

MICHIGAN DEPARTMENT OF ENVIRONMENT, GREAT LAKES, AND ENERGY

INTEROFFICE COMMUNICATION

TO: File for Subtilisins (CAS #9014-01-1) and products containing proteases with CAS Nos. 1395-21-7, 9001-92-7 and 37259-58-8 and amylase with CAS #9000-90-2

FROM: Doreen Lehner, Toxics Unit, Air Quality Division

DATE: June 25, 2024

SUBJECT: Screening Level for Subtilisins

Summary

The initial threshold screening level (ITSL) for subtilisins is 0.0006 µg/m³ (1-hour averaging time).

Uses and Physical Chemical Properties

Subtilisins are monomeric proteins of 268 – 275 amino acid residues with high and low affinity calcium binding sites. Subtilisins is a protease (a protein-digesting enzyme) initially obtained from *Bacillus subtilis*. “[N]ative enzymes (i.e., those obtained from natural sources) currently hardly have any practical applications because of the decisive advantages of the enzymes modified by genetic engineering and having better properties: e.g., thermal stability, general resistance to detergents and specific resistance to various oxidants, high activity in various temperature ranges, independence from metal ions, and stability in the absence of calcium. The vast majority of subtilisin proteases are expressed in producer strains belonging to different species of the genus *Bacillus*. Meanwhile, there is an effort to adapt the expression of these enzymes to other microbes, in particular species of the yeast *Pichia pastoris*” (Rozanov et al., 2021). Subtilisins are used: as a detergent; in food processing and packaging; in the synthesis of inhibitory peptides; in therapeutic applications; and in waste management applications.

Table 1. Physical/Chemical Properties of Subtilisins

Structure



CAS Number	9014-01-1 other products containing proteases with CAS Nos. 1395-21-7, 9001-92-7 and 37259-58-8 and amylase with 9000-90-2
Synonyms	Selenosubtilisin, Nattokinase, Bioprotease, Subtilopeptidase, Thermoase, Bacillopeptidase, Alkase, and Biopraxe

Literature Search

The literature was searched to find relevant data to assess the toxicity of subtilisins. The following references or databases were searched: U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS), Registry for Toxic Effects of Chemical Substances (RTECS), American Conference of Governmental and Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs), National Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Levels (RELs), International Agency for Research on Cancer (IARC) Monographs, Chemical Abstract Service (CAS) SciFinder (searched 6/10/2024), U.S. EPA ChemView, California Office of Environmental Health Hazard Assessment (OEHHA), the U.S. Department of Health and Human Services Agency for Toxic Substances and Disease Registry (ATSDR), European Chemical Agency (ECHA), and the U.S. National Toxicology Program (NTP).

Key Study

ACGIH set a Threshold Limit Value-Ceiling (TLV-Ceiling) of 0.00006 mg/m³ (0.06 µg/m³) “as 100% crystalline active pure enzyme (CAPE) is recommended for occupational exposure to proteolytic enzymes derived from *Bacillus subtilis* or closely related organisms commonly called subtilisins. The TLV-Ceiling is intended to minimize the potential for sore throat, nasal congestion, headache, persistent cough, wheezing, dermal irritation, particularly in body areas of high perspiration, and more severe effects that can include airway obstruction (reduced forced expiratory volume in one second [FEV₁]), pulmonary edema, and allergic respiratory sensitization” (ACGIH, 2001).

A TLV-Ceiling is defined as “the concentration that should not be exceeded during any part of the working exposure” (ACGIH). When TLV-Ceiling values are used, the most appropriate averaging time would be a 1-hour averaging time (Hultin, 1997).

ITSL Derivation

The ACGIH TLV-Ceiling of 0.06 µg/m³ was used to derive an ITSL using the Rule 232(1)(c) equation below:

$$ITSL = \frac{\text{occupational exposure level}}{100} = \frac{0.06 \mu\text{g}/\text{m}^3}{100} = 0.0006 \mu\text{g}/\text{m}^3$$

As stated above, the most appropriate averaging time for a TLV-Ceiling is 1-hour, therefore the initial threshold screening level (ITSL) for subtilisins is 0.0006 µg/m³ (1-hour averaging time).

References

ACGIH. 2001. Subtilisins CAS number: 1395-21-7 (*Bacillus subtilis* BPN) and CAS number: 9014-01-1 (*Bacillus subtilis* Carlsberg). TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices. ACGIH Worldwide Signature Publications.

Act 451 of 1994, Natural Resources and Environmental Protection Act and Air Pollution Control Rules, Michigan Department of Environment, Great Lakes, and Energy.

Hultin. 1997. Files for BIOSAM TP-1.5 (product contains proteases with CAS Nos. 9001-92-7 and 37259-58-8 and amylase with CAS #9000-90-2) and PURAFECT 4000G (contains *Bacillus subtilis* with CAS #9014-01-1). From Mary Lee Hultin, Toxics Unit, Air Quality Division. Averaging time for Subtilisins. Dated January 7, 1997.

Rozanov AS, Shekhovtsov SV, Bogancheva NV, Pershina EG, Ryapolova AV, Bytyak DS, and Peltek SE. 2021. Production of subtilisin proteases in bacteria and yeast. Vavilov Journal of Genetics and Breeding 25 (1):125-134.

DL:lh
Attachment

MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

January 7, 1997

TO: Files for BIOSAM TP-1.5 (product contains proteases with CAS#'s 9001-92-7 and 37259-58-8 and amylase with CAS# 9000-90-2) and PURAFECT 4000G (contains Bacillus subtilis with CAS# 9014-01-1)

FROM: Mary Lee Hultin, Toxics Unit, Air Quality Division

SUBJECT: Averaging time for Subtilisins

The averaging times in previous derivations of screening levels for subtilisin compounds were listed with 8 hour averaging. It has been determined that the averaging time should be changed to 1 hour. The basis for the screening level derivation is found in ACGIH, 1991 which lists a TLV CEILING for subtilisins as 100% crystalline active pure enzyme and equivalent TLV values for variations of percent enzyme in products. Initially, the TLV values for various preparations were thought to represent 8 hour time-weighted average values. However, after further review of the document and communication with ACGIH, it was noted that the various formulations are also TLV-CEILING values and not 8 hour time weighted averages. In order to protect against short-term health impacts, it has been determined that screening level derivations using TLV-CEILING values will be associated with a one hour averaging time. Thus, subtilisin and related compounds, including compounds with CAS Nos. 9001-92-7, 37259-58-8, and 9000-90-2 and 9014-01-1 and any others determined by staff of the toxics unit to meet the definition of subtilisins listed in the ACGIH documentation, will have an associated averaging time of 1 hour.

MLH:slb

MICHIGAN DEPARTMENT OF NATURAL RESOURCES

INTEROFFICE COMMUNICATION

September 9, 1994

TO: Files for BIOSAM TP-1.5 (product contains proteases with CAS#'s 9001-92-7 and 37259-58-8 and amylase with CAS# 9000-90-2) and PURAFECT 4000G (contains Bacillus subtilis with CAS# 9014-01-1)

FROM: Mary Lee Hultin, Toxics Unit

SUBJECT: Screening levels for BIOSAM TP-1.5 and PURAFECT 4000G

Screening levels for these products have been derived in response to a request from AMWAY for Permit 140-94. The initial request was for a product called alkaline stable protease enzyme. No toxicity information was available for this product. A material safety data sheet was obtained from the company which identified the product as BIOSAM TP-1.5 containing proteases with CAS#'s 9001-92-7 and 37259-58-8 and amylase with CAS# 9000-90-2. A second product was subsequently added to the permit request. This product, called PURAFECT 4000G, contains a subtilisin with CAS# 9014-01-1. Literature searches were performed for toxicity data on these compounds including the following references:

RTECS
EPA IRIS
DNR EPB and NUTSHELL
NIOSH
ACGIH TLV
CAS Online
NLM Toxline database
AQD files and screening level databases
HEAST
NTP Management Status Report
IARC

The RTECS name for the BIOSAM product protease CAS# 9001-92-7 is Bacillus subtilis neutral protease. The Purafect product subtilisin is listed as Bacillus subtilis Carlsburg in the RTECS databases. While there is not sufficient data for derivation of inhalation reference concentrations for the individual components in these products, the subtilisins have an ACGIH TLV. The TLV varies depending on the enzyme content of product preparation. The TLV documentation for subtilisins lists CAS numbers for Bacillus subtilis Carlsberg, Bacillus subtilis BPN, as well as various product trade names. The document states, "The term subtilisins refers to

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a group of proteolytic enzymes derived from Bacillus subtilis or closely related organisms."

Information obtained from AMWAY indicates that the appropriate TLV associated with the enzyme preparations is 0.002 mg/m³ listed in the TLV documentation as "As Received" Enzyme. The TLV clearly applies to the Purafect product since the CAS# is listed in the documentation. As mentioned above, the TLV documentation notes that the exposure level applies to proteolytic enzymes derived from Bacillus subtilis or "closely related organisms". Studies on the Bacillus subtilis neutral protease CAS# 9001-92-7 and alpha-amylase contained in the BIOSAM product (see AQD INTERIM CHEMICAL EVALUATIONS for these compounds) indicate that their mode of toxic action is similar (i.e., pulmonary sensitization) to other subtilisins. Therefore, the TLV value for subtilisins will be applied to the BIOSAM product.

Since both products will be emitted and both have pulmonary sensitizing properties, the combined impacts from these emissions must meet the following Initial Threshold Screening Level (ITSL), as per Rule 230 (9)(b):

TLV = 0.002 mg/m³ based on 8 hour averaging

$$ITSL = \frac{0.002 \frac{mg}{m^3}}{100} = 2 \times 10^{-5} \frac{mg}{m^3} = 0.02 \frac{ug}{m^3} \text{ based on 8 hour averaging}$$

MLH:ma