GreenScreen® Assessment for Hydroxyapatite (nano) (1306-06-5)

Method Version: GreenScreen® Version 1.2[1]

Assessment Type[2]: Authorized

GreenScreen® Assessment Prepared By:	**GreenScreen® Assessment Quality Control Performed By:**
Name: Jennifer Sass; Nina Hwang	Name:
Title: Senior Scientist; Research Assistant	Title:
Organization: NRDC	Organization:
Date: May 9, 2016	Date:
Assessor Type (Licensed GreenScreen Profiler, Authorized GreenScreen Practitioner or Unaccredited): Authorized; Unaccredited	

[1] Use GreenScreen® Assessment Procedure (Guidance) V1.2
[2] GreenScreen reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen Practitioner), "CERTIFIED" (by Licensed GreenScreen Profiler or equivalent) or "CERTIFIED WITH VERIFICATION" (Certified or Authorized assessment that has passed GreenScreen Verification Program)

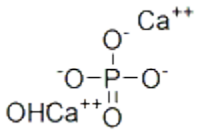
Confirm application of the *Disclosure and Assessment Rules and Best Practice*[3]: (List disclosure threshold and any deviations)

Chemical Name (CAS #): Hydroxyapatite (nano) (1306-06-5)

Also Called:
HA, Hydroxylapatite, Calcium phosphatetribasic, Calcium hydroxyphosphate

Suitable analogs or moieties of chemicals used in this assessment (CAS #'s):
No surrogates were used in the assessment.

Chemical Structure(s):
$Ca_5(PO_4)_3(OH)$



HA forms a hexagonal crystal structure.

Notes related to production specific attributes[4]:

Identify Applications/Functional Uses:
(e.g., Cleaning product, TV casing)
Hydroxyapatite (HA) is a naturally occurring compound found in bone mineral and teeth. HA can be taken as an oral supplement or added to infant formula as a source of calcium. It can also be added to oral care products such as toothpaste and mouthwash. HA has biomedical applications such as drug delivery matrices, bone repair, and coating implants.

Nano-HA has been used for all above-mentioned applications as well.

GreenScreen Benchmark Score and Hazard Summary Table:[5,6,7,8] Nano-hydroxyapatite (specifically needle-like nano-hydroxyapatite) has a preliminary BM score of 2 (based on moderate systemic toxicity and very high persistence; and moderate mutagenicity) and a final Benchmark score of Unknown, after Data Gap analysis.

[3] See GreenScreen Guidance V1.2
[4] Note any composition or hazard attributes of the chemical product relevant to how it is manufactured. For example, certain synthetic pathways or processes result in typical contaminants, by-products or transformation products. Explain any differences between the manufactured chemical product and the GreenScreen assessment of the generic chemical by CAS #.
[5] See Appendix A for a glossary of hazard endpoint acronyms
[6] See Appendix B for alternative GreenScreen Hazard Summary Table (Classification presented by exposure route)
[7] For inorganic chemicals only, see GreenScreen Guidance V1.2 Section 14.4. (Exceptions for Persistence)
[8] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen Guidance V1.2 Section 9.3.

Green Screen Hazard Ratings: Hydroxyapatite (nano)																			
Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeated *	single	repeated *										
DG	*M*	DG	DG	DG	L	*M*	*M*	DG	DG	*L*	DG	*L*	*M*	DG	DG	vH	DG	*L*	*L*

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in **BOLD** font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M and L) instead of three (i.e., H, M and L), and are based on single exposures instead of repeated exposures.

Environmental Transformation Products and Ratings[9]:
Identify feasible and relevant environmental transformation products (i.e., dissociation products, transformation products, valence states) and/or moieties of concern[10]

Hydroxyapatite is an inert, inorganic chemical. Transformation information on nano-hydroxyapatite specifically is not available.

Introduction

Nano-hydroxyapatite (needle-like) has been detected in commercially available infant formula produced by popular brands. Nano-hydroxyapatite is also marketed as an oral supplement and added to oral care products such as toothpaste and mouthwash.

While hydroxyapatite is a naturally occurring mineral critical for strong bones and teeth, significantly less health data is available for engineered nanoparticles. Because it is a mineral, nano-hydroxyapatite exists in various phases that may have different health impacts.

Nano-hydroxyapatite was assessed against GreenScreen® Version 1.2 (CPA 2013).

Toxicokinetics

While non-nano hydroxyapatite particles has low oral bioavailability, hydroxyapatite becomes increasingly soluble under acidic conditions. A screening study using needle-like nano-hydroxyapatite found some indication for penetration of mucosa-like human corneal epithelial cells. Using the few *in vivo* toxicity studies available, it can be extrapolated that nano-hydroxyapatite particles can be distributed to liver, kidneys and lungs.

Hazard Classification Summary Section:

For all hazard endpoints:

[9] See GreenScreen Guidance V1.2 Section 13
[10] A moiety is a discrete chemical entity that is a constituent part or component of a substance. A moiety of concern is often the parent substance itself for organic compounds. For inorganic compounds, the moiety of concern is typically a dissociated component of the substance or a transformation product.

- **Search all GreenScreen specified lists. Report relevant results either in each hazard endpoint section or attach to the end of the report.**
- **Always indicate if suitable analogs or models were used.**
- **Attach modeling results (See Appendix C).**
- **Include all references either in each hazard endpoint section or at the end of the report.**

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M or L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for carcinogenicity based on a lack of studies or listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

Mutagenicity/Genotoxicity (M) Score (H, M or L): *M*

Nano-hydroxyapatite was assigned a score of *Moderate* (low confidence) for mutagenicity. This is based on an *in vitro* study that found dose-dependent increases in sister chromatid exchanges, micronuclei, chromosome aberration rates, and 8-oxo-2-deoxyguanosine levels in human lymphocyte cells exposed to needle-like particles. Confidence is low due to poor material description study design limitations.

- Authoritative and Screening Lists: none

- *In vitro* and/or non-mammalian

Ames test was negative both with and without activation for two types of nano-hydroxyapatite (number 1#: rod-like; 10 – 20 nm in diameter; 30 – 50 nm in length; number 4#: rod-like, 20 – 40 nm in diameter; 70 – 90 nm in length) at a dose of 0.1g/mL (0.1 mL of extract). Ames tests may not be suitable for screening nanomaterials for genotoxicity and mutagenicity due to the size of bacteria, bacterial cell wall, and limited uptake of nanoparticles by bacteria.

Mouse lymphoma assay (OECD TG 476) was performed using two types of nano-hydroxyapatite (number 1#: rod-like; 10 – 20 nm in diameter; 30 – 50 nm in length; number 4#: rod-like, 20 – 40 nm in diameter; 70 – 90 nm in length). Incubation time was 3 hr with S9 and 24 hr without S9. No further information on the source of S9 was given. The assays were negative for mutagenicity both with and without metabolic activation.

A study investigated sister chromatid exchanges, micronucleus formation, chromosome aberration and 8-oxo-2-deoxyguanosine formation by **needle-shaped nanohydroxyapatite** (10-50nm) nm in cultured peripheral blood lymphocytes from 6 human donors. In **comparison to untreated cultures, dose-dependent increases - in part of statistical significance - of sister chromatid exchanges, micronuclei, chromosome aberration rates and 8-oxo-2-deoxyguanosine levels were observed in treated cultures**.

Reproductive Toxicity (R) Score (H, M, or L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for reproductive toxicity based on a lack of studies or listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M or L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for developmental toxicity based on a lack of studies or listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

Endocrine Activity (E) Score (H, M or L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for developmental toxicity based on a lack of studies or listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

Group II and II* Human Health Effects (Group II and II* Human)

Acute Mammalian Toxicity (AT) Group II Score: L

Nano-hydroxyapatite was assigned of score of **Low** (high confidence) for acute mammalian toxicity based on experimental data (the most conservative acute risk estimate is the oral rat LD50). The confidence is high because it is based on experimental data.

- Authoritative and Screening Lists: none
- Oral
  - LD50 for rats: 20,800 mg/kg. Defined as "Hydroxyapatite 5%, aqueous solution, 20 nm"
- IV
  - LD50 for mice >50 ml/kg bw (highest dose tested). Defined as "Hydroxyapatite, 0.9% Sodium Chloride in water"

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)

(ST-single) Group II Score (single dose: vH, H, M or L): *M*

Nano-hydroxyapatite was assigned a score of *Moderate* (low confidence) for systemic toxicity/organ effects of a single oral exposure based on animal data. A rat study found biochemical changes and oxidative damage in the liver following exposure via intra-peritoneal injection. These effects are further supported by inflammation observed in supplemental *in vitro* studies. Confidence is low because the animal study was poorly reported and the material was poorly characterized.

- Authoritative and Screening Lists: none

In vivo

- Oral

Poorly reported study exposed rats via gavage to nano hydroxyapatite 5% in an aquous solution at a dose range between 1600 and 36000 mg/kg bw. Death of the animals occurred from 10,000 mg/kg bw in both sexes as a result of cardiac and respiratory arrest. Animals that survived throughout the 2 week observation period had lung vessels that were **moderately congested, alveoli filled with air, and had signs of fatty degeneration of the liver**.

- Inhalation

Single study observing mice is too poorly reported.

- Injection

Rats were exposed via a single ip injection of 50 mg/kg bw nano-hydroxyapatite (**needle-like**; long diameter: 80 nm, short diameter: 20 nm; hydrodynamic diameter in physiological saline: 245.1 nm (i.e. agglomeration in physiological saline) and monitored for 48h. Compared to control livers which had normal histology, the liver tissues from the rats exposed to nanohydroxyapatite showed **inflammatory cell infiltration at the portal areas of the liver**. Haematological analysis demonstrated **increased white blood cells, elevated levels of the inflammatory cytokine TNF-α, and increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bile acid (TBA), cholesterol, uric acid, lactate dehydrogenase (LDH) and low density lipoprotein (LDL)** compared to controls. In the livers**, increased levels of H2O2 and MDA (malondialdehyde) and decreased levels of glutathione (p<0.05) were observed**.

Mice were exposed via a single iv injection of 3.8 g/kg bw and were not adversely affected within an observation period of 3 days.

Mice were exposed via a single ip injection of 50 mg/kg bw nano-hydroxyapatite extracted in sesame oil and observed for 3 days. Treatment did not adversely affect body weight.

In vitro

Informational only: Commercially available nano-hydroxyapatite of spherical shape, with a size of 51.1 ± 12.1 nm and a Zetapotential of -5.41±0.59 mV in DMEM/F12 biological medium, was used in an assay with commercially available human buccal epithelial cells TR146. Cells were cultivated for 12 hr with 125 and 1250 µM of FITC-labelled nano-hydroxyapatite, cytosolic and membrane fractions were prepared and fluorescence measurements performed. Cells at the higher treatment concentration were also analyzed microscopically. Intracellular reactive oxygen species was investigated by the 2′,7′-dichlorofluorescein diacetate assay (DFCH-DA assay) after cultivating cells for 24 hr in the presence of 0, 62.5, 125, 250, 500, and 1250 µM nano-hydroxyapatite. A **significant, concentration dependent increase in ROS formation** was observed reaching a 40% increase at 1250 µM. Counterstaining with MitoSox suggested that nano-hydroxyapatite stimulates **mitochondrial superoxide production in TR146 cells**. In the concentration range investigated, nanohydroxyapatite significantly **increased IL-6** (up to 4-fold) expression and NF-κB transcriptional activity compared to untreated controls.

A study on the effect of nano-hydroxyapatite (rod-like crystals, smallest parts 20 – 30 nm; less than 10% > 100 nm) on steroid hormone production and apoptosis was conducted in human ovarian granulosa cells. TEM results confirmed **uptake of nano-hydroxyapatite into granulosa cells and distribution** into membrane-bound compartments, including lysosomes, mitochondria and intracellular vesicles. The increased percentage of cells in S phase when cultured with nanoparticles indicated that there was an **arrest at the checkpoint from phase S-to-G2/**M (from 6.28 ± 1.55% to 11.18 ± 1.73%, $p < 0.05$). The increased ratio of S/(G2/M) implied the **inhibition of DNA synthesis and/or impairment in the transition from the S phase**. The **apoptosis rate of normal granulosa cells was 7.83 ± 2.67% and increased to 16.53 ± 5.56% ($p < 0.05$)** after the cells had been treated with 100 µM nano-hydroxyapatite for 48 hours.

There are no relevant case reports of people with adverse effects from nano-hydroxyapatite exposure.

(ST-repeat) Group II* Score (repeated dose: H, M, L): *M*

Nano-hydroxyapatite was assigned a score of *Moderate* (low confidence) for systemic toxicity/organ effects based on repeated exposure based on an animal study that found adverse effects on the liver and lung.

- Authoritative and Screening Lists: None

Neurotoxicity (N)

(N-single) Group II Score (single dose: vH, H, M or L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for neurotoxicity based on a lack of studies or listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

(N-repeat) Group II* Score (repeated dose: H, M, L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for neurotoxicity based on repeated exposure due to a lack of studies.

- Authoritative and Screening Lists: None

Skin Sensitization (SnS) Group II* Score (H, M or L): *L*

Nano-hydroxyapatite was assigned a score of *Low* (low confidence) for skin sensitization based on a negative study. Confidence is low because the test material is insufficiently characterized.

- Authoritative and Screening Lists: None
- *In vivo* mammalian
  Guinea pigs were exposed to needle-shaped nano-hydroxyapatite particles (<50nm) for 6 hours, 3x a week, 3 weeks. No indications for skin sensitization were observed in the study. The test material is insufficiently characterized.

Respiratory Sensitization (SnR) Group II* Score (H, M or L): DG

Nano-hydroxyapatite was assigned a score of DG for respiratory sensitization based on a lack of studies or listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

Skin Irritation/Corrosivity (IrS) Group II Score (vH, H, M or L): *L*

Nano-hydroxyapatite was assigned a score of *Low* (low confidence) skin irritation and corrosion based on negative results in voluntarily-exposed humans. The classification is low confidence because the study material was poorly categorized.

- Authoritative and Screening Lists: None

- *In vivo*, mammalian
  NZ rabbits were dermally exposed to nano-hydroxyapatite extracted in 0.9% sodium chloride or sesame oil via intracutaneous injections. Erythema or oedema were not observed.

Acute cutaneous irritation test (patch test) was performed on 10 Caucasion volunteers of both genders and consisted of a single application of material 1 at 25% (aqueous solution with adjusted pH of 5.2) for 48 hours. Erythema score 1 was reported in 2 volunteers after deduction of negative control score (no further information). Dryness of skin was reported for 5/10 volunteers. The study authors concluded that material 1 (Company B) 25% adjusted to pH 5.2 is nonirritating.

Eye Irritation/Corrosivity (IrE) Group II Score (vH, H, M or L): *M*

Nano-hydroxyapatite was assigned a score of *Moderate* (low confidence) based on animal data suggesting mild, transient irritation. Confidence is low because the materials are poorly characterized and the studies are poorly reported.

- Authoritative and Screening Lists: None
- *In vivo* – mammalian
  The eye irritation potential of material 1 was investigated in the Hen`s Egg Chorioallantoic Test. According to the HET-CAM, 25% of the material was **considered weakly or slightly irritant** with a score of 2.8 on the CAM, based on hyperaemia at the 2 min score. Three fertilised hen's eggs were incubated with each substance respectively (material 1 at a concentration of 25%, diluted in water, and 2 positive controls (1% SDS and 0.1M NaOH) and 1 negative control (0.9% NaCl)) for nine days, and on the 10th day, the eggs were opened and the CAM (chorioallantoic membrane) exposed. 0.3 g of the test substance was applied to the surface of the CAM and after a 20-second exposure period, the CAM was rinsed with 5 ml of sterile Milli-Q water.

  A material termed as "Hydroxyapatite 5%, aqueous solution, 20 nm" was investigated for effects on mucous membrane of the eye. 2 drops of substance were instilled into the conjunctival sac of a rabbit (apparently one animal, no further information) which was observed for 1 month. From the study it was concluded that the test material **causes slight and reversible irritation.**

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M or L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for acute aquatic toxicity based on a lack of studies and listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

Chronic Aquatic Toxicity (CA) Score (vH, H, M or L): DG

Nano-hydroxyapatite was assigned a score of Data Gap for chronic aquatic toxicity based on a lack of studies and listing by authoritative or screening bodies.

- Authoritative and Screening Lists: None

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vH

Nano-hydroxyapatite was assigned a score of **very high** (high confidence) for persistence based on the fact that it is an inorganic chemical. Confidence is high because it is listed on a Screening list.

- Authoritative and Screening Lists:
    - Screening List: CEPA DSL- Persistent

- Nano-hydroxyapatite is water insoluble.

Bioaccumulation (B) Score (vH, H, M, L, or vL): DG

Nano-hydroxyapatite was assigned a score of data gap for bioaccumulation based on a lack of any studies or listing by authoritative or screening bodies.

- Authoritative and Screening Lists: none

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M or L): *L*

Nano-hydroxyapatite was assigned a score of *Low* (low confidence) due to its NFPA reactivity rating of 0. It is not believed to be reactive.

- Authoritative and Screening Lists: none

The Safety Data Sheet (SDS) identifies "no data" for reactivity and "no data" for possibility of hazardous reactions. Incompatible materials are strong oxidizing agents. Under the firefighting measures, the SDS identifies "oxides of phosphorous, calcium oxide" as special hazards arising from the chemical. (Sigma Aldrich SDS 2014)

Flammability (F) Score (vH, H, M or L): *L*

Nano-hydroxyapatite was assigned a score of *Low* (low confidence) for flammability due to its NFPA flammability rating of 0.

- Authoritative and Screening Lists: none

The Safety Data Sheet (SDS) identifies "no data" for flammability (Sigma Aldrich MSDS 2014).

References (may be provided under each hazard endpoint or at the end of document)

Scientific Committee on Consumer Safety (SCCS) OPINION ON Hydroxyapatite (nano) SCCS/1566/15 Revision of 16 March 2016 http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_191.pdf

MSDS (2014) Hydroxyapetite, nanopowder <200 nm. Sigma-Aldrich. Material Safety Data Sheet. http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=677418&brand=ALDRICH&PageToGoToURL=http%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Faldrich%2F677418%3Flang%3Den

APPENDIX A: Hazard Benchmark Acronyms

(alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(Cr) Corrosion/ Irritation (Skin/ Eye)

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

Appendix B
Optional Hazard Summary Table

Exposure Route	GreenScreen Hazard Ratings: [*Chemical Name*]																			
	Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
	C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
							single	repeate	single	repeated*										
oral																				
dermal																				
inhalation																				

Appendix C
Modeling Results

Attach:

- **EPISuite Results for Chemical Name (CAS #)**
- **ECOSAR Results for Chemical Name (CAS #)**
- **Other**