

MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

January 14, 1997

TO: File for *Bacillus subtilis* neutral protease [BSNP]  
(CAS # 9001-92-7)

FROM: Dan O'Brien, Toxics Unit, Air Quality Division

SUBJECT: Initial Threshold Screening Level for BSNP

The initial threshold screening level (ITSL) for *Bacillus subtilis* neutral protease is  $0.02 \mu\text{g}/\text{m}^3$  based on a 1 hour averaging time.

The following references or databases were searched to identify data to determine the ITSL: AQD chemical files, IRIS, HEAST, ACGIH TLV Booklet, NIOSH Pocket Guide to Chemical Hazards, RTECS, NTP Management Status Report, EPB Library, IARC Monographs, CAS On-line and NLM/Toxline (1967 -December 20, 1996), Handbook of Environmental Data on Organic Chemicals, Patty's Industrial Hygiene and Toxicology, Merck Index and the Condensed Chemical Dictionary.

BSNP is one of a number of proteolytic enzymes produced by microorganisms for use in laundry detergents. Very little specific toxicology data was found in the course of our searches. With respect to acute toxicity data, the only citation, from RTECS (1996), refers to an intraperitoneal (i.p.)  $\text{LD}_{50}$  in mice from a Chinese journal. Since studies using the i.p. route of exposure are generally not considered representative of the toxicity that can be anticipated upon inhalation exposure, this data is not appropriate as the basis for derivation of a screening level.

Search of the AQD Chemical Files found a file already established for BSNP, with information showing that its inhalation toxicity had been considered previously for another permit, in 1994. In that context, BSNP was a component of an enzyme preparation called Biosam TP-1.5, a mixture of BSNP, another protease (37259-58-8) and  $\alpha$ -amylase (9000-90-2). A screening level for Biosam TP-1.5 was developed at that time based on the American Conference of Governmental Industrial Hygienists Threshold Limit Value (ACGIH TLV) for subtilisins, per R232(1)(c). The TLV documentation (ACGIH, 1992) defines subtilisins as "a group of proteolytic enzymes derived from *Bacillus subtilis* or closely related organisms". Using this definition, BSNP fits the definition of a subtilisin, although its CAS number is not specifically listed in the TLV documentation. Given the facts that 1) a complete review of the toxicological literature pertaining to BSNP was performed in 1994 (see

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the AQD Chemical File for BSNP)<sup>1</sup> and 2) at that time, the most appropriate foundation for a human health-based limit for BSNP (as present in Biosam TP-1.5) was considered to be the TLV for subtilisins, it is concluded that unless significant new literature on the toxicity of BSNP has been published since that time, the TLV for subtilisins will be considered the most appropriate basis for the screening level for BSNP as well.<sup>2</sup>

Of the citations found in our searches of the literature between 1994 and the present, only one is worthy of note as potentially providing information of significant use in the derivation of a screening level. The Chemical Abstract Service (CAS) On-line search lists a recent review article (Gajiyev, 1993) on effects of BSNP in humans and experimental animals. However, this article was in Russian and unavailable for our review.

Thus, since no new information was found that suggests a more appropriate scientific basis for a screening level for BSNP which will also be sufficiently protective of human health<sup>2</sup>, the ACGIH TLV for subtilisins<sup>3</sup> is used as the basis for the ITSL for BSNP.

*Derivation of the ITSL:* Per Rule 232(1)(c), part 55, of Act 451:

$$\text{ITSL} = \text{OEL} \times \frac{1}{100} = 0.002 \text{ mg/m}^3 \times \frac{1}{100} = 0.00002 \text{ mg/m}^3 \times \frac{1000 \text{ } \mu\text{g}}{1 \text{ mg}} = 0.02 \text{ } \mu\text{g/m}^3$$

where the factor of 1/100 is a safety factor to account for: 1) differences in susceptibility between the healthy, adult worker population as compared to the general population which may include individuals or subpopulations more sensitive to the effects of

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<sup>1</sup> The critical human health effects of enzyme preparations such as subtilisins and  $\alpha$ -amylase are respiratory and skin sensitization (ACGIH, 1992). Following initial immunologic sensitization of an exposed individual, subsequent exposure to these enzymes can result in hypersensitivity reactions (e.g., dyspnea, bronchoconstriction, and other asthmatic signs) which can be life-threatening without provision of immediate therapy. In some cases, even minute exposures can elicit severe clinical signs in sensitized persons. Skin irritation and hypersensitivity reactions have also been reported.

<sup>2</sup> With respect to the inhalation toxicity of enzymes such as BSNP, there is evidence that the default trace (0.04  $\mu\text{g/m}^3$ , annual averaging) concentration normally employed in the situation where there is insufficient toxicity data per R232(1)(i) may not protect against adverse health effects (viz., respiratory sensitization) in persons exposed to that concentration. Note that the trace concentration is higher than an ITSL based on the TLV for subtilisins (0.02  $\mu\text{g/m}^3$ , 1 hour averaging).

<sup>3</sup> ACGIH lists several values for the TLV; which is considered appropriate depends on the percent pure enzyme content (PEC) of the preparation. The TLV of 0.002  $\text{mg/m}^3$  employed here assumes the BSNP preparation is best characterized as "As Received" enzyme, reflecting a PEC of approximately 2.4% [This "As Received" characterization wa

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exposure to BSNP and 2) the difference in exposure duration for the worker population as opposed to the general population. The factor is derived as follows:

$$\text{Safety factor} = \frac{40 \text{ hours}}{168 \text{ hours}} \times \frac{30 \text{ years}}{70 \text{ years}} \times \frac{1}{10} = \frac{1}{100}$$

The first term adjusts for the difference between a 40 hour work week and the total hours in a week; the second factor adjusts for the difference between an assumed working life of 30 years and an assumed total lifespan of 70 years; and the third factor is a standard ten-fold uncertainty factor to extrapolate from the healthy worker to sensitive individuals in the general population.

Future review of the appropriateness of this screening level will be warranted as more recent data of better quality and specific to the toxicity of BSNP become available.

Consistent with 232(2)(a), since the TLV used here is based on a ceiling threshold limit value, a 1 hour averaging time applies.

#### REFERENCES

- ACGIH (1991). Subtilisins (1395-21-7 and 9014-01-1). In: Documentation of Threshold Limit Values and Biological Exposure Indices, American Conference of Governmental Industrial Hygienists, Cincinnati, pp. 1447-1448.
- Gajiyev, A.S. (1993). [Effect of alkaline protease on humans and experimental animals] (in Russian). *Med Tr Prom Ekol* 9-10:34-35.
- RTECS (1996). Protease (9001-92-7). In: Registry of Toxic Effects and Chemical Substances Database. National Institute for Occupational Safety and Health, Public Health Service, Centers for Disease Control, U.S. Department of Health and Human Services, and Canadian Centre for Occupational Safety and Health.

DO:slb

cc: Asad Khan, Permits