MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

January 14, 1997

TO: File for α-amylase (CAS # 9000-90-2)

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

SUBJECT: Initial Threshold Screening Level for α-amylase

The initial threshold screening level (ITSL) for α-amylase is 0.02 $\mu g/m^3$ 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.

α-amylase is an enzyme produced by microorganisms for use in laundry detergents. It also finds application in starch processing; brewing, baking and distilling; dry cleaning and textile desizing; in sewage treatment and in animal feeds (Merck, 1983; Hawley, 1981). Limited specific toxicology data was found in the course of our searches. Search of the AQD Chemical Files found a file already established for α-amylase, with information showing that its inhalation toxicity had been considered previously for another permit, in 1994. In that context, α-amylase was a component of an enzyme preparation called Biosam TP-1.5, a mixture of *Bacillus subtilis* neutral protease (9001-92-7), another protease (37259-58-8) and α-amylase. 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, α-amylase does not strictly fit the definition of a subtilisin. Moreover, its CAS number is not specifically listed in the TLV documentation. However, α-amylase has very similar mechanisms of action and toxicity as those of the subtilisins, and like the subtilisins, is a microbial enzyme. The critical human health effects of enzyme preparations such as subtilisins and α-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. Given the facts that 1) a complete review of the toxicological literature pertaining to α-amylase was performed in 1994 (see the AQD Chemical File

for α-amylase) and 2) at that time, the most appropriate foundation for a human health-based limit for α-amylase (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 α-amylase has been published since that time, the TLV for subtilisins will be considered the most appropriate basis for the screening level for α-amylase as well.[1]

Of the citations found in our searches of the literature between 1994 and the present, several were worthy of note as providing information of sufficient value to warrant inclusion in the screening level documentation. Houba and coworkers (Houba *et al.*, 1996) examined the relationship between α-amylase allergen exposure and the prevalence of work-related respiratory allergies. In a cross-sectional study of 178 bakery workers, work-related respiratory symptoms and skin prick tests were associated with exposures to fungal origin α-amylase as measured in personal dust samples by sandwich enzyme immunoassay. Twenty five percent of all workers had one or more job-related symptoms, and as much as 9% of the workers registered positive skin prick tests to α-amylase. Positive skin prick results were positively associated with work-related respiratory symptoms. α-Amylase-specific IgE was demonstrated in eight percent of these workers. Alpha amylase exposure and atopy appeared to be the most important determinants of skin sensitization, with prevalence ratios (95% confidence intervals) for atopy of 20.8 (2.74-258) and for medium and high α-amylase exposure groups of 8.6 (1.01-74) and 15.9 (1.95-129), respectively. The authors concluded that there was a strong and positive relationship between α-amylase allergen exposure levels in bakeries and specific sensitization in bakery workers. An elevated risk of work-related allergic respiratory disease in bakers due to α-amylase exposure was also found by DeZotti *et al.* (1994*a*), although skin prick tests did not indicate that bakers were significantly more at risk of skin sensitization than controls. Reports from a case series of eight asthmatic subjects with positive reactions to α-amylase on bronchial provocation showed skin prick tests to be sensitive in 88% of subjects. However, skin prick tests are not always specific for α-amylase sensitization, since some asthmatic subjects with positive skin tests were shown to be negative on bronchial provocation (Moneo *et al.*, 1995). A significant association between symptoms and atopy by prick tests (Odds Ratio [OR] 17.2 (5.27-56.4) was also noted in a cross-sectional study of 144 trainee bakers (DeZotti *et al.*, 1995). Those authors concluded that the presence of asthma or other severe allergy should be grounds for exclusion of individuals from training as bakers if found on pre-employment screening. Other similar recent prevalence reports were published by DeZotti *et al.* (1994*b*) and Moneo *et al.* (1994).

While α-amylase can function as the sole causative agent in bakers' asthma (Valdivieso *et al.*, 1994), the problem of confounding exposure to other allergens in sensitized bakery workers has received attention as well (Cullinan *et al.*, 1994). Despite an elaborate exposure assessment scheme (Nieuwenhuijsen *et al.*, 1994) set up for use in a cohort study, they could

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[1] With respect to the inhalation toxicity of enzymes such as α-amylase, there is evidence that the default trace (0.04 $\mu g/m^3$, 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 $\mu g/m^3$, 1 hour averaging).

not isolate the specific effects of exposure to α-amylase from those due to flour aeroallergens as a group. However, these authors did find a strong association between intensity of exposure to flour aeroallergens as a group and occurrence of eye/nose and skin symptoms that was independent of atopic status and cigarette smoking.

Two other points seem worthy of mention. First, it is of interest that ingestion of α-amylase can elicit respiratory signs (including severe bronchoconstriction) once an individual has become sensitized (Baur and Czuppon, 1995; Kenny and Moneret-Vautrin, 1995). In the former report, a 34 year old woman developed asthma specific to α-amylase during a period spent working as a baker, which caused her to leave that job. Later, oral exposure to α-amylase in bread in a double-blind placebo controlled trial led to severe bronchoconstriction which required medication for relief. Second, immunologic investigations with bakers, 32% of whom were sensitized to α-amylase, led Baur *et al.* (1994) to conclude that active *Aspergillus oryzae* α-amylase was "the allergenic component of widely used commercially available baking enzyme products". This conclusion was based on the fact that *Aspergillus oryzae* α-amylase was the component which was exclusively or predominantly bound by specific IgE antibodies in the blood of symptomatic bakers. With respect to the derivation of a screening level, the significance of this second point is that it implicates exposure specifically to *fungal* α-amylase in bakers' asthma, and, strictly speaking, it is not known (based on the data available to us) that identical toxicity would be brought on by exposure to bacterial origin α-amylase, such as that derived from *Bacillus subtilis*. Yet, other reports (Garcia Casado *et al.*, 1995; DeZotti *et al.*, 1994*b*; Sandiford *et al.*, 1994; Blanco Carmona *et al.*, 1991) suggest that α-amylase from non-fungal origins (cereal, animal and bacterial) can also ellicit hypersensitivity reactions very similar in character and severity to those in baker's asthma.

Thus, despite valuable recent research on the role of α-amylase in the etiology of respiratory hypersensitivity, no new information was found that can be used in the quantitative derivation of a screening level. Since no evidence was found which suggests a more appropriate scientific basis for a screening level that will also be sufficiently protective of human health, the ACGIH TLV for subtilisins[2] is used as the basis for the ITSL for α-amylase.

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

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

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 exposure to α-amylase and 2) the

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[2] 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 mg/m^3 employed here assumes the α-amylase preparation is best characterized as "As Received" enzyme, reflecting a PEC of approximately 2.4% [This "As Received" characterization was confirmed for permit 825-77a by phone conversation with Ms. Loretta Campbell-Jones of Amway Corporation on 1/7/96, 7:25 a.m.].

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 α-amylase 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.

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