium Silicate

Litton Bionetics, Inc. (1974) conducted a study in which FDA compound 71-41, hydrated Calcium Silicate, was suspended in 0.85% saline at concentrations of 1000, 500, 200, 100, and 10 μg/ml and applied to WI-38 cells in a logarithmic phase of growth. The cells were observed for cytopathic effects (CPEs) and the presence of mitosis at 24 and 48 h. Inhibition of mitosis was observed at all concentrations except 100 and 10 μg/ml. A closer range of concentrations, 200, 150, 100, 75, and 50 μg/ml, were employed and tested for the same findings. Mitosis was stopped only in the cells dosed at 200 μg/ml.

FDA compound 71-41, hydrated Calcium Silicate, was also tested for mutagenic properties in a host-mediated assay using the microorganisms *Salmonella* TA-1530 and G-46 and *Saccharomyces* D3. These experiments were carried out in mice orally administered (acute and subacute) 15, 150, and 1500 mg/kg of Calcium Silicate. No increased mutation frequencies were seen in *Salmonella* TA-1530 or G-46. *Saccharomyces* D3 had no significant increase in recombinant activity. In fact, a reduction in recombinant activity was produced by the compound. In a second host-mediated assay, Calcium Silicate was administered at 5000 mg/kg to mice against *Salmonella* TA-1530 and G46 and *Saccharomyces* D3. All tests were negative.

Cytogenetic studies in vivo examined bone marrow cells arrested in C-metaphase from rats exposed to FDA compound 71-41, Calcium Silicate. Rats were administered 15, 150, and 1500 mg/kg doses. The positive-control was triethylene melamine (TEM) and the negative-control was saline. The chromosomal abnormalities observed in the positive-control animals were significantly greater than those of either the negative control or the compound. The maximum effect of the positive control was observed at 48 h after administration. Calcium Silicate produced breaks in the range of 1% to 3% in all three acute dosage levels. However, these were not significantly higher than the negative controls. The subacute dose of 150 mg/kg produced breaks at 3%. The negative-control breaks were consistent with those of other experiments.

These same cytogenetic tests were observed in vitro. Cells (not specified) were observed in anaphase for chromosomal aberrations such as bridges, psuedochiasmata, multipolar cells, acentric fragments, etc. Doses of Calcium Silicate were as follows: 1.0, 10.0, and 100.0 μg/ml. Controls, both positive and negative, were the same as reported above. The positive control produced significantly greater percentages of chromosomal aberrations than the negative control or test compound. There were no aberrations observed due to Calcium Silicate.

In a third cytogenetic test, Calcium Silicate was administered to male rats in one dose and in five doses of 5000 mg/kg. A positive-control, TEM, and a negative-control, saline, were also tested. Metaphase spreads were prepared from the bone marrow cells of these animals and scored for chromosomal aberrations. Neither the variety nor the number of the aberrations differed significantly from the negative controls. Calcium Silicate was nonmutagenic.

Dominant lethal assays were carried out in male rats administered FDA compound 71-41, hydrated Calcium Silicate, at doses of 15, 150, and 1500 mg/kg, both as one dose and as five doses. Also tested were the negative saline control and a positive TEM control. This assay measures the amount and type of fetal wastage that may occur following administration of a potential mutagen. Each treated male rat was mated with two virgin female rats each week for eight (acute) or seven (subacute) doses. Two weeks after mating, the female rats were sacrificed and the fertility index, preimplantation loss, and lethal effects were determined and compared with the same parameters calculated from the negative and positive controls. No significant findings were observed in the fertility index or preimplantation loss. The test compound was also administered at a dose of 5000 mg/kg. The protocol was the same as listed above. All parameter values did not differ significantly from that of the negative control. Comparing the data of both experiments indicates that hydrated Calcium Silicate does not induce dominant lethal mutations (Litton Bionetics, Inc., 1974).

Hectorite

Hectorite suspended in dimethylsulfoxide (DMSO) at concentrations of 10 to 3000 μg/plate was subjected to spot test using five mutant strains of *Salmonella typhimurium* LT2, hisTA98, hisTA100, hisTA1535, hisTA1537, and hisTA1538, with and without metabolic activation. Positive controls were carried out utilizing Aroclor 1254. Hectorite was nonmutagenic in all five test strains (Inveresk Research International 1995).

Magnesium Aluminum Silicate

MAS was subjected to spot test using five mutant strains of *S. typhimurium* LT2, hisTA98, hisTA100, hisTA1535, hisTA1537, and hisTA1538. Positive and negative controls were carried out utilizing S9 mitochondrial preparations from the livers of Sprague-Dawley rats and 2-aminoanthracene. MAS was found to be nonmutagenic in all five test strains (Blevins and Taylor 1982).

Zeolite

Durnev et al. (1993) tested the clastogenic potential of Zeolite particles <10 μm in length in peripheral human blood lymphocytes. Chrysotile fibers were used as a positive control. Both fibers produced statistically significant increases in the percentage of aberrant metaphases, mostly from chromatid breaks. Superoxide dismutase (50 μg/ml) protected against the induction of aberrant metaphases by chrysotile asbestos, but not by Zeolite. However, catalase (20 μg/ml) protected against induction of aberrant metaphases by Zeolite, but not by chrysotile asbestos.

Chromosomal aberrations in cells of C57BL/6 mice were also investigated. The cells were collected by peritoneal lavage and

from the bone marrow of mice and were sampled at 1, 2, 7, and 28 days after the intraperitoneal injection of 100 μg/mouse natural Zeolite particles. Chrysotile asbestos was used as a positive control. The lavage sample contained 20% lymphocytes, 20% to 30% macrophages, and 50% to 60% PMN leukocytes. The injection of the Zeolite induced a statistically significant increase in aberrant metaphases after 7 and 28 days in the peritoneal lavage cells. Chrysotile induced the aberrant metaphases at all times in both the peritoneal lavage and bone marrow cells (Durnev et al. 1993).

Valatina, Pylev, and Lemjasev (1994), tested the clastogenic effect on bone marrow cells of five dust samples from Zeolite tuffs. Presterilized dusts were administered intraperitoneally to BALB/C mice. The known clastogen mitomycin C was used as a positive control and 0.5 ml of saline as a negative control. The animals were killed 24 h after administration and mice bone marrow samples were taken. Polychromatophilic erythrocytes (PCEs), which contain micronuclei that are formed during mitosis on acentric fragments of the chromosomes as a result of clastogenic actions, were counted. Many of the dust samples were as potent a clastogenic agent as mitomycin C. A summary of the results is listed in Table 21.

CARCINOGENICITY

The IARC (1997) has placed Attapulgite fibers >5 μm in Group 2B, *possibly carcinogenic to humans*. Fibers <5 μm *cannot be classified as to their carcinogenicity to humans* and were classified in group 3. The Utrecht University's Institute for Earth Sciences and Vening Meinesz Institute for Geodynamic Research (Englehard 1998) analyzed Engelhard's Attapulgite clay by transmission electron microscopy to determine the fiber length. The transmission electron microscopic analytical results was <5 μm.

TABLE 21
Micronuclei induced by Zeolite tuffs (Valatina, Pylev, and Lemjasev 1994)

Administered substance	Dose (mg/g)	Amount of PCEs with micronuclei (per 1000 PCEs)
Dust 1	2.0	8.33 ± 0.5
	0.8	5.83 ± 0.5
Dust 2	1.4	2.83 ± 0.3
	2.1	3.83 ± 0.6
Dust 3	3.15	0.5 ± 0.8
	1.26	3.8 ± 0.5
Dust 4	2.15	6.7 ± 0.5
	.86	5.2 ± 0.5
Dust 5	3.25	4.83 ± 0
	1.3	3.66 ± 0.5
Mitomycin C	0.16 mg/kg	7.70 ± 0.3
Saline control	0.5 ml	2.70 ± 0.03

Clinoptilolite, Phillipsite, Mordenite, Nonfibrous Japanese Zeolite, and synthetic Zeolites *cannot be evaluated as to their carcinogenicity to humans* (group 3) according to the IARC (1997).

Table 22 is a summary of carcinogenicity data, which were detailed earlier in the section *Animal Toxicology*.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation

Magnesium Aluminum Silicate

Applications of 2 g of VEEGUM were made to the skin of two human subjects in an 1-inch area daily for 1 week. No effects were noted and no other details were given (Munch 1944).

Inhalation

Aluminum Silicate

Musk et al. (1980) surveyed 17 workers exposed to the Aluminum Silicate dust, alunite. Respiratory questionnaires and occupational history, pulmonary function testing, and posterioanterior chest radiographs were obtained. The alunite chemical analysis was that 48.5% of it was Al_2O_3 and 35.0% was SiO_2. The average age of the subjects was 29.1 years. The mean transfer factor for carbon monoxide (T_L) predicted for the whole group was 85.8% and the mean ratio of T_L to effective alveolar volume (V_A) was 83.8%. The actual group T_L and T_L/V_A was less than predicted. Overall, the group had comparable predicted levels of forced expiratory volume (FEV) in 1 second, vital capacity (VC), and total lung capacity (TLC). Two subjects had small irregular opacities on chest films. Neither of these subjects had previous exposure.

Attapulgite

Churg (1983) surveyed the total pulmonary nonasbestos mineral content in 20 patients who had no occupational dust exposure. The lungs were autopsied and 3- to 5-g pieces were dissolved in bleach and the treated sediment was transferred to a electron microscope grid. Mineral fibers were identified using electron diffraction and energy dispersive x-ray spectroscopy. No correlations were between numbers or types of fibers and age, sex, or smoking. Attapulgite was identified in 12/20 patients and approximately 8400/106000 fibers (7.9%) were Attapulgite. Further mineralogical analysis revealed 100% of the Attapulgite fibers were 1 to 4.9 μm in length.

Kaolin

Churg (1983) surveyed the total pulmonary nonasbestos mineral content in 20 patients who had no occupational dust exposure. The lungs were autopsied and 3- to 5-g pieces were dissolved in bleach and the treated sediment was transferred to an electron microscope grid. Mineral fibers were identified using electron diffraction and energy dispersive x-ray spectroscopy. No correlations were between numbers or types of fibers and

TABLE 22
Summary of carcinogenicity data

Procedure	Dose/concentration	Result	Reference
	Aluminum Silicate		
Single intrapleural injections of four samples into rats (lived life span)	20 mg (0–40 μm)	3 malignant mesotheliomas (1 pleural and 2 peritoneal)	Pigott and Ishmael 1992
	Calcium Silicate		
Single intraperitoneal injections into rats (lived life span)	25 mg	Little dust or dust-related fibrosis was visible; no mesotheliomas	Bolton et al. 1986
Chronic inhalation exposure for 1 year in rats	10 mg/m^3	Interstitial fibrosis, 1 small squamous cell carcinoma, 1 adenoma in lungs	Bolton et al. 1986
	Attapulgite		
Single intraperitoneal injections into rats	25 mg	Tumor incidence rate was 67%	Pott, Huth, and Friedrichs 1974
Single direct pleural application to left pleural surface of rats (killed 2 years later)	40 mg	17/615 of treated rats developed pleural sarcomas	Stanton et al. 1981
Single intrapleural injections into rats (lived life span)	20 mg/ml of 0.9% NaCl (0.77 μm)	No mesothelial neoplasms in either control or treated rats	Jaurand et al. 1987
Single intraperitoneal injections into rats (lived life span)	No concentrations given (fiber lengths ranged from 0 to 25 μm)	46 mesotheliomas	Wagner, Griffiths, and Munday 1987
Single intrapleural injections into rats (lived life span)	20 mg (0.77 μm)	No mesotheliomas	Renier et al. 1989
Single intrapleural injections into rats (lived life span)	0.5, 2, 4, 8, 16, or 32 mg (<1 μm)	2/140 had mesotheliomas	Coffin, Cook, and Creason 1992
3 samples were injected one time each week for 9 weeks into rats (surviving animals were killed at 2.5 years)	60 mg (0.04 to 0.8 μm)	Noncarcinogenic results for all three samples	Pott et al. 1987
Single intraperitoneal injections were administered for 3 weeks in rats (killed at 2.5 years)	2, 4, and 4 mg (1.3 and 0.07 μm)	40% of 30 rats had neoplasms	Pott et al. 1987
Inhalation chamber exposure to rats for 6 h/day for 5 day/week (killed at 3, 6, and 12 months)	10 mg/m^3	2 mesotheliomas, 2 peritoneal mesotheliomas, 1 malignant alveolar neoplasm, 2 benign alveolar neoplasms, 11 bronchoalveolar hyperplasias	Wagner, Griffiths, and Munday 1987
	Zeolite		
Oral administration for 104 weeks in rats	1, 10, 100, or 1000 mg/kg	No incidence of neoplastic changes	Gloxhuber et al. 1983
Single intratracheal instillations into rats (killed at end of study)	30 and 60 mg (< 5 μm)	No significant increase in the incidence of any specific neoplasm	Tatrai and Ungv'ary 1983
Single intraperitoneally injections into mice (10 month study)	10 or 30 mg (< 5 μm)	No neoplastic changes were observed	Suzuki 1982
Single intraperitoneal injection into mice	10 mg (<3 μm)	Mild peritoneal fibrosis but no neoplasms	Suzuki and Kohyama 1984

(Continued on next page)

TABLE 22
Summary of carcinogenicity data *(Continued)*

Procedure	Dose/concentration	Result	Reference
Single intraperitoneal injections into mice (7–23-month exposure)	10 mg (2.24 μm)	No mesotheliomas observed	Suzuki and Kohyama 1984
Single intrapleural injection into rats (chronic study)	20 mg	1 pleural and 1 peritoneal mesothelioma	Wagner et al. 1985
Single intraperitoneal injections into rats (141 weeks)	25 mg	1 peritoneal mesothelioma	Maltoni and Minardi 1988
Single intrapleural injections in rats	25 μm	No difference in tumor incidence between control and treated groups	Maltoni and Minardi 1988
Single subcutaneous injections	25 μm	No difference in tumor incidence between control and treated groups	Maltoni and Minardi 1988
3 intrapleural injections were given in monthly increments to rats	20 mg (3 to 500 μm)	2 mesotheliomas and 1 bronchial carcinoma/93 treated animals	Pyev et al. 1986
3 intrapleural injections were given in monthly increments to rats	20 mg (5 to 100 μm)	Neoplasms were found in 41/101 animals	Pyev et al. 1986
Inhalation exposure to rats for 7 h/day, 5 days/week for 1 year (lived life span)	10 mg/m^3	1 mesothelioma and 1 pulmonary adenocarcinoma	Wagner et al. 1985

age, sex, or smoking. Kaolin was identified in 12/20 patients and approximately 3500/106000 (3.3%) fibers were Kaolin. Further mineralogical analysis revealed 94% of the Kaolin fibers were 1 to 4.9 μm in length.

Morgan et al. (1988) surveyed and studied the prevalence of ventilatory impairment, chest symptoms, and radiographic abnormalities in over 2000 Kaolin workers representing over 95% of the current employees in the industry. Of the participants, 19% admitted having a cough. Of those participants with a cough, 17% had an abnormal FEV and 14% had an abnormal VC. Of those without a cough, 5.5% had an abnormal FEV and 7% had an abnormal VC. Also, 18% of the participants admitted to chronic sputum production. Of those with sputum production, 16% had abnormal FEV, and 12.5% had abnormal VC. Of those without the production, 6% had an abnormal FEV, and 7.5% had an abnormal VC. About 30% of the participants complained of shortness of breath, 3.1% was 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. Of these cases, 3.16% had category 2 pneumoconiosis, 1.0% had category 5, and 0.25% had category 3. Eighteen subjects (0.89%) had complicated pneumoconiosis. Of these cases, five had stage A, eight had stage B, and five had stage C. 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 FEV and forced vital capacity (FVC), 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 FEV 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 (Morgan et al. 1988).

Waxweiler et al. (1988) evaluated the possible health effects of occupational exposure to Attapulgite. A cohort study of 2302 men employed for at least 1 month at an Attapulgite mining and milling facility was followed through 1975. A significant deficit of mortality from nonmalignant respiratory disease (NMRD) was observed based on age, calendar year, and rates was observed. A marked deficit of NMRD 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 years in high-exposure-level jobs. An increased mortality was observed for gastric cancer (six observed) and a deficit due to nonmalignant respiratory disease was observed (nine observed).

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 (Wagner et al. 1996). Fifty-four of the 62 men worked with china clay or china stone. All the test subjects were employed in the mining industry. Test subjects were divided into groups according to their contact with the minerals: dusty china clay; wet, nondusty china clay; china stone; other dusty environments. The authors of this publication define china clay as "consisting mainly of the mineral kaolinite and in most other countries it is referred to as Kaolin." China stone "consists essentially of a mixture of quartz, feldspars, micas, and amorphous silicon dioxide." 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.

Mineralological analysis of the 42 cases revealed two separate groups of mineral composition and one miscellaneous group. The china clay group was composed of $\geq$90% Kaolinite in its samples consisted of 16 cases. The other distinct group, the clay and stone group, was composed of <90%; Kaolinite and greater contents of subsidiary components including quartz comprised 16 cases. The other group had a large variation of mineral composition. Lung-dust concentrations were greatest in the china clay group as shown in Table 23.

The grades of nodular fibrosis ranged in the china clay group from 0 (none) to 2 (moderate—up to 7 nodules/section or nodules of 3 to 6 mm in diameter). In china stone/clay group half, 8 of 16, were grade 3 (severe—more than 7 nodules/section or 6 to 10 mm in diameter). An increasing quartz concentration appears to be related to nodular fibrosis. Interstitial fibrosis in group ranged from 1 (slight—fibrosis located around respiratory bronchioles, which may extend into alveolar ducts and adjacent alveoli, but with areas remaining free of fibrosis between adjacent respiratory bronchioles) to 3 (severe—widespread diffuse fibrosis with few recognizable alveoli; honeycomb may or may not be present). No correlation was found between Kaolinite concentration and interstitial fibrosis grades; however, the china clay group had little exposure to anything but china clay. The degree of interstitial fibrosis appears to be more related to dust lung concentrations, although these results failed to reach statistical significance (Wagner et al. 1996).

TABLE 23
Dust concentrations in lung tissue of deceased men who worked in the mining industry (Wagner et al. 1996)

	Lung dust concentrations (mg/g)		
Mineral group	Minimum	Maximum	Median
China Clay (a)	7.6	289.3	40.0
China Stone/Clay (b)	4.1	44.8	15.0
Miscellaneous (c)	1.6	28.7	6.5

The ACGIH does not classify Kaolin as a human carcinogen and gives a TLV-TWA of 2 mg/m^3 for respirable dust and total dust (ACGIH 1997).

Zhang, Zhang, and Song (1997) reported the results of environmental monitoring and health surveillance performed on 781 Pyrophyllite miners and Pyrophyllite dust carvers from the years of 1954 to 1986. Routine radiographs of the workers lungs were studied for lesions of pneumoconiosis. The PM workers were divided into three groups, manual drillers (A), mechanical dry drillers (B), and mechanical wet drillers (C). The PCM workers were divided in two groups, carvers in factories (A) and carvers working at home (B).

PM workers, group B, had a greater incidence (43.5%) of pneumoconiosis than all other groups. In order to exclude the effect of the duration of exposure (DE), the DE-adjusted prevalence rate was calculated. The DE-adjusted rates are as follows, PM groups, 36.6% and PCM groups, 14.4% of pneumoconiosis (Zhang, Zhang, and Song 1997).

Case Reports

Aluminum Silicate

Sherwin (1979) found abnormal numbers of birefringent particles in the lungs of seven patients: five vineyard workers, one farmer, and one rural resident. A spectrum of early-to-late interstitial inflammation and fibrosis were seen. Nodular granulomas seen in silicosis were absent. Mineralogical analysis revealed mostly silicates, i.e., aluminum and potassium silicate.

Musk, Greville, and Tribe (1980) reported a case of a 42-year-old woman who had no history of previous exposure to Aluminum Silicate dust until she started working at an alunite-residue bagging mill. Chemical analysis of the alunite-residue showed 48.5% of constituents to be Al_2O_3 and 35.0% to be SiO_2. Eight months after working, she noticed the onset of dry cough and shortness of breath. Within 3 months these signs lasted throughout the day. She remained working for 18 months and after leaving work, the cough completely subsided within 3 months. She also complained of pain and morning stiffness in joints, wrists, elbows, and right knee. Corticosteroid treatment was started after a lung biopsy. A chest film taken 3 months after the onset of symptoms had lesions of diffuse small irregular opacities throughout both lungs. Subsequently, pulmonary function tests revealed a decrease in transfer factor for carbon monoxide (TL) and effective alveolar volume (TL/VA) and abnormal transpulmonary pressure–lung volume relationships. Pulmonary lesions included examination interstitial infiltration with small round cells, variable fibrosis, and scattered granulomas. Alveoli were distorted and the granulomas were moderately well formed with multinucleate giant cells and epithelioid histiocytes. After corticosteroid treatment, no increase in severity of the lung lesions was seen.

Calcium Silicate

A 23-year-old man was involved in the bagging process of a food additive. The food additive produced a white thin layer of powder that continuously covered the work floor. An antibiotic, carboxymethylcellulose, and Calcium Silicate comprised the food additive. On the third day of working, the patient experienced an itchy eruption on his face, neck, and forearms. The rash was erythematopapular with no vesicles. The redness was not diffuse and patches of erythema and papules were confluent on the neck and forearms. All signs faded the following morning. The rash occurred again when the patient returned to work. Patch tests were performed using the food additive, an antibiotic, carboxymethylcellulose, and Calcium Silicate. All tests were negative and there were no clinical signs of irritation at the test sites. No late reaction was recorded either. A sample of the food additive was examined under the microscope. Analysis revealed sharp-edged particles corresponding to Calcium Silicate. It was determined that the Calcium Silicate dust caused an "airborne irritant contact reaction." The problem was eliminated by increasing the humidity in the workplace and aspirating the air (Lachapelle 1984).

Bentonite

Phibbs, Sundin, and Mitchell (1971) reported many case studies involving Bentonite workers. Some milling plants had dangerous concentrations of silica that ranged from 2 to 10 times the safe maximal concentration according to the U.S. Bureau of Mines. Silicotuberculosis developed in four patients studied.

Austin and Doughman (1980) reported a 20-year-old dental assistant who noted a foreign body in her right eye after using a drill to polish a patient's teeth with Prophypaste. Immediately she noticed decreased vision and photophobia. Several opaque deposits superficially embedded in her right cornea were removed within 2 h. There was no evidence of corneal perforation or iritis. A residual superficial corneal infiltrate was noted paracentrally. An anterior uveitis developed and was treated. One month after the injury, the cornea was edematous with a superficial, peripheral ringlike stromal infiltrate and a deep inferior stromal infiltrate. A retrocorneal abscess was present. There was no eyelid edema present. Culture results were negative. Anterior segment inflammation, progression of the corneal edema, and an enlarged ring abscess in the corneal stroma continued. There was complete loss of red reflex and iris detail. The diagnosis was infectious endophthalmitis and anterior chamber and vitreous aspirations were performed. No organisms were seen but a few PMN leukocytes were present in the aspirations. These authors undertook the toxicity studies in rabbits presented in the ocular animal toxicity section under Bentonite. They concluded that the similarity of the findings in animals after injection of Bentonite with the findings in this case report suggested that Bentonite was the responsible agent in the dental assistant's symptoms.

Fuller's Earth

Tonning (1949) reported a man having worked in a Fuller's Earth plant as a young man. The length of employment was estimated at no more than 15 years. He was diagnosed with terminal aspiration pneumonia, pneumoconiosis due to Fuller's Earth exposure, bilateral emphysema, and fibrous pleural adhesions. 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%).

Sakula (1961) reported two cases of pneumoconiosis due to Fuller's Earth (Table 24). Mineralogical analysis of the Fuller's Earth established Montmorillonite as the major component.

Kaolin

Lynch, Harrison, and Nagelschmidt (1954) investigated two case studies of men who worked in a Kaolin-processing plant for many years. The lungs of the two persons and chest x-ray films were evaluated. The first case was a 36-year-old man who worked on the plant for 17 years. 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-year-old man who worked in a Kaolin-processing plant for 21 years. Within his last 3 years, he had dyspnea and a slight cough with small

TABLE 24
Pneumoconiosis cases reportedly linked to exposure to Fuller's Earth (Sakula 1961)

Patient	Symptoms
Male who worked in a Fuller's Earth processing plant for 42 years	Fine to medium miliary mottling of both lungs; sputum examinations were negative for *M. tuberculosis*; slowly deteriorating pulmonary function; recurrent bronchitis
Male who worked for 28 years in milling	Chronic cough and sputum; fine miliary mottling throughout both lungs; increasing dyspnea

TABLE 25
Pneumoconiosis cases reportedly linked to exposure to Kaolin (Hale et al. 1956)

Patient	Symptoms	Diagnosis
44-year-old man; worked in a Kaolin mill for 28-years	Cough with thick white sputum; easily dysponeic on slight exertion; well-marked nodulation of silicotic type with coalesence of the nodules in several areas and emphysema	Pneumoconiosis
67-year-old man; worked in china clay bagging for nearly his entire life	Several years of a productive cough; emphysema; massive fibrosis on both sides; no evidence of neoplasm	Pneumoconiosis
44-year-old man; worked in china clay bagging for nearly his entire life	Diffuse nodular mottling with considerable attenuation of the bronchovascular markings	Pneumoconiosis
39-year-old man; worked 14 years with clay	Fine miliary mottling in both lungs; well-marked calcification at the left hilum	Pneumoconiosis
73-year-old man; worked 12 years in open limestone quarries	Small discrete nodular mottling with an increase in the root shadows and the lung markings	Pneumoconiosis
64-year-old man; 43 years loading china clay	Cough and shortness of breath; emphysema; definite nodular mottling	Pneumoconiosis

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).

Hale et al. (1956) reported six cases of pneumoconiosis due to Kaolin. These are given in Table 25 and not further discussed here.

Butz (1970) reported that a 47-year-old man who was a chronic intravenous drug user died from tetanus. The man had been injecting paregoric, a camphorated opium tincture containing 35 to 46 mg of morphine per 100 ml. Paregoric can be found in proprietary preparations that do not require prescriptions; intravenous drug users often attempt to separate the paregoric from the Kaolin. Often the injection of Kaolin, either through shunts in the lung of an intravenous drug user with obliterative pulmonary arteritis and angiomatoid formations or by extrusion from the arterial lumen and transfer to the pulmonary veins, allows the Kaolin crystals to go into the peripheral circulation. In this patient, numerous skin abcesses were noted on the neck, shoulders, upper extremities, chest, thighs, and lower extremities. In skin sections, the lesions were multiple foreign body granulomata and large birefringent crystals. Adhesions over the pleural surface of the lungs were also noticed. At microscopic examination the lungs had foreign body granulomata within the pulmonary arterioles. Extensive pulmonary edema and masses of pigmented histiocytes filled the alveolar spaces. Extensive periportal fibrosis was seen in the liver. The central nervous system lesions were extremely fine, double refractile particles in nerve bundles entering the anterior roots in the central region.

Herman, Olscamp, and Weisbord (1982), reported a patient with multiple pulmonary Kaolin granulomas. 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.

Lapenas and Gale (1983) reported that a 35-year-old man who worked at a Kaolin-processing plant for 17 years complained of chest pain and was hospitalized. For the previous 2 years 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 $8 \times 12 \times 10$-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.

Lapenas et al. (1984) obtained pulmonary tissue from five Kaolin workers with advanced pneumoconiosis. 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 comprised 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.

Levin et al. (1996) investigated the death of a 62-year-old man who worked in a cotton textile mill for 43 years. 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 bronchiolectasis, 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.

Magnesium Trisilicate

Lee et al. (1993) reported a case of a 30-year-old female with a long-term history of ingesting trisilicate-containing antacids. The patient had repeated attacks of renal colic but the presence of calculi could not be determined by intravenous pyelography nor ureteroscopy. X-ray diffraction did detect a silicate stone. The patient stopped taking trisilicate containing products. The frequency of stone passage decreased and the renal colic was relieved.

Montmorillonite

A 73-year-old Montmorillonite worker developed signs of pneumoconiosis. A chest radiograph was taken 2 years before his death and a bilateral fine reticulonodular shadowing was observed. The man died of acute gastrointestinal hemorrhage from a benign gastric ulcer. A few weeks before his death another chest radiograph 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 to 5 mm in diameter that occupied most of the lungs were found. 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. There was a slight degree of fibrosis associated with the dust lesions and the neoplasm was a poorly differentiated adenocarcinoma containing giant cell areas. Mineralogical analysis showed a large amount of calcium Montmorillonite (Gibbs and Pooley 1994).

Zeolite

Casey et al. (1985) reported a patient living in the Nevada desert who developed extensive pleural thickening and interstitial fibrous associated with the pulmonary deposition of Zeolite. An open biopsy of the right lung and pleura was performed on the 52-year-old man. Mycobacterial and fungal cultures were negative. Histopathological evaluation established lesions of chronic inflammation and fibrosis and presence of many fibrous and nonfibrous particles. The particles were analyzed by SEM and were identified as aluminum silicates. The analytic pattern was characteristic of Zeolites. No asbestos fibers were found and exposure to these fibers was unlikely.

Zirconium Silicate

A nonsmoking 25-year-old woman developed a worsening dry cough and dyspnea after 3.5 years as a tile sorter and glazer. The woman had a history of atopic dermatitis and at age 13 developed pneumonia. An open lung biopsy specimen had lesions of a severe granulomatous interstitial pneumonia with mild fibrosis and numerous very small birefringent crystals around the terminal airways and occasionally in the granulomas. Pulmonary particle analysis established a dust burden almost 100 times the normal. The particles consisted mainly of clay minerals and Zirconium Silicate (Lippo et al. 1993).

SUMMARY

This report provides a review of the safety of Aluminum, Calcium, Lithium Magnesium, Lithium Magnesium Sodium, Magnesium Aluminum, Magnesium, Sodium Magnesium, and Zirconium Silicates, Magnesium Trisilicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite. These ingredients are termed silicates because they contain silicon, oxygen, and one or more metals. Many silicates occur naturally and are mined; yet others are made synthetically.

Typical cosmetic uses of silicates include abrasive, opacifying agent, viscosity-increasing agent, anticaking agent, emulsion stabilizer, binder, and suspending agent. Clay silicates (silicates containing water in their structure) primarily function as adsorbents, opacifiers, and viscosity-increasing agents. Pyrophyllite is also used as a colorant. Current concentrations of use range from as low as 0.01% for Zeolite to a high of 84% for Kaolin. Some ingredients with no uses reported to FDA in 1998 have current concentrations of use reported by the industry, so it is assumed they are in use.

Aluminum Silicate is approved as an indirect food additive in the Code of Federal Regulations (21 CFR 177.2600 and 21 CFR 177.1200). VEEGUM, a tradename for Magnesium Aluminum Silicate, has been designated by the FDA as a raw material with the following number: FD CRMCS no. R0010045 and has an individual Chemical Abstract Registry number, 12199-37-0. According to the European Cosmetic Directive (EU reference no. 391 Annex II), zirconium and its compounds are listed under substances that must not form part of the composition of cosmetic products, with the exception of complexes in Annex III, Part I. IARC has ruled Attapulgite fibers >5 μm as group 2B, *possibly carcinogenic to humans*, and fibers <5 μm as group 3, *not classified as to their carcinogenicity to humans* (IARC 1997). Bentonite is considered GRAS as a direct food additive (21 CFR 184.1155). Kaolin is considered GRAS as an indirect

food additive (21 CFR 186.1256). Pyrophyllite is listed as a naturally occurring color additive in the Code of Federal Regulations (21 CFR 73.1400). The natural Zeolites (Clinoptilolite, Phillipsite, Mordenite, Nonfibrous Japanese Zeolite) and synthetic Zeolites *cannot be classified as to their carcinogenicity to humans* (group 3) according to IARC (1997). Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Trisilicate, Attapulgite, Hectorite, and Kaolin are all used in over-the-counter products.

Hectorite and Montmorillonite catalyzed glycine and diglycine oligomerization reactions; oligomers were formed by self-condensation of both purines and pyrimidines in the presence of Montmorillonite treated with Na^+. Under UV light, adenosine monophosphate molecules were absorbed onto Kaolin and the products were hydrolyzed by phosphodiesterase.

All silicates have the great ability to absorb, especially the clays. Reports describe drugs, bacteria, viruses, and toxins absorbed to clays due to the physical structure of clays and their cationic nature.

No statistically significant absorption of aluminum and elevated levels of silicon were recorded in assayed plasma samples of dogs given Magnesium Trisilicate and Zeolite orally. The urinary excretion of silica was 5.2% in males given 20 g of Magnesium Trisilicate. Ten percent Bentonite in the diets of rats overcame T-2 toxicosis completely. Various Zeolites were added to the diets of pigs. No adverse effects were noted by the supplementation.

A sample of Aluminum Silicate was toxic to pulmonary alveolar macrophages and LDH activity and β-GAL release were increased. Aluminum Silicate had relatively no effect on the hemolysis of rat RBCs. Synthetic Calcium Silicate samples and higher concentrations of Calcium Silicate caused increased hemolysis of human RBCs; a greater fibrous character of Calcium Silicate samples caused increased LDH and β-GAL release. Many clays (Attapulgite, Bentonite, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite) demonstrated cytotoxicity to several macrophage type cell lines and have hemolytic activity towards several species' RBCs. Particle size, fibrogenicity, concentration, and mineral composition had the greatest effect on toxicity. Larger particle size and longer and wider fibers cause more adverse effects. In most of the studies, a dose-dependent effect on cytotoxicity or lysis was observed. Most mineral samples were not 100% pure and many samples already contained toxic dusts or minerals like quartz or cristobalite.

The following are a list of acute oral LD_{50} determinations: Calcium Silicate, 3400 mg/kg in rats; Magnesium Aluminum Silicate, 50000 mg/kg in mice; Zirconium Silicate, $>$200 g/kg in mice; Hectorite, $>$5 g/kg in rats; Kaolin, 149 g/kg in rats (death due to bowel obstruction); 15 natural Zeolites, 10 g/kg in rats. In short-term oral toxicity studies, no adverse effects were seen in mice or rabbits dosed up to 5 g/kg Magnesium Aluminum Silicate; beagle dogs and rats fed Aluminum Silicate had no renal lesions. Dogs and rats fed Magnesium Trisilicate for 4 weeks had polydypsia and polyuria, and all dogs had renal cortical lesions. Guinea pigs had renal lesions after 4 months of drinking Magnesium Trisilicate in their tap water. Rats fed 10% Magnesium Aluminum Silicate had slightly elevated silicon levels of the spleen and dogs and rats fed 10% VEEGUM had no negative responses in 90-day feeding studies. No lesions were found in rats dosed up to 1000 mg/kg for 104 weeks.

The following results are from acute parenteral injection studies. Intratracheal injections of Aluminum Silicate caused lesions in a dose-dependent manner and the intrapleural injections of four different Aluminum Silicate samples all resulted in lesions. One aluminosilicate injection caused three malignant mesotheliomas, one pleural and two peritoneal. No mesotheliomas developed in rats injected intraperitoneally with 25 mg of Calcium Silicate dust. Subcutaneous injection into the oral mucosa and into the back, periosteal injections into periosteal tissue, and intramuscular injections into the thigh of rats and guinea pigs with Zirconium Silicate resulted in mild inflammatory reactions. Attapulgite was injected intraperitoneally, intrapleurally, and intratracheally in various studies. Most studies reported that lesions and mesotheliomas were dependent on fiber length. Samples with a longer length caused greater numbers of mesotheliomas. Subplantar injections of Bentonite caused granulomas. Intratracheal injections of Bentonite and group C *Streptococcus* species caused an 85% mortality compared to a 5% control mortality in mice; another intratracheal injection caused loose reticulin fibrils with no collagen. Kaolin injected with the *Streptococcus* species caused statistically significant but modest mortality in mice. In a series of intrapleural injections, Kaolin was used as a negative control. Heat treated Montmorillonite dosed to rats by means of intratracheal instillation was restricted to alveoli within and adjacent to alveolar ducts. Minor inflammatory reactions, but no lesions, were found in rats given intratracheal injections of Clinoptilolite, and intraperitoneal injections of Mordenite, Synthetic Zeolite 4A, and synthetic Zeolite MS5A (one mesothelioma was seen in rats given MS4A). An intrapleural injection of Nonfibrous Japanese Zeolite caused two mesotheliomas in rats.

Small primary neoplastic lesions were found in two rats exposed to a Calcium Silicate sample in an inhalation chamber. The mass of silicate measured in the lungs ranged from 0.1 to 0.8 mg. Lebrija and Leichester Attapulgite samples caused one peritoneal mesothelioma, one adenocarcinoma, and three bronchoalveolar hyperplasia and two mesotheliomas, one peritoneal mesothelioma, one malignant alveolar tumor and eight bronchoalveolar hyperplasia (inhalation route) in rats, respectively. Both samples contained long fibers. Moderate to extensive respiratory disease was noted in rats chronically exposed to Synthetic Zeolite A by inhalation methods.

The acute dermal LD_{50} was $>$3.5 g/kg for rabbits exposed to VEEGUM. Magnesium Aluminum Silicate (4%) was a weak primary skin irritant in rabbits and had no cumulative skin irritation in guinea pigs. No gross effects were reported in any of these studies. Sodium Magnesium Silicate (4%) had no primary skin irritation in rabbits and had no cumulative skin irritation in

guinea pigs. Hectorite was nonirritating to the skin of rabbits in a Draize primary skin irritation study.

A 4% solution of Magnesium Aluminum Silicate and a 4% solution of Sodium Magnesium Silicate caused minimal eye irritation in a Draize eye irritation test. Bentonite caused severe iritis after injection into the anterior chamber of the eyes of rabbits. When injected intralamellarly, widespread corneal infiltrates and retrocorneal membranes were recorded. In a primary eye irritation study in rabbits, Hectorite was moderately irritating without washing and practically nonirritating to the eye with a washout. Rats tolerated a single dose of Zeolite A without any adverse reaction in the eye.

Calcium Silicate (250 to 1600 mg/kg) had no discernible effect on nidation or on maternal or fetal survival in rabbits. Magnesium Aluminum Silicate (6000 mg/kg) had neither a teratogenic nor adverse effects on the mouse fetus. Female rats receiving a 20% Kaolin diet exhibited maternal anemia but no significant reduction in birth weight of the pups was recorded. Type A Zeolite produced no adverse effects on the dam, embryo, or fetus in either rats or rabbits at any dose level (74 or 1600 mg/kg). Clinoptilolite had no effect on female rat reproductive performance.

No increase mutation frequencies were seen in the *Salmonella* TA-1530 or G-46 assay and no significant increase in recombinant activity in the *Saccharomyces* D3 assay treated with Calcium Silicate. A subacute dose of 150 mg/kg of Calcium Silicate produced 3% breaks in bone marrow cells arrested in c-metaphase. In a metaphase spread of bone marrow cells, Calcium Silicate produced no significant increase in the number of aberrations compared to controls and in a dominant lethal assay did not induce any dominant lethal mutations. In the *S. typhimurium* LT2 spot test (TA98, TA100, TA1535, TA1537, and TA1538) with or without metabolic activation, Magnesium Aluminum Silicate and Hectorite were found nonmutagenic. In primary hepatocyte cultures, the addition of Attapulgite had no significant unscheduled DNA synthesis (UDS) response or modulated response to AAF (a positive control); Attapulgite at 10 μg/cm^2 caused significant increases in UDS in rat pleural mesothelial cells. Zeolite particles ($<$10 μm) produced statistically significant increase in the percentage of aberrant metaphases, mostly chromatid breaks.

Applications of 2 g of VEEGUM made to the skin of two humans daily for 1 week caused no effects.

Occupational exposure to mineral dusts has been studied extensively. Fibrosis and pneumoconiosis has been documented in workers involved in the mining and processing of Aluminum Silicate, Calcium Silicate, Zirconium Silicate, Fuller's Earth, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite.

DISCUSSION

The CIR Expert Panel determined that the data provided in this report are sufficient to assess the safety of the tested ingredients: Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite. The Panel did note a concern about inhalation of these ingredients due to reported cases of pneumoconiosis and fibrosis in humans and pulmonary lesions in animals. However, extensive pulmonary damage in humans was the result of direct occupational inhalation of the dusts and lesions seen in animals were affected by particle size, fiber length, and concentration. The Panel recognizes that most of the formulations are not respirable and of the preparations that are respirable, the concentration of the ingredient is very low. Even so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation.

Note: The cosmetic ingredient, *Talc*, is a hydrated magnesium silicate with the chemical composition of $Mg_3Si_4O_{10}(OH)_2$. Talc occurs in various forms and has a unique crystalline structure which differs from ingredients addressed in this safety assessment. Talc is not included in this report.

CONCLUSION

The CIR Expert Panel concludes that Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite are safe as used in cosmetic products.

REFERENCES

Adachi, S., K. Kawamura, S. Yoshida, and K. Takemoto. 1992. Oxidative damage on DNA induced by asbestos and man-made fibers in vitro. *Int. Arch. Occup. Environ. Health* 63:553–557.

Adamis, Z., M. Timar, L. Koefler, E. Tatari, and G. Ungari. 1986. Biological effects of the respirable dusts from ore mines. *Environ. Res.* 41:319–326.

Akers, M. J., J. L. Lach, and L. J. Fischer. 1973. Alterations in adsorption of dicumarol by various excipient materials. *J. Pharm. Sci.* 62:391–395.

American Conference on Governmental Industrial Hygienists (ACGIH). 1997. *Threshold limit values and biological exposure indices for 1997*. Cincinnati, OH: ACGIH.

American Minerals, Inc. 1998. Material safety data sheet on zirconium silicate. Unpublished data submitted by CTFA. 4 pages.[2]

Angino, E. E. 1964. Far-infared spectra of montmorillonite, kaolin, and illite. *Nature* 204:569–571.

Armstrong, N. A., and C. D. Clarke. 1971. The adsorption of crystal violet by Kaolin. *J. Pharm. Pharmacol.* 23:95S–100S.

Austin, P. S., and D. J. Doughman. 1980. Reaction to introcular penetration of bentonite. *Am. J. Ophthalmol.* 89:719–723.

Babhair, S. A., and M. Tariq. 1983. Effect of magnesium trisilicate and kaolin-pectin on the bioavailability of trimethoprim. *Res. Commun. Chem. Pathol. Pharmacol.* 40:165–168.

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036–4702, USA.

Banin, E., and H. Meiri. 1990. Toxic effects on alumino-silicates on nerve cell. *Neuroscience* 39:171–178.

Barr, M. 1963. General characteristics and applications of the montmorillonite hydrocolloids. *Am. Perfumer Cosmet.* 78:2–37.

Barr, M., and E. S. Arnista. 1957. Adsorption studies on clay I. The adsorption of two alkaloids by activated attapulgite, halloysite, and kaolin. *J. Am. Pharm. Assoc.* 46:486–489.

Bartko, P., L. Vrgula, M. Prosbova', and J. Blazovsky'. 1983. The effect of the administration of zeolite (clinoptilolite) on the health condition of sheep. *Vet. Med.* 28:481–492.

Beck, E. G., and J. Bignon. eds. 1985. In vitro *effects of mineral dusts.* NATO ASI Series, vol. G3. Berlin: Springer-Verlag.

Be'gin, R., S. Masse', M. Rola-Pleszcynski, M. Geoffroy, M. Martel, Y. Desmarais, and P. Sebastien. 1987. The lung biological activity of American attapulgite. *Environ. Res.* 42:328–339.

Belmonte, A. A. 1994. Bentonite. In *Handbook of Pharmaceutical Excipients*, 2nd ed., ed. A. Wade and P. J. Weller, 24–26. Washington, DC: American Pharmaceutical Association.

Benke, G. M., and T. W. Osborn. 1979. Urinary silicon excretion by rats following oral administration of silicon compounds. *Food Cosmet. Toxicol.* 17:123–127.

Beveridge, A., and W. F. Pickering. 1983. The influence of surfactants on the adsorption of heavy metal ions by clays. *Water Res.* 17:215–226.

Bish, D. L., and G. D. Guthrie Jr. 1993. Mineralogy of clay and zeolite dusts (exclusive of 1:1 layer silicates). In *Reviews in mineralogy, vol. 28, Health effects of mineral dusts*, ed. G. D. Guthrie Jr., and B. T. Mossman, 163–181. Chelsea, MI: Brook Crafters.

Blevins, R. D., and D. E. Taylor. 1982. Mutagenicity screening of twenty-five cosmetic ingredients with salmonella/microsome test. *J. Environ. Sci. Health Part A* 17:217–239.

Bolton, R. E., J. Addison, M. G. Davis, K. Donaldson, A. D. Jones, B. G. Miller, and A. Wright. 1986. Effects of the inhalation of dusts from calcium silicate insulation materials in laboratory rats. *Environ. Res.* 39:26–43.

Brouillard, M. Y., and J. G. Rateau. 1989. Adsorption potency of 2 clays, smectite and kaolin on bacterial endotoxins. In vitro study in cell culture and the intestine of newborn mice. *Gastroenterol. Clin. Biol.* 13:18–24.

Brown, R. C., M. Chamberlain, R. Davies, and G. T. Sutton. 1980. The in vitro activities of pathogenic mineral dusts. *Toxicology* 17:143–147.

Browne, J. E., J. R. Feldkamp, J. L. White, and S. L. Hem. 1980. Characterization and adsorptive properties of pharmaceutical grade clays. *J. Pharm. Sci.* 69:816–823.

Budavari, S., ed. 1989. *The Merck index. An encyclopedia of chemicals, drugs, and biologicals*, 11th ed. Rahway, NJ: Merck & Co.

Bujdak, J., and B. M. Rode. 1996. The effect on smectite composition on the catalysis of peptide bond formation. *J. Mol. Evol.* 43:326–333.

Butz, W. C. 1970. Disseminated magnesium and aluminum silicate associated with paregoric addiction. *J. Forensci. Sci.* 15:581–587.

Carlson, B. C. 1977. Veegum in cosmetic gels and sticks. *Cosmet. Tolietries* 92:81–86.

Carrol, D. 1959. Ion exchange in clays and other minerals. *Bull. Geol. Soc. Am.* 70:749–780.

Carson, M. S., and T. K. Smith. 1982. Effect of non-nutritive mineral additives and fibers on T-2 toxicosis in male weanling rats. *J. Animal Science* 53:284.

Carson, M. S., and T. K. Smith. 1983. Role of bentonite in prevention of T-2 toxicosis in rats. *J. Anim. Sci.* 57:1498–1506.

Casey, K. R., J. W. Shigeoka, W. N. Rom, and F. Moatamed. 1985. Zeolite exposure and associated pneumoconiosis. *Chest* 87:837–840.

Cefali, E. A., J. C. Nolan, W. R. McConnell, and D. L. Walters. 1995. Pharmacokinetic study of zeolite A, sodium aluminosilicate, magnesium silicate and aluminum hydroxide in dogs. *Pharm. Res.* 12:270–274.

Cefali, E. A., J. C. Nolan, W. R. McConnell, and D. L. Walters. 1996. Bioavailability of silicon and aluminum from zeolite A in dogs. *Int. J. Pharm.* 127:147–154.

Chamberlain, M., R. Davies, R. C. Brown, and D. M. Griffiths. 1982. In vitro tests for the pathogenicity of mineral dusts. *Ann. Occup. Hyg.* 26:583–592.

Churg, A. 1983. Nonasbestos pulmonary mineral fibers in the general population. *Environ. Res.* 31:189–200.

Coffin, D. L., P. M. Cook, and J. P. Creason. 1992. Relative mesotheliomas induction in rats by mineral fibers: Comparison with residual pulmonary mineral fiber number and epidemiology. *Inhal. Toxicol.* 4:273–300.

Cosmetics Directive of the European Union. 1995. Updated version—Incorporating all amendments until August 1, 1995. Dir 76/768EEC, Annex III, 12.

Cosmetic Ingredient Review (CIR). 1980. Final report on the safety assessment of Quaternium-18 Hectorite, Quaternium-18, and Quaternium-18 Bentonite. Washington: CIR. 25 pages.[2]

Cosmetic, Tolietry, and Fragrance Association (CTFA). 1970a. Safety data of magnesium aluminum silicate. Unpublished data submitted by CTFA. 4 pages.[2]

CTFA. 1970b. Safety data of Sodium Magnesium Silicate. Unpublished data submitted by CTFA. 4 pages.[2]

CTFA. 1999a. Concentrations of use of cosmetic ingredients. Unpublished data submitted by CTFA.[2]

CTFA 1999b. VEEGUM is nontoxic and nonirritating. Unpublished data submitted by CTFA. 1 page.[2]

Davies, R., D. M. Griffiths, N. F. Johnson, A. W. Preece, and D. C. Livingston. 1984. The cytotoxicty of kaolin toward macrophages in vitro. *Br. J. Exp. Pathol.* 65:453–466.

Davies, R., and A. W. Preece. 1983. The electrophoretic mobilities of minerals determined by laser Doppler velocimetry and their relationship with the biological effect of dusts toward macrophages. *Clin. Phys. Physiol. Meas.* 4:129–140.

Denizeau, F. M., G. Marion, G. Chevalier, and M. G. Cote. 1985a. Ultrastructural study of mineral fiber uptake by hepatocytes in vitro. 26:119–126.

Denizeau, F. M., G. Marion, G. Chevalier, and M. G. Cote. 1985b. Absence of genotoxic effects of nonasbestos mineral fibers. *Cell Biol. Toxicol.* 1:23–32.

Ditter, B., R. Urbaschek, and B. Urbascek. 1983. Ability of various adsorbents to bind endotoxins in vitro and to prevent orally induced endotoxemia in mice. *Gastroenterology* 84:1547–1552.

Dobbie, J. W., and M. J. Smith. 1982. Silicate nephrotoxicity in the experimental animal: The missing factor in analgesic nephropathy. *Scot. Med. J.* 27: 10–16.

Dougherty, S. H., V. D. Fiegel, R. D. Nelson, G. T. Rodeheaver, and R. L. Simmons. 1985. Effects of soil infection potentiating facots on neutrophils in vitro. *Am. J. Surg.* 150:306–311.

Drachman, S. R., G. E. Roch, and M. E. Smith. 1997. Solid state NMR characterization of the thermal transformation of Fuller's Earth. *Solid State Nucl. Magn. Reson*. 9:257–267.

Drucker, M. M., J. Goldhar, P. L. Ogra, and E. Neter. 1977. The effect of attapulgite and charcoal on enterotoxicity of *Vibrio cholerae* and *Escherichia coli* entertoxins in rabbits. *Infection* 5:211–213.

Durnev, A. D., N. O. Dauger-Dauge, L. G. Korkina, and S. B. Seredenin. 1993. Peculiarities of the clastogenic properties of chrysotile-asbestos fibers and zeolite particles. *Mutat. Res.* 319:303–308.

Dvora'k, M. 1989. Ability of bentonite and natural zeolite to absorb aflatoxin from liquid media. *Vet. Med. (Praha.)* 34:307–316.

Edwards, M. S., M. S. Harrison, M. R. Jalks-Miller, N. Nakayama, M. S. Berger, and D. H. Glick. 1984. Kaolin-induced congenital hydrocephalus *in utero* in fetal lambs and rhesus monkeys. *J. Neurosurg.* 60:115–122.

El-Nakeeb, M. A., and R. T. Youssef. 1968. Influence on various materials in antibiotics in liquid pharmaceutical preparations. *Acta. Pharm*. 5:1–8.

Englemard. 1998. Dear customer letter re fiber length for Englehard attapulgite products. Unpublished data submitted by Englehard Corporation. 1 page.[2]

Ertem, G., and J. P. Ferris. 1998. Formation of RNA oligomers on montmorillonite site of catalysis. *Orig. Life Evol. Biosph.* 28:485–499.

Evcim, N., and M. Barr. 1955. Adsorption of some alkaloids by different clays. *J. Am. Pharm. Assoc. Sci. Edn.* 44:570–573.

Federation of American Societies for Experimental Biology. 1977. Evaluation of the health aspects of bentonite and clay(kaolin) as food ingredients. NTIS report No. PB276416.

Ferreira, J. M., and Y. M. Freitas. 1976. Microbiological surveys of talcum powders and raw materials. *Cosmet. Toiletries* 91:19–26.

Ferris, J. P., A. R. Hill, R. Liu, and L. E. Orgel. 1996. Synthesis of long prebiotic oligomers on mineral surfaces. *Nature* 381:59–61.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. Washington, DC: FDA.

FDA. 1994. *OTC Drug Review Ingredient Status Report.* September 1, 1994.

FDA. 1998. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Food and Drug Research Labs. (FDRL), Inc. 1958a. 90-Day feeding study using rats. Unpublished data submitted by R. T. Vanderbilt Co., Inc. 16 pages.[2]

FDRL, Inc. 1958b. 90-Day feeding study using dogs. Unpublished data submitted by R. T. Vanderbilt Co., Inc. 7 pages.[2]

FDRL, Inc. 1973. Tetraologic evaluation of FDA 71-41 (hydrated calcium silicate). NTIS report No. PB223829.

FDRL, Inc. 1980a. Primary skin irritation study in rabbits. Unpublished data submitted by Rheox, Inc. 9 pages.[2]

FDRL, Inc. 1980b. Acute oral toxicity in rats study. Unpublished data submitted by Rheox, Inc. 12 pages.[2]

FDRL, Inc. 1981. Primary eye irritation study with hectorite. Unpublished data submitted by Rheox, Inc. 15 pages.[2]

Fugiyoshi, T., I. Hayashi, and S. Oh-ishi. 1989. Kaolin-induced writhing response in mice activation of the plasma kallidrein-kinin system by kaolin. *J. Pharmacobiol. Dyn.* 12:483–487.

Gamble, J. F. 1986. Silicate pneumoconiosis. In *Occupational respiratory diseases*, ed. J. A. Merchant, NIOSH publication no. 86–102.

Gantzer, C., F. Quignon, and L. Schwartzbrod. 1994. Poliovirus-1 adsorption onto and desorption from montmorillonite in seawater. survival of the adsorbed virus. *Environ. Technol.* 15:271–278.

Garcia, J. G. N., R. F. Dodson, and D. S. Callahan. 1989. Effect of environmental particulates on cultured human and bovine endothelium. *Lab. Invest.* 61:53–61.

Ghazy, F. S., A. A. Kassem, and S. H. Shalaby. 1984. Adsorption characteristics of certain antibiotics to Veegum and a charcoal. *Pharmazie* 39:821–823.

Gibbs, A. R., and F. D. Pooley. 1994. Fuller's earth (montmorillonite) pneumoconiosis. *Occup. Environ. Med.* 51:644–646.

Gormley, I. P., and J. Addison. 1983. The in vitro toxicity of some standard clay mineral dusts of respirable size. *Clay Minerals* 18:153–163.

Gormely, I. P., M. J. Kowolik, and R. T. Cullen. 1985. The chemiluminescent response of human phagocytic cells to mineral dusts. *Br. J. Exp. Pathol.* 66:409–416.

Gamble, J. F. 1986. *Silicate pneumoconiosis. Occupational respiratory diseases*, ed. J. A. Merchant, 243–285. Appalachian Laboratory for Occupational Safety and Health (NIOSH), U.S. Department of Health and Human Services, DHHS (NIOSH), Publication no. 86-102.

Gloxhuber, C., M. Potokar, W. Pittermann, S. Wallat, F. Bartnik, H. Reuter, and S. Braig. 1983. Zeolite A—a phosphate substitute for detergents: Toxicological investigation. *Food Chem. Toxicol.* 21:209–220.

Grim, R. E., ed. 1972. *Clay mineralogy*, 2nd ed. New York: McGraw-Hill Book Co.

Hale, L. W., J. Gough, E. J. King, G. Nagelschmidt. 1956. Pneumoconiosis of kaolin workers. *Br. J. Ind. Med.* 13:251–259.

Hansen, D., and B. T. Mossman. 1987. Generation of superoxide formation (O_2^-) from alveolar macrophages exposed to asbestiform and nonfibrous particles. *Cancer Res.* 47:1681–1686.

Harding, H. E. 1948. The toxicology of Zircon: Preliminary report. *Br. J. Ind. Med*. 5:73–76.

Harvey, G., M. Page, and L. Dumas. 1984. Binding of environmental carcinogens to asbestos and mineral fibers. *Br. J. Ind. Med.* 41:396–400.

Hatch, G. E., E. Boykin, J. A. Graham, J. Lewtas, F. Pott, K. Loud, and J. L. Mumford. 1985. Inhalable particles and pulmonary host defense: *In vivo* and *in vitro* effects of ambient air and combustion particles. *Environ. Res.* 36:67–80.

Hazelton Laboratories, Inc. 1968. Acute ocular and dermal testing with magnesium aluminum silicate. Unpublished data submitted by R. T. Vanderbilt Co., Inc.17 pages.[2]

Healy, D. P., A. B. Dansereau, A. B. Dunn, C. E. Clenedning, A. W. Mounts, and G. S. Deepe Jr. 1997. Reduced tetracycline bioavailabilty caused by magnesium aluminum silicate in liquid form of bismuth subsalicylate. *Ann. Pharmacother.* 31:1460–1464.

Herman, S. J., G. C. Olscamp, and G. L. Weisbord. 1982. Pulmonary kaolin granulomas. *J. Can. Assoc. Radiol.* 33:279–280.

Hevilin, F. G., and Murray, H. H. 1994. Clays. Hormites: palygorskite (attapulgite) and sepiolite. In *Industrial minerals and rocks*, 6th ed., ed. D. D. Carr, 159–173. Littleton, CO: Society for Mining, Metalurgy, and Exploration.

Hunt, H., F. D. Pooley, and R. J. Richards. 1981. Biological activity of calcium silicate composites-in vitro studies. *Environ. Res.* 26:51–68.

International Agency for Research on Cancer (IARC). 1997. *IARC monographs on the evaluation of carcinogenic risks of chemicals to humans. Silica and some silicates*, vol. 47. Lyon, France: IARC.

Informatics, Inc. 1974. Scientific literature reviews on generally recognized as safe (GRAS) food ingredients—bentonite and clay. NTIS report no. PB234893.

Inveresk Research International. 1995. Mutagenicity assay of hectorite with five strains of *S. typhimurium* bacteria. Unpublished data submitted by Rheox, Inc. 40 pages.[2]

Jaurand, M. C., J. Fleury, G. Monchaux, M. Nebut, and J. Bignon. 1987. Pleural carcinogenic potency of mineral fibers asbestos attapulgite and their cytotoxicity in cultured cells. *J. Natl. Cancer Inst.* 79:797–804.

Keeting, P. E., M. J. Oursler, K. E. Wiegand, S. K. Bonde, T. C. Spelsberg, and B. L. Riggs. 1992. Zeolite A increases proliferation, differentiation, and transforming growth factor production in normal adult human osteoblast-like cells in vitro. *J. Bone Min. Res.* 7:1281–1289.

Keller, W. D. 1979. Clays. In *Kirk-Othmer encyclopedia of chemical technology*, vol. 6, 3rd ed., ed. M. Grayson, 202. New York: John Wiley & Sons.

Kelse, J. W. 1997. Crytalline silica risk information. Unpublished data submitted by R. T. Vanderbilt Co., Inc. 14 pages.[2]

Khali, S. A. H., L. M. Mortada, and M. El-Khawas. 1984a. Uptake of ampiciliin and amoxycillin by some adsorbents. *Int. J. Pharm.* 18:157–167.

Khali, S. A. H., L. M. Mortada, and M. El-Khawas. 1984b. Decreased bioavailability of ampicillin and amoxycillin in presence of kaolin. *Int. J. Pharm.* 19:233–238.

Kleber, C. J., and M. S. Putt. 1986. Plaque removal by chewing gum containing zirconium silicate. *Compend. Contin. Educ. Dent.* 7:681–685.

Korkina, L. G., T. B. Suslova, S. I. Nikolova, G. N. Kirov, and B. T. Velichkovsky. 1984. The mechanism of cytotoxic action of the natural zeolite clinoptilotile. *Farmakol. Toksikol.* 47:63–67.

Kruglikov, G. G., B. T. Velichkovsky, and T. I. Garmash. 1990. Morphology of pneumoconiosis induced with the natural zeolite. *Gig. Tr. prof. Zabol.* 5:14–17.

Kruglikov, G. G., B. T. Velichkovsky, T. I. Garmash, and V. M. Volkogonova. 1992. Functional and structure changes in macrophages of lungs during the phagocytosis of the natural zeolite clinoptilolite. *Gig. Tr. prof. Zabol.* 11–12:44–46.

Kukita, T., A. Yamaguchi, A. Okamoto, and M. Nemoto. 1992. Interaction between polyethylene films and bromohexine HCL in solid dosage form. IV. Prevention of the sorption by the addition of magnesium aluminum silicate. *Chem. Pharm. Bull. (Tokyo)* 40:1257–560.

Lachapelle, J. M. 1984. Occupational airborne irritant contact reaction to the dust of a food additive. *Contact Dermatitis* 10:250–254.

Lapenas, D. J., and P. N. Gale. 1983. Kaolin pneumoconiosis. A case report. *Arch. Pathol. Lab. Med.* 107:650–653.

Lapenas, D. J., P. Gale, T. Kennedy, W. Rawlings, and P. Dietrich. 1984. Kaolin pneumoconiosis: Radiological, pathological, and mineralogical findings. *Am. Rev. Respir. Dis.* 130:282–288.

Lee, M. H., Y. H. Lee, T. H. Hsu, M. T. Chen, and L. Chang. 1993. Silica stone development due to long time oral trisilicate intake. *Am. J. Ind. Med.* 27:267–269.

Lemaire, I. 1991. Selective differences in macrophage populations and monokine production in resolving pulmonary granuloma and fibrosis. *Am. J. Pathol.* 138:487–495.

Lemaire, I., P. G. Dionne, D. Nadeau, and J. Dunnigan. 1989. Rat lung reactivity to natural and man-made fibrous silicates following short-term exposure. *Environ. Res.* 48:193–210.

Levin, J. L., A. L. Frank, M. G. Williams, et al. 1996. Kaoliniosis in a cotton mill worker. *Am. J. Ind. Med.* 29:215–221.

Lewis, R. J., Sr. 1993. *Hawley's condensed chemical dictionary*, 12th ed. New York: van Nostrand Reinhold.

Lide, D. R., ed. 1993. *CRC handbook of chemistry and physics*, 74th ed. Boca Raton, FL: CRC Press, Inc.

Lippo, K. K., A. L. Anttila, O. Taikina-Aho, et al. 1993. Hypersensitivity pneumonitis and exposure to zirconium silicate in a young ceramic tile worker. *Am. Rev. Respir. Dis.* 148:1089–1092.

Lipson, S. M., and G. Stotzky. 1984. Effect of proteins on reovirus adsorption to clay minerals. *Appl. Environ. Microbiol.* 48:525–530.

Lipson, S. M., and G. Stotzky. 1985. Specificity of virus adsorption to clay minerals. *Can. J. Microbiol.* 31:50–53.

Litton Bionetics, Inc. 1974. Mutagenic evaluation of Compound FDA 71-41, calcium silicate. NTIS report No. PB245457.

Lynch, K. M., C. V. Harrison, and G. Nagelschmidt. 1954. Pneumoconiosis from exposure to kaolin dust: kaolinosis. *Am. J. Pathol.* 30:1117–1122.

Maltoni, C., and F. Minardi. 1988. First available results of long-term carcinogenicity bioassay on detergency zeolites (MS 4A and MS 5A). In *Living in a Chemical World*, vol. 534, ed. C. Maltoni and I. J. Selikoff, 978–985. New York: New York Academy of Sciences.

M'anyai, S., J. Kabai, J. Kis, E. Suveges, and M. Timar. 1969. The in vitro hemolytic effect of various clay minerals. *Med. Lav.* 60:331–342.

M'anyai, S., J. Kabai, J. Kis, E. Suveges, and M. Timar. 1970. The effect of heat treatment on the surface of kaolin and its in vitro hemolytic activity. *Environ. Res.* 3:187–198.

Marek, J., and V. Blaha. 1985. Some methological and morphological aspects of bentonite-induced inflammatory reaction in the rat. *Acta Univ. Palacke Olkmuc. Fac. Med.* 108:151–170.

Martin, J. C., H. Daniel, and L. Le Bouffant. 1975. Short-term and long-term experimental study of the toxicity of coal-mine dust and its constituents. *Inhal. Part.* 4:361–371.

McClurg, H. J., R. D. Beck, and P. Powers. 1980. The effect of a kaolin-pectin adsorbent on stool losses of sodium, potassium, and fat during a lactose-intolerance diarrhea in rats. *J. Pediatr.* 96:769–771.

McCollum, F. T., and M. L. Galyean. 1983. Effects of clinoptilolite on rumen fermentation, digestion and feedlot performance in beef steers fed high concentrate diets. *J. Anim. Sci.* 56:517–524.

McGinity, J. W., and J. L. Lach. 1976. In vitro adsorption of various pharmaceuticals to montmorillonite. *J. Pharm. Sci.* 65:896–902.

Morgan, W. K., A. Donner, I. T. T. Higgins, et al. 1988. The effects of kaolin on the lung. *Am. Rev. Respir. Dis.* 138:813–820.

Mossman, B. T., and R. O. Be'gin. eds. 1989. In vitro *effects of mineral dusts.* NATO ASI Series, vol. H30. Berlin: Springer-Verlag.

Mossman, B. T., and J. E. Craighead. 1982. Comparative carcinogenic effects of crocidolite asbestos, hematite, kaolin, and carbon in implanted tracheal organ cultures. *Ann. of Occup. Hyg.* 26:553–567.

Munch, J. C. 1944. Oral and dermal toxicity studies on VEEGUM. Unpublished data submitted by R. T. Vanderbilt Co., Inc. 2 pages.[2]

Munch, J. C. 1945. Toxicity report on VEEGUM using mice and rabbits. Unpublished data submitted by R. T. Vanderbilt Co., Inc. 2 pages.[2]

Murphy, E. J., E. Roberts, D. K. Anderson, and L. A. Horrocks. 1993b. Cytotoxicity of aluminum silicate in primary neuronal cultures. *Neuroscience* 57:483–490.

Murphy, E. J., E. Roberts, and L. A. Horrocks. 1993a. Aluminum silicate toxicity in cell cultures. *Neuroscience* 55:597–605.

Musk, A. W., B. D. Beck, H. W. Greville, J. D. Brain, and D. E. Bohannon. 1988. Pulmonary disease from exposure to an artificial aluminum silicate: further observations. *Br. J. Ind. Med.* 45:246–250.

Musk, A. W., H. W. Greville, and A. E. Tribe. 1980. Pulmonary disease from occupational exposure to an artificial aluminum silicate used for cat litter. *Br. J. Ind. Med.* 34:367–372.

Nadeau, D., L. Fouquette-Couture, D. Paradis, J. Khorami, D. Lane, and J. Dunnigan. 1987. Cytotoxicity of respirable dusts from industrial minerals: comparison of two naturally occurring and two man-made silicates. *Drug Chem. Toxicol.* 10:40–86.

National Academy of Sciences. 1996. *Food chemicals codex*, 4th ed. Washington, DC: National Academy Press.

Nikitakis, J. M., and G. N. McEwen Jr., eds. 1990a. *CTFA compendium of cosmetic ingredient composition—Specifications*. Washington, DC: CTFA.

Nikitakis, J. M., and G. N. McEwen Jr., eds. 1990b. *CTFA Compendium of Cosmetic Ingredient Composition—Descriptions I and II*. Washington, DC: CTFA.

Nolen, G. A., and T. A. Dickerman. 1983. Test for aluminosilicate teratogenicity in rats. *Food Chem. Toxicol.* 21:697.

Nolen, R. P., A. M. Langer, and G. B. Herson. 1991. Characterization of palygorskite specimens from different geological locales for health hazard evaluation. *Br. J. Ind. Med.* 48:463–475.

Novakova, J. 1977. Effect of clays on microbe adsorption. *Zentralbl. Bakteriol. Parasitenkd. Intefektionskr. Hyg.* 132:418–422.

Oberson, D., L. Desfontaines, H. Pezerat, W. Hornebeck, P. Sebastien, and C. Lafuma. 1996. Inhibition of human leukocyte elastase by mineral dust particles. *Am. J. Physiol.* 270:761–771.

Oscarson, D. W., G. E. Van Scoyoc, and J. L. Ahlrichs. 1981. Effect of Poly-2-vinylpyridine-*N*-oxide and sucrose on silicate-induced hemolysis of erythrocytes. *J. Pharm. Sci.* 70:657–659.

Page, R. C., R. R. Heffner, and A. Frey. 1941. Urinary excretion of silica in humans following oral administration of magnesium silicate. *Am. J. Dig. Dis.* 8:13–15.

Palmieri, A. 1994. Magnesium Aluminum Silicate. In *Handbook of pharmaceutical excipients*, 2nd ed., 269–273. Washington, DC: American Pharmaceutical Association.

Patterson, E. C., and D. J. Staszak. 1977. Effects of geophagia (kaolin ingestion) on the maternal blood and embryonic development in the pregnant rat. *J. Nut.* 107:2020–2025.

Pepe, R. C., J. A. Wenninger, and G. N. McEwen Jr., eds. 2002. *International cosmetic ingredient dictionary and handbook*, 9th ed. vols. 1–3. Washington, DC: CTFA.

Perderiset, M., L. Saint Etrienne, J. Bignon, and M. C. Jaurand. 1989. Interactions of attapulgite (fibrous clay) with human red blood cells. *Toxicol. Lett.* 47:303–310.

Phibbs, B. P., R. E. Sundin, and R. S. Mitchell. 1971. Silicosis in Wyoming bentonite workers. *Am. Rev. Respir. Dis.* 103:1–17.

Pigott, G. H., and J. Ishmael. 1992. The effects of intrapleural injections of aluminum and aluminum silicate (ceramic fibers). *Int. Exp. Pathol.* 73:137–146.

Pond, W. G., and J. T. Yen. 1983a. Protection by clinoptilolite or zeolite NaA against cadmium-induced anemia in growing swine (41652). *Proc. Soc. Exp. Biol. Med.* 173:332–337.

Pond, W. G., and J. T. Yen. 1983b. Reproduction and progeny growth in rats fed clinoptilolite in the presence or absence of dietary cadmium. *Bull. Environ. Contam. Toxicol.* 31:666–672.

Pond, W. G., J. T. Yen, and J. D. Crouse. 1989. Tissue mineral element content in swine fed clinoptilolite. *Bull. Environ. Contam. Toxicol.* 42:735–742.

Porter, T. L., M. P. Eastman, M. E. Hagerman, L. B. Price, and R. F. Shand. 1998. Site-specific prebiotic oligomerization reactions of glycine on the surface of hectorite. *J. Mol. Evol.* 47:373–377.

Pott, F., F. Huth, and K. H. Friedrichs. 1974. Neoplasmigenic effect of fibrous dusts in experimental animals. *Environ. Health Perspect.* 9:313–315.

Pott, F., U. Ziem, F. J. Reiffer, F. Huth, H. Ernst, and U. Mohr. 1987. Carcinogenicity studies on fibers, metal compounds, and some other dusts in rats. *Exp. Pathol.* 32:129–152.

Pylev, L. N., R. G. Bostashvilli, T. F. Kulagina, L. A. Vasilyeva, N. F. Chelishchev, and B. G. Bernstein. 1986. Assessment of carcinogenic activity of zeolite clinoptilolite. *Gig. Tr. prof. Zabol.* 5:29–34.

Ramos, A. J., J. Fink-Gremmels, and E. Hernandez. 1996. Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. *J. Food Prod.* 59:631–641.

Registry of Toxic Effects of Chemical Substances (*RTECS*). 1999. *RTECS database*. Bestheda, MD: National Library of Medicine.

Rempe, J. L., and L. G. Santucci. 1998. *CTFA list of Japanese cosmetic ingredients*, 3rd ed. Washington, DC: CTFA.

Reiner, A., J. Fleury, G. Monchaux, M. Nebut, J. Bignon, and M. C. Jaurand. 1989. Toxicity of an attapulgite sample studied *in vivo* and *in vitro*. *IARC Sci. Publ*. 90:180–184.

Reiss, B., J. R. Millette, and G. M. Williams. 1980. The activity of environmental samples in a cell culture test for asbestos toxicity. *Environ. Res.* 22:315–321.

Renier, A., F. Levy, F. Pilliere, and M. C. Jaurand. 1989. Unscheduled DNA synthesis in rat pleural mesothelial cells treated with mineral fibers. *Mutat. Res.* 24:361–368.

Rheox Inc. 1999. The benefits of hectorite clay and safety data sheet on Bentone MA (purified hectorite). Unpublished data submitted by Rheox Inc. 3 pages.[2]

Richards, R. J., T. D. Tetley, and J. Hunt. 1981. The biological reactivity of calcium silicate composites: In vivo studies. *Environ. Res.* 26:243–257.

Rogers, R. D., and J. C. MacFarlane. 1981. Sorption of carbon tetrachloride, ethylene dibromide, and trichloroethylene on soil and clay. *Environ. Monit. Assess.* 1:155–162.

Roskill Information Services Ltd. 1988. *The economics of zeolites*, 1st ed. London: Author.

Ross, C. S., and P. F. Kerr. 1931. The Kaolin clays. *U.S. Geological Survey Profession Paper* 165E:151–175.

Sadik, F. 1971. X-ray diffraction analysis for identification of kaolin NF and bentonite USP. *J. Pharm. Sci.* 60:916–918.

Said, S., and H. Al-Shora. 1980. Adsorption of certain oral hypoglycemics on kaolin and charcoal and its relationship to hypoglycemic effects of drugs. *Int. J. Pharm.* 5:223–228.

Said, S. A., A. M. Shibal, and M. E. Abdullah. 1980. Influence of various agents on adsorption capacity of kaolin for *Pseudomonas aeruginosa* toxin. *J. Pharm. Sci.* 69:1238–1239.

Sakai, K., and K. Moriguchi. 1975. Effect of magnesium aluminosilicate administered to pregnant mice on pre- and post-natal development of offspring. *Oyo Yakuri (Pharmacometrics)* 9:704–714.

Sakula, A. 1961. Pneumoconiosis due to fuller's earth. *Thorax* 16:176–179.

Schiffenbauer, M., and G. Stotzky. 1982. Adsorption of coliphages T1 and T7 to clay minerals. *Appl. Environ. Microbiol.* 43:90–96.

Schreider, J. P., M. R. Culbertson, and O. G. Raabe. 1985. Comparative pulmonary potential of selected particles. *Environ. Res.* 38:256–274.

Sherwin, R. P. 1979. Silicate pneumoconiosis of farm workers. *Lab. Invest.* 40:576–582.

Shibayama, Y., M. Nishioto, and K. Nakata. 1993. Role of microenvironmental deterioration of the bone marrow in the development of bone atrophy in magnesium silicate-treated rats. *Exp. Toxicol. Pathol.* 45:71–74.

Shurson, G. C., P. K. Ku, E. R. Miller, and M. T. Yokoyama. 1984. Effect of zeolite A or clinoptilolite in diets of growing swine. *J. Anim. Sci.* 59:1536–1545.

Skaug, V., R. Davies, and B. Glyseth. 1984. In vitro macrophage cytotoxicity of five calcium silicates. *Br. J. Ind. Med.* 41:116–121.

Skaug, V., and B. Gyseth. 1983. Hemolytic activity of five different calcium silicates. *Environ. Health Perspect.* 51:195–203.

Smith, T. K. 1980. Influence of dietary fiber, protein, and zeolite on zearalenone toxicosis in rats and swine. *J. Anim. Sci.* 50:278–285.

Snipes, M. B., B. B. Boecker, and R. O. McClellan. 1983a. Retention of monodisperse or polydisperse aluminosilicate particles inhaled by dogs, rats, and mice. *Toxicol. Appl. Pharmacol.* 69:345–362.

Snipes, M. B., B. A. Muggenburg, and D. E. Bice. 1983b. Translocation of particles from lung lobes or the peritoneal cavity to regional lymph nodes in beagle dogs. *J. Toxicol. Environ. Health* 11:703–712.

Stanton, M. F., M. Layard, A. Tegeris, E. Miller, M. May, E. Morgan, and A. Smith. 1981. Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. *J. Natl. Cancer Inst.* 67:965–975.

Steel, R. F., and W. Anderson. 1972. The interaction between kaolinite and *Staphylococcus aureus*. *J. Pharm. Pharmcol.* 24:129.

Stookey, G. K., J. L. McGuire, S. M. Standish, and J. C. Muhler. 1967. Studies concerning the biological properties of zirconium silicate. *J. Peridontol.* 38:53–63.

Stotzky, G. 1966. Influence of clay minerals on microorganisms: II. Effect on various clay species, homoionic species, and other particles on bacteria. *Can. J. Microbiol*. 12:831–848.

Stotzky, G., and L. T. Rem. 1966. Influence of clay minerals on microorganisms: I. Montmorillonite and kaolinite on bacteria. *Can. J. Microbiol.* 12:547–562.

Stotzky, G., and L. T. Rem. 1967. Influence on clay minerals on microorganisms: IV. Montmorillonite and kaolinites on fungi. *Can. J. Microbiol.* 13:1535–1550.

Strigunkova, T. F., G. A. Lavrentiev, and V. A. Ostroshchenko. 1986. Abiogenic synthesis of oligonucleotides on kaolinite under the action of ultraviolet radiation. *J. Mol. Evol.* 23:290–293.

Suzuki, Y. 1982. Carcinogenic and fibrogenic effects of zeolites: preliminary observations. *Environ. Res.* 27:433–445.

Suzuki, Y., and N. Kohyama. 1984. Malignant mesothelioma induced by asbestos and zeolite in the mouse peritoneal cavity. *Environ. Res.* 35:277–292.

Sykes, S. E., A. Morgan, J. C. Evans, A. Holmes, and S. R. Moores. 1982. Use of an in vivo test system to investigate the acute and sub-acute responses of the rat lung to mineral dusts. *Ann. Occup. Hyg.* 26:593–605.

Syracuse Research Corporation. 1981. Information profiles on potential occupational hazards: Aluminum and compounds. Second draft (revised). NTIS report no. PB89216238.

Tatrai, E., Z. Adamis, M. Tim'ar, and G. Ung'ary. 1983. Comparative histopathological and biochemical analysis of early stages of exposure to non-silicogenic aluminum silicate and strongly siliogenic quartz-dust in rats. *Exp. Pathol.* 23:163–171.

Tatrai, E., E. Ba'csy, J. Ka'rpa'ti, and G. Ungv'ary. 1992. On the examination of the pulmonary toxicity of mordenite in rats. *Polish J. Occup. Med. Environ. Health* 5:237–243.

Tatrai, E., and G. Ungv'ary. 1983. Study on carcinogenicity of clinoptilolite type zeolite in Wistar rats. *Polish J. Occup. Med. Environ. Health* 6:27–34.

Tatrai, E., and G. Ungv'ary. 1993. Study on carcinogenicity of clinoptilolite type zeolite in wistar rats. *Polish J. Occup. Med. Environ. Health* 6:27–34.

Tatrai, E., G. Ungv'ary, Z. Adamis, and M. Tim'ar. 1985. Short term in vivo method for prediction of the fibrogenic effect of different mineral dusts. *Exp. Pathol.* 28:111–118.

Tatrai, E., I. Wojn'arovits, and G. Ungv'ary. 1991. Non-fibrous zeolite induced experimental pneumoconiosis in rats. *Exp. Pathol.* 43:41–61.

Toilet Goods Association. 1969. Concentration and product use data. Unpublished data submitted by R. T. Vanderbilt Co., Inc. 2 pages.[2]

Tonning, H. O. 1949. Pneumoconiosis from fuller's earth. *J. Ind. Hyg. Toxicol.* 31:41–45.

United States Pharmacopeial Convention, Inc. 1994. The *United States Pharmacopeia*, vol. 23, and the *National Formulary*, vol. 18. Tauton, MA: Rand McNally.

Valatina, I. E., L. N. Pylev, and M. F. Lemjasev. 1994. Mutagenicity of the zeolite dusts. *Gig. Sanit.* 4:65–67.

van Hoof, J. H. C., and J. W. Roelofsen. 1991. Techniques of zeolite characterization. In *Introduction to zeolite science and practice*, H. van Bekkum, E. M. Flanigen, and J. C. Jansen, 241–283. Amsterdam: Elsevier.

Wagner, J. C., D. M. Griffiths, and D. E. Munday. 1987. Experimental studies with palygorskite dusts. *Br. J. Ind. Med.* 44:749–763.

Wagner, J. C., F. D. Pooley, A. Gibbs, L. Lyons, G. Sheers, and C. B. Moncrieff. 1996. Inhalation of china stone and clay dusts: Relationship between the

mineralogy of dust retained in the lungs and pathological changes. *Thorax* 41:190–196.

Wagner, J. C., J. W. Skidmore, R. J. Hill, and D. M. Griffiths. 1985. Eronite exposure and mesotheliomas in rats. *Br. J. Cancer* 51:727–730.

Wallace, W. E., V. Vallyathan, M. J. Keane, and V. Robinson. 1985. In vitro biological toxicity of native and surface-modified silica and kaolin. *J. Toxicol. Environ. Health* 16:415–424.

Waxweiler, R. J., R. D. Zumwalde, G. O. Ness, and D. P. Brown. 1988. A retrospecitve cohort mortality study of males mining and milling attapulgite clay. *Am. J. Ind. Med.* 13:305–315.

Wells, I. P., R. C. V. Bhatt, and M. Flanagan. 1985. Kaolinosis a radiological review. *Clin. Radiol.* 36:579–582.

Wenninger, J. A., R. C. Canterbery, and G. N. McEwen, Jr., eds. 2000. *International Cosmetic Ingredient Dictionary and Handbook,* 8th edn., vols. 1–3. Washington, DC: CTFA.

Williams, K. C., B. J. Blaney, and R. T. Peters. 1994. Pigs fed *Fusarium*-infected maize containing zearalenone and nivalenol with sweeteners and bentonite. *Livest. Prod. Sci*. 39:275–281.

Woodworth, C. D., B. T. Mossman, and J. E. Craighead. 1982. Comparative effects of fibrous and nonfibrous minerals on cells and liposomes. *Environ. Res.* 27:190–205.

Woodworth, C. D., B. T. Mossman, and J. E. Craighead. 1983. Induction of squamous metaplasia in organ cultures of hamster trachea by natural and synthetic fibers. *Cancer Res.* 43:4906–4912.

Wright, W. E., and F. Moatamed. 1983. Characterization of zeolite fiber sizes using scanning electron microscopy. *Arch. Environ. Health* 38:99–103.

Yegles, M., X. Janson, H. Y. Dong, R. Renier, and M. C. Jaurand. 1995. Role of fiber characteristics and induction of anaphase/telophase aberrations in rat pleural mesothelial cells in vitro: Correlations with in vivo animal findings. *Carcinogenesis* 16:2751–2758.

Zaidi, S. H., K. S. Dogra, S. Khanna, and R. Shanker. 1981. Experimental ineffective pneumoconiosis: Effect of fibrous and nonfibrous silicates and *Candida albicans* on the lungs of guinea pigs. *Ind. Health* 19:85–92.

Zhang, W. C., Q. F. Zhang, and Z. F. Song. 1997. Studies on the hazardous effects and the maximum allowable concentration of pyrophyllite dust. *Biomed. Environ. Sci.* 10:377–386.

Zumwalde, R. 1976. Industrial Hygiene Study. Englehard Minerals and Chemicals Corporation. Attapulgas, GA (NIOSH 00106935), Cincinnati, OH: National Instituite for Occupational Safety and Health. August 2, 1999.