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HEALTH AND SAFETY COMMISSION

ADVISORY COMMITTEE ON TOXIC SUBSTANCES

Subtilisins – Proposal for a MEL

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Issue

1 A Maximum Exposure Limit (MEL) for subtilisins.

Timing

2 Routine

Recommendation

3 That ACTS considers the information presented and recommends to HSC that it should consult publicly on the setting of MELs for subtilisins of 0.00004 mg/m^3 (40 ng/m^3) (8-hour TWA and 15 minute reference period STEL), with a "Sen" notation.

Background

4 Subtilisins are proteolytic enzymes of bacterial origin and come in the form of light-coloured, free-flowing powders. They are derived from *Bacillus subtilis* by a fermentation process and are readily soluble in water. They are not manufactured in Great Britain but are imported for use in the manufacture of detergents and animal feeds. They are also used for food and leather processing.

5 It is estimated that up to 100 tonnes of subtilisins are imported annually, contained in up to 5,000 tonnes of granulated powder, spray-dried on a wheat substrate, or alternatively in a liquid formulation with a concentration of subtilisin of 0.5% to 10%. About 100 people are potentially exposed to subtilisins during the handling of dry concentrate and dry detergent product in the soap detergent industry. Up to 1,000 people may be potentially exposed to the 0.5% subtilisin concentrate during weighing and tipping operations during the preparation of animal feeds, while a further 15,000 people are potentially exposed to downstream animal feed containing subtilisin at a concentrate of about 0.0005%.

6 Until January 2003 subtilisins had OESs of 0.00006 mg/m^3 (60 ng/m^3) (8-hour TWA and STEL). These were withdrawn by the Health and Safety Commission in January 2003 following public consultation (Consultation Document 182). The OESs were derived from the ACGIH list of Threshold Limit Values; they became UK Recommended Limits in 1984 and were converted to OESs with the introduction of COSHH in 1989. According to the ACGIH, the TLVs for subtilisins were set at a level believed to minimise the potential for occupational asthma and allergic rhinitis in non-atopic workers. The OESs were based on static (area) sampling rather than personal exposure monitoring because the flow rates associated with personal sampling did not collect sufficient enzyme for use with the analytical methods then available. Annex 2 provides details of the recently-developed analytical method for personal sampling.

7 In 1995 a safety management consultant wrote to HSE asking why subtilisins did not warrant a MEL, given that they were recognised as respiratory sensitisers. He also queried the level at which the OESs had been set. It was therefore decided that subtilisins were a likely candidate for inclusion in the ACTS/WATCH programme of substance reviews. Subtilisins were considered by WATCH at its meeting in September 2000. WATCH agreed that the key health concerns for subtilisins are their potential to cause occupational asthma and allergic rhinitis. Subtilisins are also toxic by inhalation, causing direct effects on the lungs, haemorrhage, congestion and oedema, and there is clear evidence to show that subtilisin enzyme preparations are irritant to the eyes. Subtilisins can also irritate damaged, but not intact, skin. There is no evidence to suggest that subtilisins can induce skin sensitisation.

8 On the basis that it was not possible to establish exposure-response relationships for the induction of occupational asthmas or allergic rhinitis by subtilisins or determine where the threshold might lie, WATCH concluded that subtilisins met the criteria for the establishment of a MEL. In common with its general position on asthmagens, WATCH considered that it would be appropriate for both 8-hour TWA and STEL MELs to be established.

9 ACTS considered these recommendations at its meeting on 29 March 2001 (ACTS/05/2001), together with related proposals for bacterial alpha amylase and fungal alpha amylase. They agreed that HSE should pursue the development of a MEL for subtilisins and withdraw the existing OESs. As a result a Chemical Hazard Alert Notice (CHAN) was issued in June 2001 and HSC consulted between March and June 2002 on the removal of these OESs from EH40 “Occupational Exposure Limits”.

Argument

10 HSE recommends that public consultation should be carried out on the feasibility of setting MELs for subtilisins at 0.00004 mg/m^3 (40 ng/m^3) (8-hour TWA and 15 minute reference period STEL).

11 HSE anticipates that this change in the occupational exposure limit will result in little additional costs for exposure controls, for soap detergent manufacture or any other industry. The soap detergent manufacturing industry can control task-specific

personal exposures to subtilisins to below 40 ng/m^3 by compliance with their own established industry guidelines. Personal exposures will be further reduced because all workers involved in handling dry enzyme formulations are provided with RPE.

12 Some additional costs are likely to be encountered because the MEL proposal is based on personal monitoring rather than, as was previously the case, on static monitoring. The RIA attached at Annex 1 gives details of these estimated costs. It is estimated that the bulk of the additional costs will fall on feed millers, principally due to a need for health surveillance of workers. HSE has estimated an overall cost to this industry of £535,000 over 10 years (3.5% discount rate). It is not anticipated that the MEL would have a disproportionate effect on small businesses.

13 Annex 2 provides a summary of the personal sampling method developed by the Health and Safety Laboratory (HSL). Previously, there were no personal data for occupational exposure to subtilisins for any industry. HSE (through HSL) has now developed an assay technique for subtilisins and considers this as suitable and appropriate for analysis of personal subtilisin exposure samples. The lower limit of detection for the assay is 3 ng/m^{-3}. The sampling method is in relation to enzyme activity and not total weight (active plus inactive enzyme). Further information on this is in the RIA at Annex 1.

14 Annex 3 is a revised summary to be included in the HSE publication EH64 *Summary Criteria for Occupational Exposure Limits*.

Communication Plan

15 Subject to agreement by the Health and Safety Commission, the proposed MELs for subtilisins would be published in a Consultation Document in the usual way for limits of this nature. The eventual Consultation Document will be publicised in the usual way via a Press Notice. HSE will report the results of the consultation exercise to ACTS and to the HSC.

16 If the new OEL framework takes effect, and the MEL is implemented directly into the new OEL framework, this will not result in a lessening of worker protection. Under the current system, employers are required to reduce exposure below the MEL so far as is reasonably practicable. Under the new framework, good practice guidance, developed to help employers comply with the limit, will relate directly to the hazard – the greater the hazard, the more stringent the good practice guidance will be.

Evaluation Plan

17 As part of the Asthma Strategy, HSE plans to look at the effectiveness of measures to prevent occupational asthma.

Relevant Control Systems

18 In the soap detergent industry there is already a high degree of automation, containment, engineering controls and the use of PPE in order to control residual risks. In other industries where subtilisins are used – preparation of animal feeds,

food processing and leather processing – much of the product will be in pelletised form leading to a much lower level of dustiness.

Consultation

19 In preparing the Regulatory Impact Assessment HSE has contacted the three detergent companies operating in the UK, relevant trade associations and several individual small and medium-sized companies. It will consult widely on the implications of setting MELs at 40 ng/m^3 before reporting its conclusions back to ACTS.

Costs and Benefits

20 These are discussed in detail in the Regulatory Impact Assessment attached at Annex 1.

Environmental implications

21 None.

European implications

22 None.

Devolution

23 As with all health and safety issues, the proposed MEL for subtilisins would apply to Great Britain as a whole.

Other implications

24 HSE's policy on subtilisins may be re-visited in the future to bring it in line with the policy on other enzymes currently under consideration for a limit, principally fungal and bacterial alpha amylase.

Action

25 ACTS is asked to agree that the Health and Safety Commission be asked to consult on:

(i) the establishment of MELs (or other types of single limit) for subtilisins set at 0.00004 mg/m^3 (40 ng/m^3) (8-hour TWA and 15 minute reference period STEL)
(ii) the inclusion of a "Sen" notation for subtilisins.

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PROPOSED MAXIMUM EXPOSURE LIMIT FOR SUBTILISINS

REGULATORY IMPACT ASSESSMENT

ISSUE AND OBJECTIVE

1. Subtilisins are proteolytic enzymes of bacterial origin, derived by a fermentation process from *Bacillus subtilis*. The dry concentrate forms are readily soluble in water, light coloured in appearance and are produced as free-flowing granular powders. They are also produced in paste and liquid forms.

2. Until January 2003, subtilisins had an Occupational Exposure Standard (OES) of 60 ng/m^3 (measured using high volume static samplers) for both long-term (8-hour time-weighted average) background exposure and short-term (15 minute reference period) background exposure. Static sampling is used because the flow rates associated with personal sampling could not collect sufficient enzyme for assay using the analytical methods available at the time the OES was established.

3. The Health and Safety Commission's (HSC) Working Group on the Assessment of Toxic Chemicals (WATCH) reviewed subtilisins in September 2000. It concluded that the key health concerns for subtilisins are their potential to cause occupational asthma and allergic rhinitis, and that the available data do not allow a threshold for the induction of these conditions by subtilisins to be identified, nor is it possible to determine what the exposure-response relationship might be. WATCH concluded therefore that the criteria for setting an OES were not met, that the OESs of 60 ng/m^3 (8-hour TWA and STEL) were not sustainable, and that subtilisins met the criteria for a Maximum Exposure Limit (MEL).

4. In March 2001, the HSC's Advisory Committee on Toxic Substances (ACTS) endorsed WATCH's conclusions and agreed to the development of a new analytical technique for enzymes to underpin the new MEL. This technique was subsequently developed by the Health and Safety Laboratory (HSL) and uses highly sensitive fluorescence polarisation technology to measure the low amounts of active enzyme collected in personal sampling. ACTS agreed to the development of the new HSL technique as the basis for the MEL proposals for subtilisins because personal exposure samples are more likely to reflect an individual's exposure than static samples.

5. A Chemical Hazard Alert Notice (CHAN) was issued in June 2001 to advise industry of HSC's intention to consult on the withdrawal of the OES from 2003 and consider setting a MEL.

6. This Regulatory Impact Assessment (RIA) will inform ACTS as to the financial consequences of the assignment of a MEL, based on personal sampling, for subtilisins.

RISK ASSESSMENT

Health Effects

7. The subtilisins are high molecular weight water-soluble enzymes that may be present in the workplace either as a dry powder or in a liquid preparation. An assessment of the health effects of subtilisins was presented to WATCH at its meeting of September 2000. WATCH concluded that the key health concern for these enzymes is their potential to cause occupational asthma/allergic rhinitis. Information on the health effects of subtilisins is primarily derived from studies of workers in the detergents industry. When subtilisins were first introduced into the detergents manufacturing process there were a large number of cases of occupational asthma attributed to enzymes each year. Figures from the soap and detergent industry published by Cathcart et al., (1997) indicate that 7-39 cases of asthma per year occurred between 1968 and 1974 (total 140), 0-5 cases per year occurred between 1975 and 1980 (total 17) and 0-4 cases per year occurred between 1980 and 1992 (total 9). Unfortunately, although it is known that control of airborne subtilisins was improving during this period, personal exposure data were not gathered by the detergents industry. Hence, no data are available which would enable a threshold for the induction of occupational asthma/allergic rhinitis by subtilisins to be identified or to determine what the exposure response relationship might be. SWORD statistics for the period 1989 – 1999 show that between 1 and 15 cases of occupational asthma due to detergent enzymes still occur each year. The specific enzymes involved have not been reported. From around 1990, enzymes other than subtilisins began to be used in detergent formulations; hence it is not possible to determine how many of the cases reported to SWORD are specifically due to subtilisins.

8. In some workplaces skin prick tests to detect the presence of circulating subtilisin specific antibodies have been used as a screen to identify those who may be at risk of developing occupational asthma/allergic rhinitis. Although such tests have been carried out in detergent workers, owing to the lack of personal exposure data it is not possible to identify a threshold below which positive reactions in skin prick tests would not be elicited.

9. In respect of other toxicological endpoints, no studies have been conducted to examine the toxicokinetics of subtilisins. However, the large molecular size of these enzymes will minimize the potential for absorption directly across the respiratory tract epithelium and skin. Orally administered subtilisins will be subject to the same digestive processes as any other protein.

10. There are no human data on the effects of single exposures to subtilisins. Single exposure studies in animals indicate that subtilisins are toxic via the inhalation route, causing direct effects on the lungs, haemorrhage, congestion and oedema,

probably reflecting the proteolytic activity of these enzymes. No other tissues appear to be affected. Subtilisins are of low oral toxicity on single exposures. The effects of single dermal exposures have not been studied but, given the predicted lack of dermal absorption, systemic toxicity would not be anticipated by this route.

11. There is no evidence to suggest that subtilisins are irritant to intact skin. However, in human volunteer studies, aqueous solutions containing up to 20% of a concentrated subtilisin preparation were irritant to damaged skin. Also workers handling concentrated enzyme preparations without PPE reported skin problems mainly on the fingertips and on the wrist and neck where perspiration and chafing could exacerbate any irritation. The role of subtilisins in causing the skin problems reported in these studies has not been adequately explored. There is clear evidence to show that subtilisin enzyme preparations are irritant to the eye. No studies have specifically examined the ability of subtilisins to cause sensory irritation in the respiratory tract but findings from inhalation studies in animals suggest that subtilisins can cause inflammatory damage to the respiratory tract epithelium rather than elicit sensory irritation effects.

12. Extensive patch testing in large-scale human volunteer studies has shown no evidence for the ability of subtilisins to induce skin sensitisation. Negative results have also been obtained in patch tests in subtilisin-exposed workers. Furthermore, no confirmed cases of skin sensitisation caused by subtilisins have been identified in workers engaged in the manufacture/use of these enzymes.

13. In relation to the effects of repeated exposure, health surveillance data from the detergent industry spanning periods of up to 20 years indicate that, with the exception of occupational asthma/allergic rhinitis, there is no evidence of any adverse health effects relating to the use of subtilisin preparations. Very little additional information is available from studies in animal models. No useful inhalation data are available. However, single exposure studies suggest that on repeated inhalation exposure, chronic inflammatory damage might occur, caused by the localised proteolytic activity of these enzymes. Oral dosing studies in rats suggest that with repeated high doses, local gastro-intestinal disturbances can occur, but this has no obvious relevance to occupational exposure conditions. There are no meaningful data from repeated dermal exposure studies in animals, but the high molecular weight of subtilisin enzymes suggests dermal penetration would not occur, and hence there are no grounds for concern for systemic toxicity by this exposure route. The only predicted effects of repeated dermal exposure would be for local skin irritation.

14. There is no evidence that subtilisins have the potential to cause genotoxic, carcinogenic or adverse reproductive effects.

PRODUCTION

15. Subtilisins are not manufactured in Great Britain but are imported by less than five major enzyme suppliers. Approximately 100 tonnes/year of the enzyme are imported contained in up to 5,000 tonnes of granulated powder, wheat substrate or liquid formulation with a concentration of subtilisins of up to 10%.

16. Enzyme in granulated dry concentrate form is encapsulated in a coarse granule (>150 micron) of phosphate base using a tacky non-ionic detergent to make it less dusty. The enzyme is made even less dusty by forming 'prills' which are beads containing enzyme embedded in a non-dusty matrix. The result is non-dusty solid beads containing up to 10% subtilisins.

17. Liquid formulations of subtilisins tend to be stabilised with additives to prevent microbial spoilage and to decrease the vapour pressure and thus reduce the volatility of the liquid formulation.

USE

18. Subtilisins are used in the manufacture of detergents and animal feeds: also for food and leather processing. Dry concentrate forms are supplied to users in 25 kg bags, 50 kg kegs or 1 tonne bags. Liquid formulations are delivered in either 25 litre containers or 1 tonne intermediate bulk containers (IBCs).

Manufacture of detergents

19. Enzymes are used in soap detergent manufacture to produce 'biological' washing powders to enhance stain removal properties. The most commonly used enzymes are subtilisins. The enzymes are added at the final stage of product manufacture by tipping and blending dry or liquid enzyme concentrate into the detergent product.

20. Work practices in the soap detergent manufacturing industry include a high degree of automation, containment, local exhaust ventilation (LEV) and use of respiratory protective equipment (RPE) to control residual risks. Liquid handling processes are unlikely to generate aerosol as they are carried out within full containment. The potential for inhalation and dermal exposure is most likely to arise during the handling and tipping of dry concentrated enzyme formulations and any subsequent handling of dry detergent product. The volatility and dustiness of the dry concentrate is low however.

21. About 100 people are exposed to subtilisins during the handling of dry concentrate and dry detergent product in the soap detergent manufacturing industry. This occupational group have the highest risk of exposure to subtilisins as exposures may occur continually throughout an 8-hour shift.

22. HSE has therefore carried out sampling and monitoring visits at three soap detergent manufacturers in order to establish typical personal exposures to subtilisins in dry form under the good working practices identified in the industry guidelines[2]. Enzyme granulate containing up to 5% subtilisins was added to the detergent at a rate of up to 1% giving a maximum concentration of 0.05% subtilisin in the final product. The measured mean task specific exposure to subtilisins for all tasks was generally kept below 40 ng/m^3.

Preparation of animal feeds

23. Animal feed sometimes contains enzyme supplements such as subtilisins to aid digestion in the gut. Subtilisins are typically added to feeds for pigs and poultry. The enzymes are added at the final stage of feed preparation by tipping and blending dry or liquid enzyme concentrate into the feed.

24. Liquid handling processes are unlikely to generate aerosol as they are carried out within full containment. The potential for inhalation and dermal exposure is most likely to arise during the handling and tipping of dry concentrated enzyme formulations and any subsequent handling of dry feed preparation. The enzyme concentrate contains up to 0.5% subtilisins whilst the final product of animal feed preparation typically contains up to 0.0005% subtilisins.

25. About 1,000 people at about 100 feed mills may be potentially exposed to the dry 0.5% subtilisins concentrate during weighing and/or tipping, and up to 15,000 people may be exposed to the 'downstream' animal feed preparation containing subtilisins at about 0.0005%.

26. Workers exposed to the 0.5% subtilisins concentrate during weighing and/or tipping are the occupational group at greatest risk of exposure to subtilisins with typically twelve intermittent exposures, each of 5 minutes duration, spread throughout an 8-hour shift.

27. HSE has no subtilisin exposure data for this industry.

Food processing

28. Subtilisins are used in food processing to hydrolyse soya bean, gelatin and yeast. Up to 25 tonnes of both liquid and dry formulations with concentrations of up to 10% enzyme are used annually for these applications. The potential for inhalation and dermal exposure to subtilisins will occur at the point of dispensing the enzyme concentrate and adding it to the liquid food preparation in a reactor vessel. However, the processes are unlikely to generate aerosol and the dustiness of the dry formulations will be low. It is estimated that less than 50 workers at no more than 15 workplaces may be potentially exposed to the enzyme during food processing.

29. Typically, exposures to the 10% subtilisins concentrate during dispensing will occur during three intermittent 5 minute periods spread throughout an 8-hour shift and will therefore not exceed 15 minutes in total per shift.

Leather processing

30. Subtilisins are used in leather processing to process skin and hides into leather. Up to 20 tonnes of dry formulation with concentrations of up to 10% subtilisin are used annually for this application. The enzyme formulation is added to an aqueous solution in an enclosed mixing drum to a concentration of about 0.1% subtilisin. The skin and hides are then gently mixed and soaked in the aqueous solution in the drum. The potential for exposure to subtilisins occurs at the point of weighing and tipping the dry enzyme concentrate into the mixing drum. However, the dustiness of the dry concentrate will be low. It is estimated that less than 40 workers at no more than 15 workplaces are exposed to subtilisins during leather processing.

31. Typically, exposures to the enzyme concentrate will occur during three intermittent 5 minute periods spread throughout an 8-hour shift and will therefore not exceed 15 minutes in total per shift.

OCCUPATIONAL EXPOSURE AND MEASUREMENT

32. Previously, there were no personal data for occupational exposure to subtilisins for any industry. HSE (through HSL) have now developed an assay technique for subtilisins and consider this as suitable and appropriate for analysis of personal subtilisin exposure samples.

33. The lower limit of detection for the assay is 3 ng/m^3. This was determined by analysing a number of field blank samples and taking the mean value of these, plus 2.5 times the standard deviation to arrive at the lower limit.

34. HSE visited three workplaces during summer 2002 to carry out personal exposure sampling and monitoring for subtilisins. The new exposure data obtained is presented in this paper and has not previously been seen by either WATCH or ACTS.

35. Several exposures in excess of 30 ng/m^3 were recorded. However, a number of samples obtained at activities where subtilisins were known to be in use were found to be below the limit of detection, 3 ng/m^3.

36. Samples found to be below the limit of detection were recorded as assumed exposures of 1.5 ng/m^3 to provide them with a numerical value. This value was selected as it was considered to be close enough to zero and sufficiently lower than measured data values in excess of 30 ng/m^3 to ensure there were sufficient data points recorded to populate the lower end of the exposure distribution.

37. This approach to interpretation of data found to be below the limit of detection has not previously been proposed to either WATCH or ACTS but has been accepted by the Advisory Committee on Pesticides (ACP).

Measured Occupational Exposure Data

Manufacture of detergents

38. HSE visited three soap detergent manufacturers during summer 2002 to carry out personal exposure sampling and monitoring for subtilisins and total dust at work activities involving the handling and transfer of dry enzyme concentrate and dry detergent product. The purpose of this exercise was to identify typical personal exposure data where work practices were found to comply with established industry guidelines[2] for exposure control, and be in accordance with recognised good practice for the safe handling of hazardous substances. Dry enzyme concentrate is supplied to large users in 1 tonne bags and to smaller users in 50 kg kegs.

39. Dry enzyme concentrate supplied in 1 tonne bags is emptied into a central storage hopper by gravity feed. The 1 tonne bag is hoisted into position above a

hopper feed chute located within a laminar flow booth or room. The sealed bag is coupled onto the feed chute and then opened to provide sealed transfer of the enzyme concentrate into the hopper below.

40. It takes about ten minutes for one worker to carry out this activity and when the bag is empty, it is safely folded up and sealed before removal from the laminar flow booth or room. Workers wear full face air-fed visors during this activity. Exposures to subtilisins at this stage were found to be below the limit of detection (3 ng/m^3) and an assumed exposure of 1.5 ng/m^3 was taken. Measured mean exposures to total dust were recorded as 4.1 mg/m^3.

41. These data reflect both the effectiveness of the control measures in place and the low dust potential of the enzyme concentrate in granulate form. The granulate form would thus appear not to have been physically damaged by this method of transfer. This working practice is already in use by industry and may thus be taken as being reasonable and practicable and representative of good practice.

42. Dry enzyme concentrate supplied to smaller users in 50 kg kegs, however, was transferred into a central storage hopper by vacuum transfer. The sealed keg was placed in a laminar flow booth, opened and a suction lance inserted. The lance was 'worked' into position to the base of the keg and the enzyme concentrate was then drawn out by vacuum and transferred via a sealed pipeline to a central hopper

43. It takes about ten minutes for one worker to carry out this activity and when the keg is empty, it is sealed before removal from the laminar flow booth. Workers wear full face air-fed visors during this activity. The measured task specific exposure to subtilisins for this activity was 63.9 ng/m^3 whilst the measured exposure to total dust was 0.2 mg/m^3.

44. These data show that although the laminar flow booth was effective in controlling total dust exposure, exposure to subtilisins was much higher than for emptying 1 tonne bags by gravity feed. This higher exposure may well be caused by 'working' the lance into the enzyme concentrate thereby breaking up the granular form and creating a more dusty solid.

45. These results are shown at Table 1 and indicate that vacuum transfer cannot be regarded as reasonable and practicable and representative of good working practice.

Table 1: Short term task specific mean occupational exposures to subtilisins in detergent manufacture, measured by HSE

Work Activity	**Task specific mean personal exposure**				
	Number of samples	**Approx sampling period**	**Subtilisins ng/m^3**	**Total dust mg/m^3**	**Subtilisins in total dust sample**
Addition of enzyme concentrate (5%			Below limit		

subtilisins conc.) to process by gravity feed (10 minute work activity)	2	10 mins	of detection. Assumed as 1.5	4.1	0.00004%
Addition of enzyme concentrate (5% subtilisins conc.) to process by vacuum transfer (10 minute work activity)	1	10 mins	63.9	0.2	0.03%

46. Industry transfers blended dry detergent product into 1 tonne bags for on-site buffer storage, prior to final packing or for transit to another workplace for final packing. Bags are filled at a dedicated fill-point housed within a laminar flow booth, or located in the open factory but provided with LEV. A worker attaches a clean empty bag to the powder delivery chute and an inflatable seal between chute and bag is then actuated before powder delivery commences. Bag filling is automatically metered and when a bag is full, the worker releases the bag from the fill point and ties up the open neck before moving the bag away from the fill point.

47. Typically, workers can spend a full shift carrying out these activities. Workers wear high efficiency particulate respirators whenever they access fill points housed within laminar flow booths. Workers who access fill point operations in the open factory wear full-face air-fed visors.

48. Measured mean exposures to subtilisins for this activity, when undertaken in a laminar flow booth, were found to be 35.3 ng/m^3 and mean total dust exposures were 6.1 mg/m^3. The measured exposure to subtilisins for this activity when carried out at a fill point in the open factory provided with LEV was 53.2 ng/m^3 and total dust exposure was 8.5 mg/m^3.

49. These data show that exposure to subtilisins and total dust were lower for work carried out within a laminar flow booth than for similar work carried out in the open factory with LEV provided. This indicates that bag filling in the open factory cannot be regarded as good working practice and it is reasonable and practicable to house fill points within laminar flow booths or provide other equally effective control measures.

50. The maximum measured mean exposures to subtilisins at all further activities downstream of this point were found to be 5.1 ng/m^3 and the mean total dust exposure for specific work activities did not exceed 1.1 mg/m^3.

51. These results are shown at Table 2 and indicate that full shift exposures to subtilisins can be kept below 40 ng/m^3 and mean total dust exposures for specific work activities do not exceed 6.1 mg/m^3 where good practice techniques are used when handling enzyme formulations.

Table 2: Full shift activity mean occupational exposures to subtilisins in detergent manufacture, measured by HSE

Work Activity	Task specific mean personal exposure				
	Number of samples	**Approx sampling period**	**Subtilisins ng/m^3**	**Total dust mg/m^3**	**Subtilisins in total dust sample**
Bagging blended product containing 0.05% conc. subtilisins within a laminar flow booth (full shift activity)	2	2 hours	35.3	6.1	0.0006%
Bagging blended product containing 0.05% conc. subtilisins in open factory with LEV at point of fill. (full shift activity)	1	2 hours	53.2	8.5	0.0006%
Transfer of bags of blended product to buffer store by truck drivers (full shift activity)	4	2 hours	5.1	0.9	0.0006%
Deliver blended product to packing line and pack into boxes for end user (full shift activity)	11	2 hours	Below limit of detection Assumed as 1.5	1.1	0.0001%

52. The percentage of subtilisins in the total dust exposure at the bag fill and bag transfer activities was 0.0006%. This indicates the effectiveness of the granulate form in reducing dustiness as the granulate subtilisin content in the dry detergent product handled is 0.05%.

53. Exposures at bag filling and bag transfer occurred as a result of product residue spilling from the transfer chute onto the outside surface of the bag as it was manually coupled up to and subsequently removed from the workstation.

54. One plant visited can deliver blended product to 1 tonne containers and transfer them to buffer store by fully automated process where there is no operator intervention. This eliminated exposure at those stages but cost in excess of £0.25 million. Not all workplaces can afford this option.

55. Packing of dry detergent product is carried out continually throughout the shift within fully contained automated workstations. Exposures to subtilisins at this activity were found to be below the limit of detection (3 ng/m^3) and an assumed exposure of 1.5 ng/m^3 was taken for this activity.

Predicted Occupational Exposure Data

Other Industries

56. HSE has only measured personal exposure data for soap detergent manufacturing. For other industries, i.e. animal feeds and food and leather processing, we can only predict exposure data.

57. Dry enzyme concentrate is supplied in 25kg or 1 tonne bags to the animal feed, food and leather processing sectors. When required, bags are opened and tipped directly into a mixer. The mixer is held under negative pressure and provided with local exhaust ventilation (LEV) at the tipping point.

58. HSE has measured exposures to enzyme concentrate (alpha amylase in similar granular formulation) and total dust during bag tipping in the food sector. The workplace inspected did not use good practice to carry out this task as no control measures other than RPE were provided. The worker wore a medium efficiency particle respirator and slit open and tipped two 25kg bags of 10% concentration enzyme additive in granulate form into an open container. A total dust exposure (shown at Table 3 below) of 12.7 mg/m^3 was measured over ten minutes but no enzyme was detected in the sample. This indicates, even under poor conditions of dust control, the low dust potential of enzyme in granulate form.

59. Industry exposure data shown at Table 3 from the animal feeds sector indicates that the adoption of good working practice techniques can be used to control task specific total dust exposures during tipping of dry product to within the range 2.2 to 6.6 mg/m^3.

60. Table 3 also shows that by extrapolation from measured exposure data for dry product containing 10% concentration enzyme additive (measured at bag tipping in the food processing sector) the task specific exposure to subtilisins can be anticipated to be less than 1.5 ng/m^3 when good working practices are applied during this operation.

Table 3: Anticipated task-specific occupational exposures to subtilisins in other industries.

Work Activity	**No of samples**	**Sampling period**	**Total dust mg/m^3**	**Subtilisins ng/m^3**
Opening and tipping bags of 10% enzyme concentrate in food processing	1	10 mins	12.7	Below limit of detection, assumed value 1.5
Opening and tipping bags of 0.5% enzyme concentrate in animal feed mills	3	4 hours	Within range 2.2 to 6.6	Anticipated to be less than 1.5 (extrapolated from food sector data)

OPTIONS CONSIDERED

61. Because of the unsafe nature of the previous OES, and based on the advice of WATCH, the Health and Safety Executive did not consider that retention of the OES was a viable option in this case. It would have been feasible to have simply withdrawn the OES and issued a Chemical Hazard Alert Notice (CHAN) but this was not considered to be a viable option because of the risks to health arising out of exposure to subtilisins. The options lie in the level of the MEL to be set and what is reasonable and practicable for industry to comply with it.

INFORMATION SOURCES AND ASSUMPTIONS

62. Information used in this RIA was obtained from the following sources:

- Britain's three detergent companies
- The Food and Drink Federation
- The United Kingdom Association of Agricultural Supplies Trade Association
- The British Leather Confederation
- The Textile Services Association
- Several individual small and medium sized companies
- Various HSE and other government publications referred to in footnotes.

The base year for the cost and benefit estimates is 2003. Costs have been discounted over ten years using both the Treasury's old discount rate of 6% (1997 Green Book) and the Treasury's new rate of 3.5%. This approach aids comparison with previous relevant RIAs (where the 6% rate was used), and also conforms to the new rate.

COSTS TO COMPLY WITH A MEL

Business sectors affected

63. This MEL would affect three businesses within the detergent industry, an estimated fifteen businesses in the food processing industry, approximately 100 businesses in the feed milling sector and approximately 15 businesses in the leather and textiles industries.

64. The greatest risk of exposure to subtilisins for workers occurs when handling dry enzyme concentrate and dry detergent product during soap detergent manufacture. The soap detergent manufacturing industry can control mean task specific exposures to subtilisins to below 40 ng/m^3 and mean total dust exposures for specific work activities to not exceed 6.1 mg/m^3 by compliance with their own established industry guidelines[2].

65. Mean task specific exposures to subtilisins in other sectors are not expected to exceed 1.5 ng/m^3 whilst task specific total dust exposures are expected to lie in the range 2.2 to 6.6 mg/m^3.

66. Personal exposures to subtilisins will be further attenuated as all workers involved in handling dry enzyme formulations are provided with RPE. If a MEL is set at 40 ng/m^3 for both short term (15 minute reference period) and long term (8-hour time weighted average) then no additional costs for exposure controls are anticipated

for soap detergent manufacture or any other industry. Such a limit would thus be reasonable and practicable.

Compliance costs to business, charities and voluntary organisations

67. Compliance costs are considered under the following headings: Familiarisation costs; additional monitoring; health surveillance; and actions required to reduce worker exposure.

Familiarisation costs

68. HSE has assumed that one production/HSE manager at each workplace will require two hours to become familiar with the MEL and then develop a plan in response. Production managers are assumed to be paid £23.17 per hour[1]. HSE has estimated that there are 3 workplaces in the detergent industry, 100 in the feed milling industry, 15 in the food processing industry, and 15 in the leather and textiles industries. Total costs, occurring on a one-off basis in the first year of compliance, are estimated to be £6,000.

Additional monitoring

69. Under the previous Occupational Exposure Standard, businesses were encouraged to conduct static sampling of workplaces. Although the OES was withdrawn by the Health and Safety Commission in January 2003, HSE has used the OES as the default baseline for assessing the incremental monitoring compliance costs associated with the MEL. In the detergent industry, static sampling is used to detect the range of enzymes used in detergent manufacturing. This practice would continue after the introduction of the subtilisins MEL in order to detect concentrations of non-subtilisin enzymes. The detergent industry's MEL compliance costs would therefore be the full cost of introducing personal monitoring, assumed to be £800 per 10 workers. Detergent businesses would be encouraged to conduct personal monitoring on one occasion in order to establish the baseline, and maintain plant and machinery in an appropriate state thereafter. In cases where there are significant process changes, the business involved would need to conduct another baseline personal monitoring exercise. However, these events are assumed to be very rare and therefore have not been costed in this RIA. HSE has assumed that 100 workers are exposed to subtilisins within the detergent industry. The additional monitoring cost to the detergent industry, occurring on a one-off basis in the first year of compliance, is estimated to be £8,000. One detergent manufacturer has indicated to HSE that monitoring will cost the company £50,000. However, this estimate is based on much stricter monitoring practices than required under the MEL, and HSE therefore judges that its lower estimate is more appropriate.

70. The other industries affected by the MEL already conduct personal sampling, although they do not test the dust for enzyme content (in any case the technology to do this has only recently become available). The compliance cost to these industries of conducting personal sampling for subtilisins is therefore assumed to be a one-off £400 per ten workers, or in other words, half the full cost of personal sampling for subtilisins. HSE has assumed that 1,000 workers are exposed to subtilisins in the feed milling industry, 50 workers in the food-processing industry and 40 workers in the leather and textiles industries. The additional monitoring costs to these

[1] £17.82 taken from the New Earnings Survey (SOC110) plus 30% for non-salary costs.

industries, occurring on a one-off basis in the first year of compliance, are estimated to be £44,000.

71. The total cost to industry from additional monitoring is therefore estimated to be £52,000.

Health Surveillance

72. The previous OES did not require businesses to conduct health surveillance for workers exposed to subtilisins. However, currently followed good practice in the detergent industry specifies the need for regular health surveillance, and hence HSE has assumed no incremental health surveillance costs arising from the MEL. The other affected industries have not provided health surveillance and hence would incur the medical fees determined under The Health and Safety (Fees) Regulation 2002 for medical examinations under COSHH. The examinations would be annual and the fee is currently £57. The annual costs arising from the need to provide health surveillance in the non-detergent industries is estimated to be £62,000. Using the Treasury's old discount rate of 6%, the ten year present value of health surveillance costs is £485,000. Using the Treasury's proposed discount rate of 3.5%, the present value (PV) is £535,000.

Actions Required to Reduce Worker Exposure

73. Although sampling evidence gathered by HSE in 2002 suggests that there were some instances of workers in the biological detergent industry being exposed to subtilisins concentrations of above the proposed MEL, information gathered since the sampling suggests that actions have already been taken to reduce exposure to below the 40ng/m^3 limit. This new information, combined with HSE's evidence that the other affected industries (feed milling, food processing, leather processing and textile manufacturing) do not expose their workers to subtilisins concentrations above the proposed MEL, indicate that no additional action would be required from industry in order to comply with the MEL. Regarding LEV maintenance, user industries already provide LEV systems at dry enzyme formulation handling points. Thus the cost of annual examination required under COSSH is already accounted for, and no incremental costs are anticipated.

Compliance costs for a 'typical' business

74. Because there are only three companies within the detergent industry that will be affected by the MEL, the notion of a typical company does not make a great deal of sense. However, for illustrative purposes only, the "average" business in this sector employs 33 workers who are exposed to subtilisins. The compliance costs faced by the notional company are as follows:

Familiarisation (£)		46
Additional monitoring (£)		2667
Health surveillance (£)		0
	Total	2746

75. Within the feed milling industry, the costs faced by a "typical" company (or more precisely, a statistically average company) are as follows:

Familiarisation (£)	46
Additional monitoring (£)	400
Annual Health surveillance (£)	570
Total first year costs	1026
Ten year NPV (6% discount rate)	4893
Ten year NPV (3.5% discount rate)	5353

Total compliance costs

76. Total compliance costs to industry in the first year of compliance will be £120,000, and ten year discounted costs of £542,000 (6% discount rate) or £593,000 (3.5% discount rate). The breakdown of costs is as following:

Table 4. Overall Summary of Costs

	One-off costs	Annual costs	Ten year costs (Present value 6% discount rate)	Ten year costs (Present value 3.5% discount rate)
Detergent industry	8000	0	8000	8000
Feed millers	45,000	57,000	489,000	535,000
Food processing	3,000	3,000	25,000	27,000
Leather and textiles	2,000	2,000	20,000	22,000
Total	58,000	62,000	542,000	593,000

Note: Totals may not add up due to rounding errors.

Impact on small businesses

77. Apart from contacting the relevant trade associations, HSE staff contacted five small companies in the leather industry in order to establish whether the MEL would have a disproportionately large effect on small businesses. None of these companies used enzymes of any description in their manufacturing processes. Furthermore, they were unaware of any other small businesses that might use enzymes. It is possible that small businesses in the feed milling industry may use products containing subtilisins, to which workers may be exposed

78. HSE anticipates that compliance will mostly involve variable costs, and very limited fixed costs (connected with familiarisation) are expected. On this basis, the subtilisins MEL should not have a disproportionate impact on small businesses.

BENEFITS

Health and safety benefits

79. Within occupational health statistics, no link is made between reported cases of occupational asthma (diagnosed by occupational physicians) and the use of subtilisins by industry in Great Britain. Occasionally, the incidence of occupational asthma in a particular workplace prompts an investigation, and, on at least one occasion within the last three years, a link between asthma and subtilisins has been established. Despite this, there remains no reliable means of estimating the number of cases that occur each year. Furthermore there is no epidemiological evidence on the degree to which introducing the proposed MEL will reduce the incidence of

occupational asthma. For these reasons, no attempt has been made to estimate the health benefits from introducing the MEL. However, in order to put the compliance costs into context, HSE has presented the costs on a "per worker exposed" basis, and compared this figure with those from analogous MELs. This analysis appears in the section on Balance of Costs and Benefits.

Other benefits

80. No other benefits have been identified.

COMPETITION ASSESSMENT

81. The biological detergent industry is highly concentrated, with three firms controlling almost 100% of the market. However the proposed MEL would not affect any one of these firms more than the others, and would therefore not affect the current market structure. New entrants to the industry would not face higher costs compared with their competitors, either through higher start-up or on-going costs.

82. All other industries affected by the MEL are not concentrated to the degree outlined in the competition filter questions. The MEL will not affect market structure even though two of the industries (feed milling, leather processing) contain a number of small and medium sized business. These will not be disproportionately affected due to the very low level of indirect costs imposed by the MEL. New entrant firms will not face higher set-up or on-going costs compared with existing firms.

83. Technology and choice will not be affected in any of the industries affected by the MEL.

Table 5. Competition filter applied to the detergent, feed milling, food processing, leather processing and textile manufacturing industries

Question	Detergent industry	All other affected industries
Does any firm have more than 10% market share?	Yes	No
Does any firm have more than 20% market share?	Yes	No
Do the largest firms together have more than 50% market share?	Yes	No
Are some firms affected substantially more than others?	No	No
Is the regulation likely to alter market structure?	No	No
Would the set-up cost be higher for new firms?	No	No
Will the on-going costs be higher for new firms?	No	No
Is there rapid technological change?	No	No
Will there be restrictions on firms' choices?	No	No

84. On the basis of these responses, a more detailed competition assessment is not required.

COSTS TO HSE

85. No incremental costs to HSE are anticipated.

OTHER COSTS

86. No other costs are anticipated.

TOTAL COSTS TO SOCIETY

87. Total costs to society are the same as the total costs to industry.

ENVIRONMENTAL IMPACTS

88. No significant environmental costs are anticipated.

BALANCE OF COSTS AND BENEFITS

89. Total costs for the subtilisins MEL can be placed in context by comparing them with total costs from other MELs involving asthmagens. This has been done in the table below on a per worker affected basis. The evidence suggests that the costs associated with the subtilisins MEL will be modest in comparison with MELs for other asthmagens.

Table 6 Comparison of MELs in terms of cost per worker

	High cost estimate (£/worker)	Low cost estimate (£/worker)
Subtilisins	456	456
Flour MEL*	10909	1549
Glutaraldehyde*	5154	3897

Note: Figures are 10 year present values, using the 6% discount rate
* Figures have been uprated from respective base years to 3rd quarter 2002 values using the ONS average earnings index

90. The "do nothing" option (which would involve not introducing the MEL in place of the withdrawn OES) might create costs associated with greater ill health. However, this would only happen if industry standards slipped to an extent that caused more cases of occupational asthma. The lack of statistical evidence on the link between subtilisins in the workplace and occupational asthma makes estimating the costs of the "do nothing" option impossible.

Uncertainties

91. There are no substantial uncertainties attached to the estimated compliance costs. Consequently, no sensitivity analysis has been conducted.

ARRANGEMENTS FOR MONITORING AND EVALUATION

92. No specific arrangements have been made to evaluate the effect of this MEL. However, the Health and Safety Executive intends to look generally at the whole issue of the use of enzymes and will include subtilisins within that review.

REFERENCES

1 WATCH Risk Assessment Document for Subtilisins, September 2000.

2 Cleaning Products Industry (CPI), formerly Soap and Detergent Industry Association (SDIA), Enzymes Occupational Exposure Working Group, Guidelines for the Safe Handling of Enzymes in Detergent Manufacturing, 2001.
3 Cathcart M., Nicholson P., Roberts D. et al. (1997). Enzyme exposure, smoking and lung function in employees in the detergent industry over 20 years. *Occup. Med*., **47(8)**, 473-478.

PERSONAL SAMPLING METHOD

This method described below for the collection and measurement of subtilisin samples is based on enzyme activity. The assay is sensitive and specific for the measurement of the active portion of subtilisin-containing products, not the total weight of product (which may contain some inactive enzyme).

Collection of inhalable dust samples

Personal samples were collected in the worker's breathing zone using IOM sampling heads with glass fibre filters (GF/A, 1.6mm, Millipore) and Gillian Gilair5 pumps at a flow rate of 2L/min. Where possible, static samples were taken at or around the areas that personal sample measurements were taken. Areas where enzymes were handled and where products were bulk bagged or emptied were also targeted. In-house static loci were mirrored where possible.

Gravimetric Analysis

The weight of total inhalable dust collected was determined as described in MDHS 14 with exposed and non-exposed filters being conditioned before and after sampling for 24 hours in the balance room. After weighing, the filters were stored at 4^0C until eluted.

Elution of Filters

Filters were allowed to reach room temperature and eluted into 2ml PBS/0.1% Tween 20 overnight by end-over-end mixing. The filters and supernatant were squeezed through a 2ml syringe and centrifuged at 600g for 10 minutes. The supernatant was removed and immediately analysed.

Fluorescence polarisation for the measurement of subtilisins.

Background

Fluorescent polarisation kits are based on the principle that a fluorescently-labelled compound when excited by plane polarised light will emit polarised fluorescent light. The level of polarisation of the emitted fluorescence is related to the speed of rotation of the complex, which is governed largely by the molecular mass. The smaller the molecular mass of a complex, the faster it can rotate and the emitted polarised fluorescence will be lower. Larger molecules will rotate at a slower speed and therefore emit higher levels of polarised fluorescent light. Therefore if a large complex with an appropriate fluorescent tag is cleaved to form two smaller molecules then there will be a measurable decrease in the levels of polarised fluorescent light emitted.

Protease assay method

The developed protease assay used BODIPY™ dye bound to α-casein protein as a substrate. The casein-BODIPYFL conjugate was prepared in-house. 10mg/ml casein

was dissolved in 0.1M sodium bicarbonate/carbonate pH9 buffer. 1mg of BODIPYFL SE. (Molecular Probes, Leiden, Netherlands) was dissolved in 1ml of DMSO, and stored at -20ºC until use. 25µl of 1mg/ml BODIPY-FL was added to 1ml of 10mg/ml casein, in a ratio of 500:1 (casein:BODIPYFL) by weight (7:1 mol:mol). This was then incubated for 15 minutes at room temperature. The reaction mixture was then run into a desalting column (Pierce, Cheshire, UK, washed with 30mls of PBS containing 0.05% sodium azide) and 1ml fractions were collected using PBS containing 0.05% sodium azide as diluent. The fluorescence of fractions was measured, (489nm:515nm) and the high initial fractions at around fractions 3 to 4 (which represent the protein conjugate) were pooled. The protein content of pooled fraction was measured by bicinchoninic acid protein assay (BCA) method and adjusted to 2 mg/ml using PBS buffer with 0.05% azide. This was then stored in small aliquots (100µl) at -20ºC or lower, until it was used as the enzyme substrate (start reagent) for the assay.

When protease is added to the enzyme substrate the α-casein is cleaved. Labelled fragments are produced which rotate faster and the plane polarised light is depolarised to a greater degree; this leads to a decrease in fluorescence polarisation (measured as millipolarisation units, mP). The method is performed on a COBAS FARA automated analyser with a fluorescent polarisation unit. Essentially the 40 µl of sample was diluted with 180 µl of an appropriate buffer, incubated at 37ºC for two minutes and a blank fluorescent polarisation reading was taken. The reaction was then initiated by the addition of 5 µl of a 1/18 dilution of the stock BODIPY-casein substrate. After 10 minutes of enzyme reaction, the final polarisation reading was taken. Subtilisin carlsberg (Sigma) was prepared at a range of concentrations between 0 and 125 ng/ml to provide standards. These were loaded on to the racks of a Cobas Fara analyser along with 100 µl aliquots of the eluted samples. Reagent racks were prepared containing cups of the diluted substrate and additional buffer. The analysis was done in duplicate for each standard and sample. Analysis was done at excitation 489nm and emission 515nm.

SUBTILISINS

(As active enzyme)

8-hour TWA: 40ng/m^3

15 minute STEL: 40ng/m^3

Notation: Sen

IDENTITY AND PROPERTIES

CAS No: 1395-21-7 (*Bacillus subtilis* BPN)
9014-01-1 (*Bacillus subtilis* Carlsberg)

Enzyme Commission No: EC.3.4.21.62

Synonyms and Trade names: Subtilisin Carlsberg, Subtilisin A, Subtilopeptidase A, Subtilisin BPN, Subtilisin B, Subtilopeptidase B, Subtilopeptidase C, Alcalase, Savinase, Maxatase, Esperase, Biozym, Milezyme, Opticlean

Molecular weight: Approx. 28,000

Subtilisin (9014-01-1) is classified in the 7th edition of the Approved Supply List under the Chemicals (Hazard Information and Packaging for Supply) Regulations 2002 (CHIP 3) and is labelled with the following risk (R) phrases to indicate its toxicological hazards:

R37/38: *Irritating to the respiratory system and skin*

R41: *Risk of serious damage to the eyes*

R42: *May cause sensitisation by inhalation*

OCCURRENCE AND USE

Subtilisins are not manufactured in Great Britain but are imported by a few major suppliers. It is estimated that, annually, up to 100 tonnes of the enzyme is imported contained in up to 5,000 tonnes of granulated powder, wheat substrate or liquid formulation with a concentration of total subtilisin up to 10%. Subtilisins are used in the manufacture of detergents and animal feeds; also for food and leather processing.

EXPOSURE AND CONTROL

Subtilisins for soap detergent use are imported as granulates, liquids or slurries. The dustiness of the dry product is controlled by "encapsulating" the enzyme in a coarse granule (>150μ) of phosphate base using a tacky non-ionic detergent. The enzyme is also supplied in the form of "prills" which are beads containing enzyme embedded in a non-dusty matrix. The result is non-dusty solid beads containing up to 5 % total enzyme.

Liquid formulations of subtilisins for all uses are stabilised with an additive which decreases the vapour pressure.

Work practices in the soap detergent industry include a high degree of automation, containment, engineering control and use of PPE to control residual risks. The potential for inhalation and dermal exposure is likely to arise during the handling and tipping of dry and liquid concentrated enzyme formulations and the packing of dry product. However, the liquid-handling processes are unlikely to generate aerosol. The dustiness of solid formulate is low. About 100 people are potentially exposed to subtilisin in the soap detergent industry, with exposure generally kept below 15 ng/m^3 active enzyme.

In the preparation of animal feeds, the potential for inhalation and dermal exposure to subtilisins occurs in filling the liquid reservoir, weighing and addition of dry enzyme concentrate formulations, plant operation, QA sampling and lorry loading. However, the liquid processes are unlikely to generate aerosol, and the dustiness of materials will be low; three-quarters of the final product will be in pelletised form. It is estimated that up to 1,000 people may be potentially exposed to the 0.5% subtilisins concentrate during weighing and/or tipping, and up to 15,000 exposed to the "downstream" animal feed containing subtilisins at about 0.0005%.

Subtilisins are used in food processing to hydrolyse soya bean, gelatin and yeast. Up to 25 tonnes of the enzyme is used annually for these applications with concentrations of up to 10% total enzyme. The potential for inhalation and dermal exposure to subtilisin will occur at the point of dispensing the enzyme and adding to the broth. However, these processes are unlikely to generate aerosol; and the dustiness of the materials will be low. It is estimated that

fewer than 50 people may be potentially exposed to the enzyme during food processing. Within leather processing, the potential for exposure to subtilisin will occur at the point of weighing the enzyme concentrate formulation and addition to the drum. However, the dustiness of the substances will be low. It has been estimated that fewer than 40 workers are exposed to subtilisin during leather processing.

MEASUREMENT

Workplace personal monitoring

The standard analytical method for measurement of airborne enzyme (based on the reaction of the enzyme with N,N-dimethylcasein (Fulwiler et al., 1972; Dunn and Brotherton, 1971) has been used for personal air sample measurements. Air samples are collected in the worker's breathing zone using IOM sampling heads with glass fibre filters (GF/A, 1.6 mm, Millipore) and Gillian Gilair5 pumps at a flow rate of 2L/min as described in MDHS 14/2 (Health and Safety Executive, 1997). Sampling times of 6 to 8 hours are required. The filters are extracted with a suitable buffer and reacted with N,N-dimethyl casein. The reaction is allowed to proceed for a fixed time under controlled conditions (in a continuous flow analyser or similar). Amino acids liberated by the action of the protease enzyme react with 2,4,6-trinitrobenzenesulphonic acid (TNBSA) to form coloured complexes. To allow for colour produced by non-enzymatic materials present in the dust a blank determination is required. Note; this is an activity –based method whereby only active enzyme is measured.

Biological Monitoring

There are no published methods available for the biological monitoring of exposure to subtilisin.

TOXICOKINETICS

No toxicokinetics studies are available. However, the following aspects of toxicokinetic behaviour can be predicted. The subtilisins are high molecular weight water-soluble proteins that may be present in the workplace either as a dry powder or in a liquid preparation. If inhaled, the large molecular size of these enzymes would minimise the potential for absorption directly across the respiratory tract epithelium. However, their proteolytic activity may enable these enzymes to damage the epithelial barrier in the lung thus increasing their potential for direct absorption. If deposition in the alveolar lung occurred then it is likely that the subtilisins will be phagocytosed by macrophages and broken into smaller peptides by proteolytic enzymes within lysosomes. Orally administered subtilisins will be subject to the same digestive processes as any other protein. In relation to dermal exposure, it is considered that absorption across intact skin would be precluded by the large molecular size of the molecule.

HEALTH EFFECTS

Studies in animals

Single exposure inhalation studies in animals indicate that subtilisins are toxic via the inhalation route, causing direct effects on the lungs, haemorrhage, congestion and oedema, probably reflecting the proteolytic activity of these enzymes. Guinea pigs appeared to be more sensitive than rats or rabbits, with mild microscopic changes reported in the lungs at 0.1 $mg.m^{-3}$ enzyme. However, no changes were observed in rats or rabbits at this concentration. Four-hour LC_{50} values of 130 or 229 $mg.m^{-3}$ were obtained in the rat for two other subtilisin preparations. Subtilisins are of low oral toxicity on single exposures. The systemic effects of single dermal exposures have not been studied but given the predicted lack of dermal absorption, systemic toxicity would not be anticipated by this route.

Mild erythema and oedema was observed in skin irritation tests with concentrated subtilisin preparations and there is clear evidence to show that subtilisin enzyme preparations are irritant to the eye. There is no evidence to suggest that subtilisins have sensory irritant properties.

In relation to skin sensitisation, very little testing has been conducted in animals; an apparently positive result was obtained in a single study in guinea pigs. However, there are doubts about whether the skin reactions observed were irritant or allergic in nature and this "positive" finding has not been confirmed in other animal studies. There is no useful information concerning the effects of repeated inhalation exposure to subtilisins in animals. However, results from single exposure studies suggest that on repeated inhalation exposure, chronic inflammatory damage could occur, caused by the localised proteolytic activity of these enzymes. Oral dosing studies in rats suggest that with repeated high doses, local gastro-intestinal disturbances can occur, but this has

no obvious relevance to occupational exposure conditions. The only predicted effects of repeated dermal exposure would be for local skin irritation.

Negative results were obtained in Ames tests with two subtilisin preparations, and *in vivo* tests in somatic and germ cells were negative for one preparation. The results support the conclusion that subtilisins are not genotoxic. No studies have been conducted to investigate carcinogenic potential but this would not be predicted for this class of substance. No studies have been conducted to investigate reproductive toxicity. Reproductive effects would not be anticipated, as systemic distribution to the reproductive organs is not likely to occur via occupational routes of exposure.

Studies in humans

There are no human data on the systemic effects of single exposures to subtilisins.
In human volunteer studies aqueous solutions containing up to 20% of a concentrated subtilisin preparation were not irritant to intact skin but were irritant to damaged skin. Workers directly handling concentrated enzyme preparations reported skin problems mainly on the fingertips, wrist and neck but the role of subtilisins in causing these problems have not been adequately explored.

Extensive patch testing in large-scale human volunteer studies has shown no evidence for the ability of subtilisins to induce skin sensitisation. Negative results have also been obtained in patch tests in subtilisin-exposed workers. Furthermore, no confirmed cases of skin sensitisation caused by subtilisins have been identified in workers engaged in the manufacture/use of these enzymes. Overall, human evidence suggests that subtilisins should not be regarded as skin sensitisers.
Evidence from bronchial and nasal challenge studies in detergent workers shows that subtilisins can cause occupational asthma and allergic rhinitis. When subtilisins were first introduced into the detergents manufacturing process there were a large number of cases of occupational asthma attributed to these enzymes each year (7-39 before 1975). It is likely that all of these cases were due to subtilisins as these were the only enzymes used in detergent manufacture over that time period. Since then, hygiene conditions have improved, and there has been a corresponding drop in the number of cases of detergent enzyme-related asthma per year (0-4 since 1980). No personal exposure data are available and there is no information concerning the exposure-response relationships for subtilisin-induced asthma/allergic rhinitis. Other than occupational asthma and allergic rhinitis, a large body of health surveillance data in detergent workers reveals no evidence for other adverse health effects relating to the use of subtilisin preparations.

BASIS FOR SETTING THE LIMIT

The key health concerns for subtilisins are their potential to cause occupational asthma and allergic rhinitis. The available data do not allow a threshold for the induction of these conditions by subtilisins to be identified nor is it possible to determine what the exposure-response relationship might be. On this basis WATCH consider that subtilisins do not meet the criteria for the establishment of an OES. In view of the potentially serious nature of occupational asthma, subtilisins meet the criteria for the establishment of MEL(s). Since both long-term repeated exposures and short-term peak exposures may be of relevance to the induction of occupational asthma and allergic rhinitis, both 8-hour TWA and STEL MELs should be established.

In relation to other risk management measures, given that there is clear evidence that subtilisins are a cause of occupational asthma, an OEL for subtilisins should be accompanied by a “Sen” notation. As dermal absorption is not an issue, a “Sk” notation is not warranted. Subtilisins do not meet the criteria for establishing a BMGV because the lack of dermal absorption indicates that an airborne limit will be sufficient to control exposure, and no suitable methods are available for biological monitoring of exposure to subtilisins

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Fifth report Soap and Detergent Industry Association, UK

OTHER USEFUL PUBLICATIONS

Subtilisins: Risk Assessment Document EH72/x
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