# SUBSTANCE EVALUATION CONCLUSION

# as required by REACH Article 48

# and

# EVALUATION REPORT

**for**

# Amylase, α-

## EC No 232-565-6

## CAS No 9000-90-2

**Evaluating Member State(s):** United Kingdom

Dated: July 2016

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# Evaluating Member State Competent Authority

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## Year of evaluation in CoRAP: 2015

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

**Further information on registered substances here:**

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

# Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

# Contents

# Part A. Conclusion

## 1. CONCERN(S) SUBJECT TO EVALUATION

Amylase, α- (alpha amylase) was originally selected for substance evaluation in order to clarify concerns about potential exposure of workers and consumers.

The CoRAP justification document states "This enzyme is classified as a respiratory sensitiser. Uses have been identified which have the potential to create exposures sufficient to produce adverse reactions in workers or consumers if suitable controls are not implemented. It is important to clarify the approach that the Registrants have taken to estimate exposure during the manufacture and use of this enzyme and the approach they have taken to derive benchmarks (DMELs) against which to judge the acceptability of exposure."

During the evaluation an additional concern was identified relating to the representativeness of the simulation studies used to support the exposure assessment for certain scenarios. This additional concern was addressed by the registrants providing additional simulation studies. In the light of the new information the eMSCA did not identify a concern for these uses.

## 2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

No occupational exposure limits have been established for amylase, α- under the Chemical Agents Directive (98/24/EC) and amylase, α- was not assessed under the Existing Substances Regulation. The eMSCA is aware of the following ongoing/previous risk assessments of amylase, α-:

- (DECOS, ongoing assessment). Dutch Expert Committee on Occupational Safety. Fungal alpha amylase (derived from the fungus *Aspergillus oryzae*). Draft recommendation for a health-based occupational exposure limit.
- Health Council of the Netherlands (2008). Prevention of work-related airway allergies. Recommended occupational exposure limits and periodic screening.
- (HERA, 2005) Human and Environmental Risk Assessment on ingredients of household cleaning products: α-amylases, cellulases and lipases.

## 3. CONCLUSION OF SUBSTANCE EVALUATION

The health concern for amylase, α- is respiratory sensitisation. It is not possible to identify a threshold for this effect from the available data. The long-term inhalation DMEL of 60 ng/m$^3$ for workers is based on the Ceiling TLV for subtilisins established by the ACGIH in the early 1970's which was the lowest level (based on measurements for the protease subtilisin) that could be achieved in the detergents industry at the time. The long-term inhalation DMEL of 15 ng/m$^3$ for consumers is based on a lack of evidence in consumer product trials that products potentially giving exposures of this magnitude cause sensitisation. No other DMELs have been derived. Although the eMSCA recognises that there is a high degree of uncertainty about the level of risk for developing respiratory sensitisation at these levels, it did not identify any information that would suggest different values would be more appropriate.

The exposure assessment provided by the Registrants is based on measured data including: high volume static sampling data from registrants' sites; high volume static sampling data from downstream user sites; simulation studies covering professional and consumer uses; a small number of personal monitoring samples covering professional hard surface cleaning and consumer product trial data. Often protease has been measured as a surrogate for amylase, α- where enzyme mixtures are present. It is possible to perform analyses for multiple enzymes from the same sampling filters using specific immunoassays or where the enzymes have very different substrate specificities. This has been done in some cases meaning that the measured data set contains some data points for amylase, α- in addition to the surrogate protease data.

The Registrants have performed a quantitative risk characterisation, based on a comparison of exposure levels against the DMEL. Typically 90$^{th}$ percentiles from data sets have been used in the risk characterisation ratio (RCR) calculations. With the exception of the measured data from registrants' sites and the detergents manufacturing sector, data sets are not large. This introduces uncertainty into the exposure estimates.

Since the risk of developing asthma following exposure at levels around the DMELs is not known, and given the uncertainty in the exposure data, rather than focus on the quantitative risk characterisation, the eMSCA has made a qualitative assessment of the suitability of the identified operating conditions (OCs) and risk management measures (RMMs) to manage the risks for respiratory sensitisation.

In relation to worker scenarios, the eMSCA concluded that generally, the measures that have been identified by the registrants to secure safe use in the workplace are suitable and adequate. However, some situations were identified where additional communication targeted at specific uses might be helpful. Also, the eMSCA is aware of some recent work by the UK Health and Safety Executive (HSE) that found inconsistencies in the way safe use information for cleaning products is being communicated along supply chains. This work was not targeted to enzyme containing products, but the eMSCA considers the findings may be relevant for products containing amylase, α-. It is therefore considering whether additional voluntary, industry-led or regulatory actions could be taken to improve supply chain communication and the implementation of the recommended measures by downstream users and will discuss this further in a risk management options analysis (RMOA) document.

The eMSCA did not identify concerns for consumer use with the exception of the possible addition of amylase, α- to dishwashing liquids supplied to consumers for manual dishwashing. The eMSCA does not have a concern for this use where such products are used for washing dishes but does have a potential concern if these products are used for activities where aerosols could be generated. Preliminary information obtained by the eMSCA suggests that enzymes are generally not included in manual dishwashing liquids supplied to consumers. The eMSCA intends to consider this potential risk further in the RMOA. There are no concerns for consumers using enzyme containing products intended for use in automatic dish washers or for hand or machine washing of laundry.

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in table 1 below.

**Table 1**

| **CONCLUSION OF SUBSTANCE EVALUATION** | |
|---|---|
| **Conclusions** | **Tick box** |
| Need for follow-up regulatory action at EU level<br>*[if a specific regulatory action is already identified then, please, select one or more of the specific follow-up actions mentioned below]* | ✓* |
| Harmonised Classification and Labelling | ✓* |

| | |
|---|---|
| Identification as SVHC (authorisation) | |
| Restrictions | |
| Other EU-wide measures | |
| No need for regulatory follow-up action at EU level | |

*This is a tentative conclusion about one possible option to improve information communication through the supply chain. This and other options, including voluntary, industry-led actions will be explored in more detail in the RMOA.

# 4. FOLLOW-UP AT EU LEVEL

## 4.1. Need for follow-up regulatory action at EU level

### 4.1.1. Harmonised Classification and Labelling

Amylase, α- is currently listed in Annex VI of the CLP Regulation with a harmonised classification of Resp. Sens 1. While it is not possible to identify a clear threshold for induction or elicitation, the evidence suggests that these processes can occur at dose levels in the ng/m$^3$ range. This raises a concern that mixtures containing amylase, α- may present a risk for respiratory sensitisation at concentrations below the generic cut off value of 1% established in CLP for classification of mixtures containing Resp. Sens. 1 substances classified as Resp. Sens. 1. Although it is possible to add warnings about respiratory sensitisation potential without formally classifying a mixture if the mixture contains 0.1% or more of a substance that has been classified as Resp. Sens. 1, given the very low exposure levels that have been linked to induction and elicitation for amylase, α-, it has been suggested that the current provisions for classifying and labelling mixtures containing this enzyme may not be adequate to ensure effective communication about the respiratory sensitisation potential of such mixtures through the supply chain. It therefore seems appropriate to consider if requirements to specifically communicate information about respiratory sensitisation potential at lower concentration thresholds would help to secure safe use of mixtures containing amylase, α- and whether action taken under the CLP regulation would be the most effective route to achieve such communication.

The CLP regulation provides two options to lower the concentration limits for communicating on respiratory sensitisation hazard. This can be done by establishing specific concentration limits for the substance in question or it can be done by making use of the Resp. Sens. 1A sub-category. The possibility of establishing specific concentration limits is discussed in section 3.4.2.1.5. of the Guidance on the Application of the CLP Criteria[2]. This states that:

"Respiratory sensitisers cannot be identified reliably on the basis of animal tests as yet, since no recognised validated test exists to determine sensitising potential and potency by inhalation. Therefore specific concentration limits (SCLs) cannot be set on the basis of animal data alone. Moreover, there is no concept available to set SCLs on the basis of human data for respiratory sensitisers."

[2] http://echa.europa.eu/documents/10162/13562/clp_en.pdf

The eMSCA has not identified information for amylase, α- that could be used to advance thinking on this point sufficiently to allow SCLs to be established for this enzyme, hence this option does not seem viable in this case.

The criteria for making use of the Resp. Sens. 1A sub-category are outlined in Table 3.1.4 of the Guidance on the Application of the CLP Criteria. Annex I: 4.5.2.1.1.3 states that substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in Table 3.4.1 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals. The relevant sections of table 3.1.4 are reproduced here for convenience.

| | |
|---|---|
| Sub-category 1A: | Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests (1). Severity of reaction may also be considered. |
| Sub-category 1B: | Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests (1). Severity of reaction may also be considered. |
| (1) At present, recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment. | |

These criteria indicate that it is the frequency of occurrence of cases of sensitisation that is the determining factor rather than the exposure levels associated with sensitisation. In relation to this, the Guidance on the Application of the CLP Criteria notes that:

"High frequency and low to moderate frequency cannot be defined as specific concentrations or percentages for human study data because when considering human evidence, it is necessary to take into account the size of the exposed population and the extent and conditions of exposure, including frequency. It is necessary, therefore, to reach a view on a case-by-case basis."

Currently, the only information available to the eMSCA from which to assess the frequency of cases is that collected by the The Health and Occupation Research Network (THOR) (see section 7.9.9. for numbers of cases associated with REACH registered uses). Given that amylase, α- is one of the enzymes included in laundry products supplied to consumers, the size of the exposed population is potentially very large. The exposed population could be limited to those potentially exposed at work, since this is the population covered by the THOR data. However, the eMSCA does not have reliable data on how many workers are potentially exposed to amylase, α-. It is therefore not clear if the information that is available would be sufficient for ECHA's Risk Assessment Committee to make a decision on whether or not allocation of amylase, α- into the Resp. Sens. 1A sub-category is justified. This will be discussed further in the RMOA alongside a consideration of the wider consequences of lowering the classification threshold.

### 4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Although respiratory sensitisers may be identified as SVHCs according to Art 57(f) the eMSCA does not think that this is an appropriate step for amylase, α- for the following reasons:

Enzymes such as amylase, α- are increasingly being used because they offer several benefits when compared with alternative substances for various processes. For example they have a very specific targeted activity meaning that good results can be achieved with small quantities without the damage to process equipment and product that can occur when harsh chemicals such as acids or alkalis are used. They allow processes to take place at lower temperatures making a significant contribution to lowering energy consumption and process wastes are less damaging to the environment. For these reasons, enzyme technologies make a valuable contribution to sustainable production initiatives and green chemistry and this should be taken into account in any regulatory action that may be taken to manage the identified risks to human health.

The clear aim of the authorisation regime is to put pressure on companies to substitute hazardous substances for less hazardous substances. Experience with this relatively new risk management tool has demonstrated that the scrutiny that is applied to companies submitting applications for authorisation can help those companies improve the way they manage very hazardous substances. However, in order to unlock the authorisation process, a substance must first be identified as an SVHC and must then be included in Annex XIV with a sunset date after which, uses that are not authorised must cease. Identification as an SVHC and setting a sunset date both send signals that this is an undesirable substance which should be phased out as soon as possible. Given the many environmental benefits that have been identified for enzyme technologies, and the fact that current alternatives do not offer the same environmental benefits, such messages seem counterproductive. If the main aim for the identification of amylase, α- as an SVHC is to allow regulatory authorities to set conditions of use, it would be better to find an alternative route that is not tied to the expectation that use will be phased out in the near future.

Another reason that identification as an SVHC and authorisation may not be a good risk management option for amylase, α- is that the greatest ill-health burdens arise in connection with uses that are not within the scope of authorisation (use in bakeries as a flour improver). Information collected by THOR in the UK suggests that up to four times as many asthma cases due to enzymes occur in bakers compared with uses that are within the scope of authorisation. By making amylase, α- subject to authorisation, regulatory authorities would be directing their resources away from those uses where there is arguably the greatest need for intervention. It would seem preferable to work with actors in the supply chain to develop tools and processes which will:

- identify best practices when working with enzymes across all uses,
- help disseminate this information to end users, and
- provide end users with the understanding to follow the best practice advice that has been given.

In this way, the benefits of intervention have the potential to spread well beyond the reach of REACH.

### 4.1.3. Restriction

Restriction may be an option to prevent uses where there appears to be an unacceptable risk. The evaluation identified a potential concern if amylase, α- is added to manual dishwashing liquids supplied to consumers. However, preliminary investigations by the eMSCA did not find evidence that this is currently being done. At this point in time, no uses have been identified which pose a sufficient level of risk to warrant the introduction of restrictions.

### 4.1.4. Other EU-wide regulatory risk management measures

Other legislative options that may help manage the risks for respiratory sensitisation will be examined in the RMOA.

# 5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

## 5.1. No need for regulatory follow-up at EU level

This is a possible outcome from the RMOA.

**Table 2**

| REASON FOR REMOVED CONCERN<br>The concern could be removed because | Tick box |
|---|---|
| Clarification of hazard properties/exposure | |
| Actions by the registrants to ensure safety, as reflected in the registration dossiers (e.g. change in supported uses, applied risk management measures, etc.) | |

## 5.2. Other actions

The potential for voluntary, industry-led activities to address the identified concerns will be considered in the RMOA.

# 6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

To address the concern that hazard and safe use information may not always be communicated effectively to end users, the eMSCA is proposing to develop an RMOA. The RMOA will look at options including voluntary, industry-led actions, to improve supply chain communication and the implementation of the recommended measures by downstream users. The RMOA will also consider whether any further regulatory action is required to address a potential concern relating to the possible use of enzymes in dishwashing liquids supplied to consumers for manual dishwashing.

**Table 3**

| FOLLOW-UP<br>Follow-up action | Date for intention | Actor |
|---|---|---|
| RMOA | Spring 2017 | UK MSCA |

# Part B. Substance evaluation

## 7. EVALUATION REPORT

This report describes the approach taken by the UK MSCA in its evaluation of the enzyme amylase, α-. Enzymes are high molecular weight substances (proteins). They are widely recognised as respiratory sensitisers and associated with immediate hypersensitivity reactions (i.e. asthma). To help the reader, some background information is provided on respiratory sensitisation and on the way exposure to enzymes is measured.

**Respiratory sensitisation**

The mechanism that underlies allergic reactions to protein-based allergens is a two-stage process. The first stage is induction in which an individual develops a population of allergen specific immunoglobulins (IgE). At this stage, an individual may not notice any change has taken place and they will be able to carry on with their life as normal. Although the individual may not be aware that they have become sensitised, this can be demonstrated using tests for the presence of allergen specific IgE, or skin prick challenge tests. For skin prick tests a small amount the suspected sensitiser is injected into the lower layers of the skin and a sensitised individual will show a functional response with a wheal and flare reaction at the injection site. This type of test can also be used to detect responses to common environmental allergens in atopic individuals, atopics are hay fever sufferers and people allergic to house dust mite.

The second stage of the process is elicitation when a sensitised individual starts to experience respiratory symptoms. The individual may notice that they have a runny nose and/or eyes and/or breathing difficulties during or after exposure to an allergen. If the allergen is something they are exposed to at work, typically symptoms will improve when they are away from work i.e. at weekends and on holiday. If the individual seeks medical advice and a skin prick test is positive (or they have raised levels of allergen specific IgE in their blood) to something they are exposed to at work, a diagnosis of occupational sensitisation can be made. If the individual is showing symptoms consistent with asthma, and which are specifically provoked by exposure to the suspected occupational allergen(s), a diagnosis of occupational asthma can be made. At this point, to prevent the asthma becoming unmanageable, these individuals often need to change their jobs to avoid continuing exposure. If they remain with their employer, the employer will need to take steps to prevent this individual being exposed to the agent that has caused the asthma.

Although substances may have the potential to cause occupational asthma, this does not mean that everyone who is exposed will develop asthma. It is likely that there is a spectrum of susceptibility across the population. However, our knowledge about the factors that make some individuals more susceptible than others is incomplete. It has been suggested that atopic individuals who suffer from hay-fever or house dust mite allergy may be more susceptible to other protein allergens (Larsen et al, 2007; Fishwick et al, 2008; Vandenplas, 2011; Green and Beezhold, 2011). Limited understanding about why some develop asthma when others with seemingly similar exposure do not, makes it difficult to identify exposure-response relationships and dose-thresholds for asthmagens. Where dose-thresholds cannot be identified, REACH Registrants have the option to calculate Derived Minimal Effect Levels (DMELs) instead of Derived No-Effect Levels (DNELs) and this has been done for amylase, α-.

Where a DMEL has been calculated instead of a DNEL, this implies that there may be a residual risk at levels of exposure below the DMEL. Where there is a lack of information about the exposure-response relationship, it is not possible to quantify the level of this residual risk. In such situations, it may be necessary to base the risk assessment on a qualitative consideration of the suitability of particular combinations of control measures to prevent or minimise exposure.

**Exposure measurements for enzymes**

The first industrial enzymes to be used commercially were proteases and the first methods to monitor workplace exposure were based on proteolytic substrate assays using total dust samples collected using static sampling devices. Static sampling devices had to be used because of the large volumes of air that needed to be sampled to collect sufficient dust for analysis. Over the years, methods to detect protease in air have been refined and standardised. As other enzymes (including genetically engineered enzymes) have been commercialised, other methods have been developed to measure these other enzymes in air samples. Although modern enzyme containing products usually contain a mixture of different enzymes, where proteases are included in enzyme mixtures it is usual to measure protease as a surrogate for all enzymes in the product. This allows companies to demonstrate compliance with the occupational exposure limits for subtilisins (a protease) that have been established in national workplace legislation in several countries. But this also means that a lot of the exposure data collected during routine workplace air monitoring and published in studies looking at the health of workers exposed to enzymes is expressed in terms of protease levels. It may also have been obtained using static sampling devices because although sufficiently sensitive analytical methods are now available to allow personal monitoring data to be collected, this is not routinely done. This use of static sampling data, rather than personal exposure monitoring, makes it difficult to define quantitative exposure-response relationships for respiratory sensitisation. Another source of uncertainty that needs to be taken into account is the fact that both active and inactive enzymes may cause the induction of immune sensitisation and elicitation of respiratory allergy. However, the functional substrate assays detect proteolytic (or other enzyme) activity only quantify active enzyme concentrations. To address this concern many organisations monitoring exposure to enzymes have developed specific and sensitive immunoassays to quantify the levels of the enzyme allergen (active and inactivated enzyme).

## 7.1. Overview of the substance evaluation performed

Amylase, α- was originally selected for substance evaluation in order to clarify concerns about:

- potential worker and consumer exposure.

This enzyme is classified as a respiratory sensitiser. Uses have been identified which have the potential to create exposures sufficient to produce adverse reactions in workers or consumers if suitable controls are not implemented. It is important to clarify the approach that the Registrants have been taken to estimate exposure during the manufacture and use of this enzyme and the approach they have taken to derive benchmarks (DMELs) against which to judge the acceptability of exposure.

During the evaluation an additional concern was identified:

- the representativeness of the simulation studies used to support the exposure assessment for certain scenarios.

**Table 4**

| EVALUATED ENDPOINTS | |
|---|---|
| **Endpoint evaluated** | **Outcome/conclusion** |
| *Human exposure assessment* | *The eMSCA considers that the Registrants have taken a suitable approach to assess exposure and are recommending appropriate measures to manage the identified risks* |
| *Basis for the Registrants' DMELs* | *The eMSCA accepts the approach taken to derive the worker and consumer DMELs* |

## 7.2. Procedure

- The evaluation focussed on the approach taken by the Registrants to calculate DMELs for amylase, α- and the human exposure assessment. **A comprehensive review of the environmental hazard and risk assessment was not carried out**.
- The evaluation was based on the information provided in the CSRs that had been submitted to ECHA by 30 March 2015, updates to CSRs submitted during the evaluation year and the study reports provided to the eMSCA by registrants on request during the evaluation year. All of the human exposure information presented in CSRs and provided to the eMSCA during the evaluation year was assessed.
- The eMSCA also took into account additional information from its previous work on fungal and bacterial amylase, α- and subtilisins, and published information where this was relevant to the uses covered in the registrations.
- During the evaluation, the eMSCA met face–to-face or held teleconferences with the lead Registrant 3 times. The purpose of these meetings was to outline the work we intended to carry out during the evaluation, to informally discuss preliminary findings from the evaluation and to provide information on the next steps once the evaluation was finalised, respectively. As a result of the informal discussions of our preliminary findings, the Registrants voluntarily conducted some new simulation studies to help clarify uncertainties about whether the current studies were properly representative for the exposure situation they were being applied to. This data was taken into account in the evaluation.

## 7.3. Identity of the substance

The substance is an enzyme and according to the REACH Guidance on Substance Identification (Section 4.3.2.3), enzyme substances are identified by:

1) the catalytic activity of the enzyme protein (IUBMB nomenclature - INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY http://www.chem.qmul.ac.uk/iubmb/) and

2) the other constituents from the fermentation.

This information is presented in Tables 5 and 6.

**Table 5**

| SUBSTANCE IDENTITY | |
|---|---|
| Public name: | Amylase, α- |
| EC number: | 232-565-6 |
| CAS number: | 9000-90-2 |
| Index number in Annex VI of the CLP Regulation: | 647-015-00-4 |
| IUBMB Name | Alpha amylase |
| Enzyme class number | 3.2.1.1 |
| Systematic name | 4-alpha-D-glucan glucanohydrolase |
| Reaction | See confidential annex |

| | |
|---|---|
| Molecular weight range: | See confidential annex |

Type of substance ☐ Mono-constituent ☐ Multi-constituent ☒ UVCB

**Structural formula:**

Not available

The constituents listed on the ECHA dissemination site are as follows;

**Table 6**

| Constituent<br>**Constituents** | **Typical concentration/ Concentration range** | **Remarks** |
|---|---|---|
| amylase, α- (EC no. 232-565-6) | Confidential | Active enzyme |
| Carbohydrates | Confidential | Constituents deriving from the fermentation or extraction process |
| Inorganic salts | Confidential | |
| Lipids | Confidential | |
| Other proteins + peptides and amino acids | Confidential | |

Further details on the concentrations of the individual constituents as reported by the Registrants are reported in the confidential Annex.

## 7.4. Physico-chemical properties

Limited information on physico-chemical properties is available in the registration dossiers. According to the Registrants the majority of physico-chemical parameters are not relevant for enzymes. The information in Table 7 is taken from the ECHA dissemination site. Further information can be found in the confidential annex.

**Table 7**

| **OVERVIEW OF PHYSICOCHEMICAL PROPERTIES**<br>**Property** | **Value** |
|---|---|
| Physical state at 20°C and 101.3 kPa | Waived |
| Vapour pressure | Waived |
| Melting point/Boiling point | Waived - Not technically feasible |
| Water solubility | Waived |
| Partition coefficient n-octanol/water (Log Kow) | <– 1.3 at 20°C pH 5.7-5.8 OECD 107 (Shake flask) (glucoamylase tested) |

| | |
|---|---|
| Surface Tension | Waived – scientifically unjustified |
| Flammability | Waived |
| Flashpoint/Auto flammability | Waived – scientifically unjustified |
| Explosive properties | Waived – scientifically unjustified |
| Oxidising properties | Waived – scientifically unjustified |
| Stability in organic solvents and identity of relevant degradation products | Waived |
| Dissociation constant | Waived - Not technically feasible |
| Relative density | 1.32 - 1.42 g/mL.<br>Published data on 20 different protein families, Registrants assume density will be in the same range. Thomas E. Creighton, Proteins: structures and molecular properties (1993) |
| Particle size distribution | Waived - Scientifically unjustified |

Whilst it should be possible to provide more information on the physico-chemical properties of the substance in the registration dossiers this information is not necessary for the purposes of this evaluation.

## 7.5. Manufacture and uses

### 7.5.1. Quantities

The information in Table 8 is taken from the ECHA dissemination site.

**Table 8**

| AGGREGATED TONNAGE (PER YEAR) | | | | |
|---|---|---|---|---|
| ☐ 1 – 10 t | ☐ 10 – 100 t | ☐ 100 – 1000 t | ☒ 1000- 10,000 t | ☐ 10,000-50,000 t |
| ☐ 50,000 – 100,000 t | ☐ 100,000 – 500,000 t | ☐ 500,000 – 1000,000 t | ☐ > 1000,000 t | ☐ Confidential |

### 7.5.2. Overview of uses

Amylases are enzymes which catalyse the hydrolysis of α-1-4 glucosidic linkages of polysaccharides such as starch, glycogen or their degradation products. Amylase, α- attacks subterminal and internal 1:4 links in the starch molecule to break the long chains into small fragments. Amylase, α- may be prepared from either bacterial or fungal sources, the choice for a particular application depends on the operating conditions in which the enzyme is required to perform. Amylase, α- derived from bacterial sources tends to be preferred for manufacture of detergents and textile processing.

Enzymes including amylase, α- are manufactured by a fermentation and recovery process which takes place in predominantly closed systems. Enzymes are not supplied as fine powders but are formulated by registrants into liquids or granulates which typically contain a maximum of 15% active enzyme protein (aep). These liquids and granulates are then used by downstream formulators to produce products which typically contain less than

0.5% aep. End users may be workers or consumers. They may be supplied with concentrated products which require dilution before use or ready-to-use formulations.

Amylase, α- containing products have applications in a range of sectors where there is a need to break down starch molecules. They are good alternatives to harsh chemicals because they are effective in small quantities and have a very specific action on starch molecules. Enzyme-based products also offer environmental benefits such as lower temperature processing. The recognised advantages over non-enzyme options has resulted in a rapid growth in the market for enzyme-based products.

In addition to the uses described by registrants, there are several food and feed uses for amylase, α-. According to REACH Article 2(5) these uses are not subject to duties to register, not covered by the substance evaluation provisions and are therefore not discussed further in this report. For the uses covered in registrations, the largest volumes are used as industrial processing aids and in laundry products supplied to consumers. Smaller volumes are supplied for laundry products intended for professional use. Small quantities are also supplied for use in dishwashing products supplied for consumer and professional use and also as a processing aid in the manufacture of pulp and paper products, as a processing aid in the manufacture of textiles, in products to clean medical devices, drain cleaning products and professional floor and hard surface cleaning products.

In laundry products, amylase, α- acts to enhance stain removal. In dishwashing and cleaning products including products supplied to clean medical devices, amylase, α- enhances the removal of solid contaminants containing starch residues. In textile manufacture, amylase, α- is used to remove the starch-based 'sizes' that are applied to warp threads to protect them during weaving. They are an effective alternative to desizing agents based on acids, bases or oxidising chemicals because they can bring about complete removal of the size without damaging the fabric. In paper and pulp manufacture, amylase is used to treat cellulose pulp, increasing fibre strength.

Table 9 lists the uses for amylase, α- that were identified by registrants in 2015.

**Table 9**

| USES | Use(s) |
|---|---|
| **Uses as intermediate** | Not applicable |
| **Manufacture** | Fermentation and recovery of enzymes |
| **Formulation** | Formulation of amylase, α-<br>Formulation of enzyme containing products at downstream user sites |
| **Uses at industrial sites** | Use as processing aid |
| **Uses by professional workers** | Processing aid used by professionals<br>Laundry products (I&I * laundry)<br>Machine dishwashing products (I&I* ware wash)<br>Manual dishwashing products<br>Cleaning of medical devices |
| **Consumer Uses** | Consumer cleaning products<br>Laundry and machine dishwashing products<br>Manual dishwashing products |
| **Article service life** | Not applicable |

* I&I – Industrial and Institutional

## 7.6. Classification and Labelling

### 7.6.1. Harmonised Classification (Annex VI of CLP)

**Table 10**

| HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Index No** | **International Chemical Identification** | **EC No** | **CAS No** | **Classification** | | **Spec. Conc. Limits, M-factors** | **Notes** |
| | | | | **Hazard Class and Category Code(s)** | **Hazard statement code(s)** | | |
| 647-015-00-4 | amylase, α- | 232-565-6 | 9000-90-2 | Resp. Sens. 1 | H334 | | |

### 7.6.2. Self-classification

- In the registration(s)*:*

See above

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

| | |
|---|---|
| Acute Tox.3 | H301 |
| Acute Tox.4 | H312 |
| Skin Corr.1B | H314 |
| Acute Tox 3 | H331 |
| Aquatic Acute 1 | H400 |
| Aquatic Chronic 1 | H410 |
| Resp. Sens. 1A | H334 |

## 7.7. Environmental fate properties

This evaluation was targeted to human health concerns and did not consider environmental fate properties.

### 7.7.1. Degradation

### 7.7.2. Environmental distribution

### 7.7.3. Bioaccumulation

## 7.8. Environmental hazard assessment

This evaluation was targeted to human health concerns and did not consider environmental hazards.

### 7.8.1. Aquatic compartment (including sediment)

7.8.1.1. **Fish**

7.8.1.2. **Aquatic invertebrates**

7.8.1.3. **Algae and aquatic plants**

7.8.1.4. **Sediment organisms**

7.8.1.5. **Other aquatic organisms**

### 7.8.2. Terrestrial compartment

### 7.8.3. Microbiological activity in sewage treatment systems

### 7.8.4. PNEC derivation and other hazard conclusions

### 7.8.5. Conclusions for classification and labelling

## 7.9. Human Health hazard assessment

The only identified human health hazard for amylase, α- is respiratory sensitisation. The eMSCA does not consider it necessary to evaluate data for other endpoints because it has not identified concerns.

### 7.9.1. Toxicokinetics

### 7.9.2. Acute toxicity and Corrosion/Irritation

### 7.9.3. Sensitisation

The eMSCA has not carried out a detailed evaluation of all of the available studies looking at the respiratory sensitisation potential of amylase, α- because the hazard is well established and widely accepted. However, it is relevant to the evaluation to consider studies that have investigated exposure-response relationships or dose-thresholds for this effect. In relation to sensitisation, there are two endpoints that may be considered for the purpose of establishing exposure-response relationships. These are the induction of a sensitised state (i.e. elevated levels of substance specific IgE) and the elicitation of clinical respiratory symptoms in those who have become sensitised. The eMSCA has not identified any studies that permit the identification of clear thresholds for induction or elicitation linked to amylase, α- of any origin.

The eMSCA is aware of an ongoing assessment of studies looking at exposure to fungal amylase, α- (FAA) derived from *Aspergillus oryzae* in the baking industry by the Dutch Expert Committee on Occupational Safety (DECOS, 2014)[3]. This committee has chosen to focus on induction (elevated levels of amylase, α-specific IgE in the absence of clinical respiratory symptoms) as the most relevant effect for deriving a health-based occupational exposure limit (OEL) because this is considered to be a precursor to the development of clinical symptoms and may be a more sensitive endpoint for FAA. Based on their draft assessment DECOS concluded that a clear threshold for induction cannot be identified.

[3] http://www.gezondheidsraad.nl/sites/default/files/2014_OCR_fungal_alpha-amylase.pdf

They provisionally identified a reference value of 0.9 ng/m$^3$ (8-hr TWA) as a level of exposure at which they consider there is an additional 1 percent risk of sensitisation to FAA. In an earlier publication, the Health Council of the Netherlands concluded that the uncertainties in studies investigating exposure-response relationships for FAA were too great to define the exact form of the exposure-response relationship or to permit estimates about where a threshold might lie (Health Council of the Netherlands, 2008).

Exposure-response relationships for amylase, α- in other industries have not been studied to the same extent. Brand et al (2009) published a study in a cohort of workers exposed to enzymes in detergent manufacture. The study compared exposure to protease with the occurrence of upper and lower respiratory disease. Although some monitoring data were available for other enzymes, the authors commented that it was not possible to establish credible exposure estimates for other enzymes in use at the factory based on the available data. The study population included all those employed in the factory between 1 January 1989 and 31 July 2002. A nested, matched case-referent analysis was used to examine exposure-response relationships between estimated protease exposure and chest symptoms and disease (chest tightness, shortness of breath or wheeze, diagnoses of asthma and new use of inhalers) or eye/nose disease (new reports of eye/nose symptoms, hay fever, diagnoses of rhinitis and use of nasal medication). Cases were matched with up to four referents who had not achieved case status by the time of the cases' identification. Cases and referents were assigned to the exposure estimate from the job exposure matrix that corresponded to their job at the time of the cases' identification (further details of the exposure assessment are provided in section 7.12.1.1.2 of this report).

Annual median estimates of protease exposure for all employees in the cohort showed wide variations between years and often a wide distribution of estimates within each year suggesting inconsistencies in the standards of control during the period covered by this study. The case-referent analyses revealed a trend for odds ratios for chest and eye/nose disease to increase with increasing exposure. For this cohort, the risk of lower respiratory disease was approximately doubled at an estimated mean exposure intensity of 8 ng/m$^3$ (odds ratio 1.87, 95% CI 1.01 to 3.48). For upper respiratory disease a significant elevation of risk was apparent with an estimated protease exposure of 2.3 ng/m$^3$ or higher (odds ratio 1.80, 95% CI 1.0.1 to 3.22). The authors note that the exposure estimates are not based on personal exposure measurements but instead are based on the possibly inaccurate assumption that the ratio of protease to dust in personal total dust samples will be the same as the ratio observed for static area samples. Given this uncertainty, the eMSCA does not think that it is appropriate to rely on these estimates of risk in the absence of corroborating evidence from other studies.

In addition to information from workers exposed to enzymes, some information has been published about consumer exposure where this has resulted in sensitisation and in a few cases respiratory symptoms. When enzymes were first introduced into consumer products in the 1960's the products were formulated using dusty enzyme preparations and a few individuals became sensitised and developed respiratory symptoms linked to detergent enzymes. Simulation studies conducted at the time these cases came to light suggested these consumers could have experienced exposures of between 100 and 400 ng/m$^3$ (average 212 ng/m$^3$) from these early dusty products (HERA, 2007). The formulations that gave rise to these cases are not relevant to the way enzymes are currently supplied for any use, this information is only included because it gives an indication of an adverse effect level for enzymes.

In a more recent study to investigate exposure to enzymes from a prototype personal cleansing bar containing a protease normally used in laundry detergents, it was found that airborne levels of between 5.7 – 11.8 ng/m$^3$ could be generated during use of the bar in a shower (SDA, 2005). During pilot clinical trials of the product, 4 out of 61 participants developed enzyme specific IgE after 4-6 months use of the product for showering. No further details were reported in the SDA publication. The eMSCA notes that exposures in this case would be via multiple routes (inhalation, mucosal tissue, hydrated skin) and it is not clear which route made the greatest contribution to the antibody reaction.

The eMSCA has not identified other relevant studies.

In conclusion, information is available suggesting that the risks for the induction of sensitisation and the elicitation of upper and lower respiratory symptoms in sensitised individuals may be increased with exposures in the low ng/$m^3$ range. Although it has been suggested there may be an additional 1% risk of sensitisation for bakers exposed to 0.9 ng/$m^3$ amylase, α- derived from *Aspergillus oryzae*, given that this is a mixed allergen exposure situation and wheat flour antigen sensitisation may influence sensitisation to other allergens such as amylase, α-, the eMSCA does not think it is appropriate to use this risk estimate for this evaluation.

The risk estimate has been derived for one form of amylase, α- used in one sector of industry whereas this evaluation covers amylase, α- derived from different fungal organisms and of bacterial origin. Although the Registrants based on their experience consider that there are not large differences between enzymes in terms of their sensitising potency, there is some evidence from studies in animals pointing towards possible differences between amylase, α- of fungal origin and amylase, α- of bacterial origin with bacterial amylase, α- being more potent than fungal amylase, α- (Sarlo et al, 1997; Robinson et al, 1998). This means that the risk estimate identified for sensitisation to FAA derived from *Aspergillus oryzae* as it is used in bakeries may not be applicable to amylase, α- derived from other sources. Rather than base regulatory decisions on unconfirmed risk estimates, the eMSCA prefers to take the view that the dose-response relationships for induction and elicitation are uncertain.

### 7.9.4. Repeated dose toxicity

### 7.9.5. Mutagenicity

### 7.9.6. Carcinogenicity

### 7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

### 7.9.8. Hazard assessment of physico-chemical properties

Not assessed

### 7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

The only endpoint of concern for amylase, α- is respiratory sensitisation. Although this is an effect for which a threshold may exist, based on current knowledge it is not possible to identify where the thresholds for either induction or elicitation are for amylase, α-. It is therefore not possible to derive a DNEL and use the risk characterisation ratio (RCR) approach to identify suitable control measures. In this situation, REACH (Annex I, section 6.5) states "*a qualitative assessment of the likelihood that effects are avoided, when implementing the exposure scenario, shall be carried out*". The Information Requirements and Chemical Safety Assessment (IR and CSA) Guidance indicates that the identification of a DMEL (derived minimal effect level) may help this process. This is a not a level where no potential effects can be foreseen but is described in the IR and CSA Guidance, Ch R8, Section R.8.1.1 as an exposure at which a tolerable level of effects may be anticipated. The basis for the DMELs recommended by the registrants has been described by Basketter et al, (2010) and is outlined below for information.

#### 7.9.9.1 Worker long-term inhalation, local effects

The worker long-term inhalation DMEL of 60 ng/m$^3$ is based on the Ceiling OEL for the protease subtilisin that has been recommended by the ACGIH (American Conference of Governmental Industrial Hygienists) TLV committee since the early 1970's and which has been used for decades by enzyme manufacturers and downstream users as a starting point to manage worker exposure. The ACGIH recommends Ceiling limits where it is necessary to avoid transient excursions above the identified limit. Where the ACGIH recommended limit has been adopted into national workplace legislation, in many cases, it has been adopted as a Ceiling limit. While some companies have adopted a limit of 60 ng/m$^3$, others have adopted lower in-house limits for different enzymes. For example, the major European detergents manufacturers use limits ranging between 5 and 15 ng/m$^3$ for amylase, α- and between 5 and 20 ng/m$^3$ for other enzymes (Basketter et al, 2010). The eMSCA is not aware of other sectors that have established their own in-house limits.

It is clear from the information available to the eMSCA that cases of sensitisation and respiratory symptoms still occur. However, the most recent data suggest that the yearly incidence rates for both sensitisation and symptoms are low. Information from health surveillance programmes operated by the European detergents industry for 2006-2010 indicates the yearly incidence rate for new cases of sensitisation is below 1% with clinical symptoms occurring in less than 1 in 10 of these, i.e. in less than 0.1% of those working with enzymes (Basketter et al, 2015). Clinical symptoms include rhinitis, conjunctivitis, and evidence of impaired lung function or asthma, not clearly linked to non-occupational causes. Only rarely is a specific bronchial challenge performed to support a diagnosis of enzyme induced asthma. Typically participation rates in these health surveillance programmes are high (around 95% participation). Non-participants include those on long-term absence for a variety of reasons including maternity leave.

The eMSCA does not have comprehensive data for other sectors, but notes that between 16 and 38 cases of occupational asthma due to enzymes and not related to work in bakeries have been reported to the THOR scheme in the UK between 2005 and 2014. THOR (The Health and Occupation Research Network) gathers information from specialist physicians, occupational physicians and general practitioners on work-related ill health. Of these reported cases, 11 worked in detergents manufacture, 2 relate to use of endoscope cleaning solutions, 1 worked in the cleaning sector and 2 cases, where the cause was specified as protease, were manufacturing process workers. These last 2 cases were reported by occupational physicians who report for 1 month in the year and hence 2 reported cases could be equivalent to up to 24 potential cases. The pattern of reporting suggests around 1-2 cases arising per year rather than isolated clusters. No information is available on the exposure situations that led to these cases.

#### 7.9.9.2 General population long-term inhalation, local effects

The value for the DMEL for the general population is derived primarily from a study in which 96 atopic volunteers (atopics are assumed to be more susceptible to developing IgE mediated reactions to high molecular weight substances) used a protease containing pre-spotter spray under exaggerated conditions in their own homes for 6 months (Weeks et al, 2011). This trial has also been reported in Basketter et al (2010) and SDA (2005). Subjects were asked to apply the product to a cloth held approximately 60 cm from their face, holding the spray approximately 15 cm from the cloth, in the area where they normally do their laundry. The product was sprayed 6 times with a single trigger pull for each spray before moving to a dry area on the cloth. This spraying protocol was repeated 5 times in total to mimic treating 5 stains. Each subject was therefore exposed to 30 sprays per day using approximately 4500 – 6500g stain removal product during the study (this level of exposure is around 10 times the level expected for heavy users based on consumer surveys). No volunteer became sensitised to the protease during the study. The level of exposure estimated for consumers in this study was 12 – 17 ng/m$^3$ based on a laboratory simulation study (see section 7.12.1.1.6 of this report for details).

Brief details from other consumer surveys/trials have been published by Sarlo et al (2010). These studies cover in total several thousand participants including both atopic and non-atopic individuals from a variety of ethnic backgrounds. The studies mainly looked for the presence of specific IgE to proteases of various origins, but some also tested for specific IgE to amylase, α- of bacterial origin. There is evidence for protease specific IgE in consumers exposed to early enzyme containing laundry products before products were formulated with encapsulated enzymes. Laboratory simulation studies suggest enzyme exposures may exceed 200 ng/m$^3$ (HERA, 2007) for products formulated with powdered enzymes. There is no evidence for specific IgE where consumers are exposed to products formulated with enzyme granulates. Enzyme exposures arising from use of products containing granulates are expected to be less than 1 ng/m$^3$ Sarlo et al (2010).

Sarlo et al (2010) also reported pre-employment screening skin prick results for 5156 employees of the Procter and Gamble Company in North America joining between 1972 and 2008. Of these, 4 gave positive results (denoting the presence of specific IgE) to a protease derived from *B. licheniformis*, one gave a positive result to amylase, α- derived from *B. licheniformis* and 2 gave positive results to cellulase derived from *H. languinosa*. This represented a baseline prevalence of just under 1.4% for the 1972 – 1992 and 1993-2002 cohorts. No cases were identified in the 2002-2008 cohort. Basketter et al (2015) also comment that in pre-employment screening tests for the detergents manufacturing sector, a very small number of individuals are found to have measureable levels of enzyme specific IgE. It is not clear how many of these prospective employees use enzyme-containing consumer products, and if all of those identified with enzyme specific IgE use enzyme containing consumer products regularly, but the eMSCA does not expect that the use pattern in prospective employees will be any different to the use pattern in the general population. This pre-employment screening information and the consumer survey/trial data support the view that the risks for sensitisation associated with current product formulations are very low.

Taking account of historic experience with the use of an occupational exposure limit of 60 ng/m$^3$ and the lack of evidence for sensitisation in consumer product trials, the DMEL of 15 ng/m$^3$ was chosen as the highest tolerable exposure level for consumers.

#### 7.9.9.3 eMSCAs conclusions on the Registrants' DMELs

The eMSCA has sufficient information to understand the approaches that have been taken by the Registrants to derive DMELs for amylase, α-. Since the risk of developing asthma following exposure at the levels of these DMELs is not known, rather than base the risk characterisation on a comparison of exposure levels with DMELs, the eMSCA has made a qualitative assessment of the suitability of the identified operating conditions (OCs) and risk management measures (RMMs) to manage the risks for respiratory sensitisation.

**Table 12**

| CRITICAL DNELS/DMELS | | | | | |
|---|---|---|---|---|---|
| **Endpoint of concern** | **Type of effect** | **Critical study(ies)** | **Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)** | **DNEL/ DMEL** | **Justification/ Remarks** |
| Worker, long-term inhalation DMEL | Respiratory sensitisation | | | 60 ng/m$^3$ | |
| Consumer, long-term inhalation DMEL | Respiratory sensitisation | | | 15 ng/m$^3$ | |

### 7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

## 7.10. Assessment of endocrine disrupting (ED) properties

This endpoint is not relevant for amylase, α-.

### 7.10.1. Endocrine disruption – Environment

### 7.10.2. Endocrine disruption - Human health

### 7.10.3. Conclusion on endocrine disrupting properties (combined/separate)

## 7.11. PBT and VPVB assessment

This endpoint is not relevant for amylase, α-.

## 7.12. Exposure assessment

This evaluation has been targeted to human health concerns. The environmental exposure assessment has not been evaluated.

### 7.12.1. Human health

The exposure assessments for workers and consumers are based on measured data taken from internal company documents produced for the registration. This includes air monitoring data from Registrants' sites, air monitoring data collected at downstream user premises and simulation studies carried out using specific products. In many cases, the number of data points for a given situation is small. This report focusses on the eMSCA's opinions on the representativeness of the data in the registration for the scenarios it is being applied to and how uncertainties in the exposure data affect the robustness of the risk characterisation. The report also provides the eMSCA's opinions on the suitability of the RMMs proposed by the Registrants. Some published information is also available and has been included where the eMSCA considers it has relevance for current conditions of use.

Since enzymes are typically present in workroom air in very low amounts (low ng/$m^3$ levels), historically it has been necessary to use high volume static sampling in order to obtain sufficient sample for analytical techniques based on enzyme activity to detect measureable quantities. More sensitive analytical techniques are now available including methods that use an immunological approach to detect specific protein domains (enzyme linked immunosorbent assay, ELISA). However, this does not entirely solve the sensitivity problems particularly for short term personal sampling. Furthermore, the results from different analytical approaches are not directly comparable because they target different aspects of the enzyme (e.g. enzyme activity vs particular protein domains in the molecule). This limits the ability to make direct comparisons between exposure levels reported for studies if different analytical methods have been used.

In most situations, high volume static sampling at defined locations within a factory remains the most practical monitoring approach since it is a useful way to ensure engineering controls such as containment and LEV are working effectively. It is the mainstay of workroom monitoring programmes for enzyme producers and large scale formulators such as detergent manufacturers. Static samplers are sited at locations close to processes where exposure to enzymes may occur. Typically static samplers will operate continuously for periods of between 1-4 hours though may be used for longer or shorter periods. Static samplers can also be used to collect short term samples of around 15 minutes duration. According to Ch14, section R.14.4.5 of the IR & CSA Guidance, static sampling data should only be used if there is information to demonstrate how this relates to personal exposures or it is expected that such data will provide a more conservative assessment than one based on personal data. The eMSCA considers it likely that the static sampling described here for processes operated in predominantly closed facilities where workers spend only a small part of their day on tasks involving direct exposure to enzymes will provide a more conservative assessment for long term exposures. The eMSCA notes that short term peak exposures are also of potential concern for respiratory sensitisers and that it is not possible to accurately assess the magnitude of short term personal peak exposures using static sampling devices. This potential source of uncertainty will be considered for each scenario where it may be relevant as will the use of static sampling data for situations where enzyme products are used in more open processes.

Often, where several enzymes are used together, samples of workroom air may be analysed for protease levels only and this used as a surrogate for other enzymes that are used in the same process at the same time. This is not always the case and it is possible to perform analyses for multiple enzymes using the same filters using specific immunoassays or where the enzymes have very different substrate specificities. The report will indicate which data sets are based on protease measurements, which are based on α-amylase measurements and, where only protease data is available, will consider if sufficient information is available to conclude that the data will be representative for amylase, α-.

Generally, the measurements quoted in the studies available for this evaluation have not been calculated for a particular reference period (e.g. 8-hr TWAs). The eMSCA has assumed that each value represents an average for the period over which the sample was collected. In the case of professional or consumer activities which have been assessed on the basis of simulation studies, the exposure values represent airborne levels that may arise during the task.

The Registrants specifically exclude use of amylase, α- in products which are intended for spray application and hard surface cleaning unless the individual product has been tested in the form it is intended to be supplied. This is to ensure that in each case, the product design (e.g. viscosity) and delivery equipment (e.g. hand held trigger spray) are suitable to ensure the user will not be exposed to levels above the DMEL under normal and exaggerated conditions of use. The product specifications form part of the exposure scenario for that product. If there are any changes to the product design or delivery equipment, the product needs to be retested to ensure the modifications do not create the potential for exposure to levels above the DMEL. The testing protocol used in simulation studies for spray products has been published and is described in more detail below.

## 7.12.1.1. **Worker**

### 7.12.1.1.1 Manufacture and formulation at registrants' sites

#### *7.12.1.1.1.1 Information provided in the Registrations*

At Registrants' sites, enzymes are manufactured and formulated into liquid preparations or low dust granulates (granulate diameters > 300 µm) containing a maximum of 15%

aep. Modern enzyme production facilities are designed as highly automated closed processes. This serves to protect workers from enzyme dust and to prevent contamination of the product. It will also limit the time that workers need to come into direct contact with enzyme containing materials, supporting the view that using static sampling data to assess long-term worker exposure at Registrant's sites will be a conservative approach. Where enclosure is not possible, local exhaust ventilation (LEV) and HEPA filtration of recirculated air is applied to limit the release of enzyme dusts into the workroom air. A strict safety policy is applied which includes worker training (the eMSCA has seen examples of the training material available to workers) and frequent (in some cases daily) use of static samplers to ensure the background concentration of enzyme protein in the workroom air is maintained below 60 ng/$m^3$. During routine operations, tasks where there is a potential for direct exposure to enzymes during manufacture are limited to sampling activities, coupling and decoupling during transfers and laboratory analysis of samples. During formulation, tasks where there is a potential for direct exposure include granulation, sieving of dry enzyme products and intermediates, coupling and decoupling during transfers and laboratory analysis of samples. Non-routine operations such as non-scheduled maintenance, trouble shooting and rework/repackaging may lead to increased exposures. Where task specific assessments identify a potential for worker exposure to concentrations greater than 60 ng/$m^3$, there is a requirement for workers to wear respiratory protection (RPE, P3 filter).

Exposure monitoring is predominantly performed using high volume static samplers using a sampling rate of 500 L/min. This allows for long and short-term (15-minute samples) monitoring of areas where there is a potential for enzymes to be released into the workroom. Samplers are typically located 1-1½m away from relevant exposure sources and at a height equivalent to a worker's breathing zone. Air is collected onto filters and analysed for enzyme content using methods that detect enzyme activity (KoneLab) or ELISA. In some cases it is the protease subtilisin that is measured and this is used as a surrogate for other enzymes that are produced in the same way. In other cases, data has been collected for amylase, α-.

Personal sampling has occasionally been performed using a flow rate of 2 L/min and sampling times in the order of 2 hours to ensure sufficient material is collected to detect levels at least 2x below the on-site OEL. Too few samples are currently available to form an opinion on the relationship between personal exposure and static sampling data.

CSRs identify single exposure values for each PROC code. The exposure values have been obtained from monitoring data collected between 2005 and 2015. Typically, the 90$^{th}$ percentile value has been used for the risk characterisation. This was not the case for some data sets which were collected from areas of the production facilities where respiratory protection is not required but include measurements during accidents and other incidental events. In these cases the 75$^{th}$ percentile has been used. Non-detects were allocated the value of the limit of quantification (LOQ) for the sampling and analysis technique which in this case is typically between 0.5 – 1 ng/$m^3$. This approach will increase the level of precaution in the exposure assessment.

Further details of the data sets used to derive the exposure values quoted in the CSR were provided to the eMSCA during the evaluation either in the form of a summary table or individual data points. The summary table provided the [arithmetic] mean and median values plus the 75$^{th}$ and 90$^{th}$ percentiles and maximum for the data sets that are applicable for each PROC code. Between 15 and 1661 results are available for each Registrant/PROC code relevant for manufacture and formulation at Registrants' sites. In some cases fewer data points are available for PROC 15. However, the eMSCA has been provided with details for individual data points including the process, sampling time, and where multiple enzymes were analysed for, the enzymes that were measured.

#### *7.12.1.1.1.2 Published data covering manufacture and formulation*

Two publications are available that provide brief summarised information about historical levels of exposure during manufacture and formulation at an enzyme manufacturing plant in Denmark (Johnsen et al, 1997 and Larsen et al, 2007). Johnsen et al summarised data collected in the 1970s and 80s. In laboratory areas, levels of protease (measured as a surrogate for all enzymes handled in the plant) in airborne dust of 50 ng/m$^3$ (50$^{th}$ percentile) to 100 ng/m$^3$ (90$^{th}$ percentile) (max. 800 ng /m$^3$) were reported. For production areas where enzyme powder was handled, protease concentrations of 100 - 1000 ng/m$^3$ (max. 2000 ng/m$^3$) were reported. At this time it was standard practice to require the use of respirators in any area where airborne enzyme concentrations were anticipated to exceed 600 ng/m$^3$. Larsen et al also provided exposure data for the 1970s and 80s and added data from the 1990s (see table 13).

Table 13. Exposure data reported by Larsen et al, 2007

| **Department** | **Decade** | **Measurements, n** | **50$^{th}$ percentile (range)*, ng/m$^3$** | **90$^{th}$ percentile (range)*, ng/m$^3$** |
|---|---|---|---|---|
| **Granulation** | 1980s | 3521 | 30 (10 - 50) | 200 (70 - 600) |
| | 1990s | 8351 | 10 (1 - 60) | 100 (10 -1000) |
| **Pilot** | 1980s | 250 | 4000 (2000 - 6000) | 30 000 (6000 – 123 000) |
| | 1990s | 86 | 5000 (8 – 215 000) | 46 000 (100 – 505 000) |
| **Recovery** | 1970s | 3607 | 400 (6 – 1000) | 1000 (20 – 19 000) |
| | 1980s | 4537 | 100 (0 - 5000) | 500 (50 – 59 000) |
| | 1990s | 2837 | 30 (0.6 -4100) | 200 (10 – 63 000) |

*These measurements are not comparable to the values reported in the CSRs because they include data from areas where use of RPE is mandatory.

Although these data include a large number of measurements (n = 23189), the data were not collected systematically. Samples were taken as required to identify areas of the plant where RPE may be required, to confirm compliance with regulatory occupational exposure limits and to track sources of exposure when cases of occupational asthma arose. Samples were collected using static samplers and this means that these data do not provide reliable information about personal exposure levels; in particular short term personal peak exposures will not be captured. These data are therefore not representative for the exposure individual workers are likely to have received during these decades. Larsen et al note that improvements were made in the working conditions over this period but do not describe these further. For these reasons, these data should be considered to reflect worst case exposures and should not be seen as indicative of the exposures likely to be experienced by workers under current conditions.

#### *7.12.1.1.1.3 Conclusions about manufacturing and formulation*

Based on all of the information available to the eMSCA about manufacturing and formulation at Registrant's sites, the eMSCA considers that the exposure values that have been identified are representative for current operating conditions. Since the exposure

measurements have been obtained using static sampling devices, there is some uncertainty about personal exposure levels. However, the eMSCA notes that RPE is mandatory for tasks where there is the potential for peak exposures above the DMEL. Given the uncertainties about levels of personal exposure, about dose-thresholds and the exposure-response relationships for induction and elicitation, the eMSCA has taken a qualitative approach to assess the recommended RMMs. In the opinion of the eMSCA, the measures described in the CSRs supported by the worker training programmes that have been described to the eMSCA are suitable and adequate.

#### 7.12.1.1.2 Formulation of enzyme containing products at downstream user sites and industrial use as a processing aid

##### *7.12.1.1.2.1 Information provided in the registration*

These scenarios cover formulation at industrial sites of liquids and granulates containing 15% aep into products containing 0.5% aep or less and use at industrial sites as a processing aid. Amylase, α- is used as processing aid in the manufacture of chemical substances/mixtures, textiles, leather and to treat waste water. It is also used in products for "*cleaning in place*" (CIP). This is a procedure that allows manufacturing plant (e.g. industrial food production equipment) to be cleaned internally without disassembly. Formulations used in these sectors typically contain 0.5% aep or less (in some cases two or three orders of magnitude less).

The exposure values reported in the CSR are based on high volume static monitoring data collected between 2006 and 2014 from 11 sites covering 5 sectors at locations where exposure to enzymes are expected. Processes across these 11 sites were divided into similar operations (analogous situations) (e.g. mixing with sample taking) and allocated to the relevant PROC code. The 90$^{th}$ percentiles of measurements for these analogous situations were used for the risk characterisation. As before, non-detects were allocated the value of the LOQ. This varied between 0.1 and 7.1 ng/m$^3$ depending on the duration of sampling. Where too few data points were available to calculate a 90$^{th}$ percentile, the worst case value was used.

Additional information provided to the eMSCA during the evaluation gives details about the individual data points including: the PROC codes that individual data points apply to; which industry sector and which enzymes were analysed for (in most cases protease, but in some cases multiple enzymes were analysed from one filter); whether a solid or liquid preparation was in use; sampling duration and the monitoring position. As a result of discussions about the representativeness of the measured data for all of the industrial sectors that it is intended to cover, the Registrants provided some additional statistical analyses of the variability in the data sets for various PROC codes showing that for most PROCs, the variability was low or moderate as defined by the IR & CSA Guidance Chapter R14, table R14-2. The one case where variability was high was for an infrequently used process and the Registrants hope to obtain more data for this process. In addition to the 90$^{th}$ percentile values calculated for each PROC code, the Registrants have calculated 90$^{th}$ percentile values for each sector based on all of the measurements for that sector. All of these 90$^{th}$ percentile values are below the worker DMEL.

Although these data have been obtained from a relatively small sample of sites, the assessment of variability in the data shows a reasonable level of consistency across sites for each PROC code suggesting that if data from additional sites were to be added, it would not radically alter the current exposure estimates. This is supported by the modest change in the exposure value for one PROC where the Registrants were able to obtain additional data points. There is a question about the use of static monitoring data. As previously discussed, the use of static monitoring for processes operated in predominantly closed facilities where workers spend only a small part of their day on tasks involving direct exposure to enzymes will most likely provide a more conservative assessment for full shift exposures, but will not provide information about short-term peak personal exposures. The eMSCA makes the following observations, the endpoint of concern for amylase, α- is

respiratory sensitisation. Respiratory sensitisation is an endpoint which is identified in the IR& CSA Guidance Part E, section 3.4.2[4] as one where a qualitative approach may be necessary owing to lack of clear thresholds for becoming sensitised and developing symptoms. Since this is the case for amylase, α-, and given the challenges to accurately measure personal exposures to very low concentrations of enzymes, the eMSCA does not consider it necessary to require the Registrants to gather additional measured data to clarify likely personal exposures. Instead, the eMSCA is taking a qualitative approach to assess the RMMs described in the exposure scenario.

The IR & CSA Guidance, Part E, Table E.1 indicates that a very high level of containment is required, supplemented by training programmes for staff and use of RPE where full containment cannot be guaranteed. The exposure scenario describes similar OCs and RMMs as those implemented at Registrant's production sites with the emphasis on use of closed processes, use of LEV where containment is not feasible, HEPA filtration of recirculated air and RPE as a secondary measure where LEV alone may not be sufficient. The eMSCA considers that these technical measures are appropriate to manage the risks providing they are implemented correctly. Following discussions between the eMSCA and the Registrants during the evaluation, the registration has been updated with additional information on the need for regular cleaning of work areas using low dust techniques and for worker training. The update includes links to training materials that are freely available via the internet[5].

#### *7.12.1.1.2.2 Historical information available to the eMSCA*

In the late 1990's and early 2000's, the UK Health and Safety Executive gathered information to support occupational exposure limits setting activities for the enzymes amylase, α- and subtilisin (HSC, 2003). The following descriptions about the detergents manufacturing process and about the use of enzymes in textiles manufacturing have been taken from this historical information.

#### *Manufacture of enzyme containing detergents*

Enzymes for detergent manufacture are supplied as liquid formulations or solid granules. Work practices in the detergents sector include a high degree of automation, containment and engineering control with use of PPE to manage residual risks.

Concentrated enzyme solutions/slurries are transported in large sealed containers with a rigid plastic interior, an outer metal casing, a vent at the top of the container which is opened during transfers to facilitate drainage and a double valve safety tap to connect to the plant equipment. Enzymes may be added batchwise or metered continuously into the product before the product is stored in a tank. Coupling the enzyme container takes about 30 minutes and takes place once per shift. Typically, this takes place in an area that is segregated from the main workroom and is maintained under negative pressure. Couplings are encased and all personnel entering this area wear respirators, overalls and gloves and eye protection. Transfer lines throughout the conveying and mixing process are enclosed, and bottles are filled and capped automatically. Low pressure water flushing is used to clean equipment. Concentrated enzyme solutions /slurries typically contain 2-5% aep and are added to the product at a rate of 0.2 – 1% giving a maximum concentration of 0.05% in the final product.

In the case of granules, these are formulated to minimise the release of enzyme dusts. Enzymes are encapsulated into a core containing inert salt, binding agents, kaolin/$CaCO_3$

[4] http://echa.europa.eu/documents/10162/13632/information_requirements_part_e_en.pdf

[5] For examples of training materials that are freely available see http://www.novozymes.tv/safety-material and https://www.aise.eu/our-activities/standards-and-industry-guidelines/safe-handling-of-enzymes.aspx.

and cellulose fibres surrounded by a coating of polyethylene glycol and kaolin. Enzyme granulates are delivered in 1000kg bags to large users but may be supplied to smaller users in 50kg kegs. Enzymes are added to powder detergents as a final stage before packaging. When required the bags are hoisted above a hopper, the outlet is untied and connected to the hopper under negative pressure and is dosed by weighfeeder prior to mixing. Emptying takes about 30 minutes and takes place once per shift. Typically it is carried out in an area which is segregated from the main workroom and is kept under negative pressure. Workers emptying bags wear respirators, overalls and gloves. Where 50kg kegs are used, these are opened in a laminar flow booth and a suction lance 'worked' into position at the base of the keg. Enzyme granulates are drawn out by vacuum and transferred to a central hopper. It takes a worker about 10 minutes to perform this activity. Empty kegs are sealed before they are removed from the booth. In all cases, transfer lines are enclosed throughout the conveying and mixing process and to the packing area. Filling and sealing of cartons is automated. Granules contain up to 5% enzyme and are added to the product at a rate of 0.2 – 1% giving a maximum concentration of 0.05% in final product. Personnel are likely to work in several areas during a shift.

In 2002, as part of its work to identify a suitable level for an occupational exposure limit for the proteases subtilisins, HSE carried out a small scale survey to collect personal subtilisins measurements during handling of granulates. It used a newly developed analytical method (a fluorescent substrate method for subtilisin) that was sufficiently sensitive to detect subtilisins in personal samples at levels greater than 3 ng/$m^3$ (see tables 14 and 15). Personal samples were collected in the workers breathing zone using IOM sampling heads with glass fibre filters and a flow rate of 2 L/min. The method of analysis detected proteolytic activity using a quenched fluorescent dye bound to a-casein and is described in HSC (2003), Annex 2.

**Table 14: Personal short term, task specific subtilisin exposure data collected by HSE from 3 UK detergents manufacturers to support occupational exposure limits setting activities (HSC, 2003)**

| | **Task specific mean personal exposure** | | | | |
|---|---|---|---|---|---|
| **Work activity** | **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% subtilisins conc.) to process by gravity feed (10 minute work activity) | 2 | 10 mins | Below LOD | 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% |

*Workers wear full-face air-fed visors during this task. The HSC paper suggested the much higher enzyme levels recorded during vacuum transfer may have been due to 'working' the vacuum transfer lance into the enzyme concentrate which could have damaged the granulates creating a more dusty solid.

**Table 15: Personal full shift, specific subtilisin exposure data collected by HSE from 3 UK detergents manufacturers to support occupational exposure limits setting activities (HSC, 2003)**

| | Task specific mean personal exposure | | | | |
|---|---|---|---|---|---|
| **Work activity** | **Number of samples** | **Approx. sampling period** | **Subtilisins ng/m³** | **Total dust mg/m³** | **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 LOD | 1.1 | 0.0001% |

*Workers accessing fill points in in laminar flow booths wear high efficiency particulate respirators and when accessing fill points in the open factory wear full-face air-fed visors. Given the higher measurements for bag filling in the open factory, the HSC report gave the opinion that bag filling in the open factory cannot be regarded as good working practice and that fill points should be housed within laminar flow booths or another equally effective control measure.

The limited number of personal samples do not provide evidence that contradicts the exposure values that have been used for the risk characterisation. They also provide some information about the levels of exposure that may be encountered for tasks where the use of RPE is mandatory. These data along with some additional unpublished data provided to HSE by the UK detergents sector demonstrated that it is possible to maintain personal exposures to subtilisins below 40 ng/m$^3$ providing good occupational practices are followed and this value was implemented as the regulatory occupational exposure limit for subtilisins in the UK.

### *Manufacture of textiles*

HSE does not have any measured exposure data relating to the use of amylase, α- in the manufacture of textiles. However, there is some descriptive information about the processes that were in use at the time of the review. Amylase, α- is used to remove the protective coating known as a 'size' that is applied to warp threads to protect the threads during the weaving process. Enzyme products for this process are supplied as liquid concentrates containing 5-15% aep in a formulation designed to avoid the formation of splashes and aerosols. Container sizes range from 25L to 225 kg drums or even 1 tonne Intermediate Bulk Containers (IBCs). The product is diluted before use, typically at a rate of 30 -350 g per 100 L in 18% sodium chloride.

The process has three stages, impregnation where the enzyme solution is absorbed into the fabric (typically at temperatures of 70°C or higher), incubation (longer periods allow lower enzyme concentrations to be used) and the after wash to remove breakdown products from the fabric. This process can be performed in a Jigger or winch, pad roll (or

pitt), J-box (continuous) or Pad-stream (continuous). At the time of the review, most UK manufacturers were thought to use a jig in which one roll of fabric is processed in an open tank. Enzymes are usually dispensed manually by direct pouring from smaller containers or via a tap at the base of larger containers. Occasionally automatic dispensers may be used. Typically, the operator will wear gloves and goggles for this activity. Jigs are emptied by a valve to the drain. The desizing process requires little agitation, there is little likelihood of aerosol production and the operator will not need to contact the roll of fabric until the after-wash stage is complete. One operator can tend 2 or 3 jigs, or up to 6 if the process is highly automated. The operator may combine desizing activities with dyeing during a shift.

Initially, the eMSCA identified an uncertainty about whether or not the exposure information for use in textiles provided in the registration was properly representative for use in the manufacture of textiles and raised this with the Registrants. The Registrants confirmed that this process description is in accordance with the processes that they have seen. They also reported that the exposure data used for textile processing in the CSR was obtained from three separate processes used in the sector and each known to be representative for that process as it is operated across the sector. On this basis, the eMSCA is satisfied that the exposure values reported in the CSR are suitable to characterise exposure to enzymes where they are used in in textile processing.

#### *7.12.1.1.2.3 Published data covering detergent manufacture*

Recent publications are available that provide relevant information on exposure during detergent manufacture (Basketter et al, 2015; Basketter et al, 2012 and Brant et al, 2009).

Basketter et al (2015) published the results of a survey to determine how effectively the EU detergent's manufacturing sector is implementing the AISE guidelines on risk management measures for handling enzymes in detergents manufacture. These guidelines (AISE, 2015) are outlined in a "guiding principles" document (AISE, 2014). These AISE documents cover all aspects of the occupational health and safety system for safe handling of enzymes. Risk assessments should be performed for all tasks to identify suitable task specific safeguards. The emphasis is placed on engineering controls and plant design to prevent or minimise exposure. LEV is recommended for operations where enclosure is not practicable, with RPE used as a secondary measure for specific tasks. The need for careful handling of enzyme granulates to avoid damaging the granulate is emphasised since this could potentially result in release of enzymes in a more dusty form. Types of equipment that should not be used for enzyme transfers unless tests have been carried out to demonstrate that significant physical damage to granulates will not occur are specified. The need for suitable working practices and worker training is identified early in the document. Advice is also provided on air monitoring as a tool to help confirm that plant controls are operating as intended and worker behaviour observations to ensure workers follow safe working practices. It is recommended that air monitoring samples are taken at random times on all shifts (day and night). Results should be compared against "action levels" and procedures should be in place to follow up deviations from acceptable performance.

The document provides recommendations for a health surveillance programme. Where workers have given a positive reaction in skin prick tests to detect enzyme specific IgE, the document does not recommend removal from further exposure but workers may be followed up at more frequent intervals. Removal of symptomatic individuals is left to the discretion of the occupational physician, but in practice such workers are usually assigned to work that does not bring them into contact with enzymes. Finally the document indicates the need for regular audits of the occupational health and safety system to ensure it remains effective.

Within the guiding principles document a DMEL of 60 ng/$m^3$ is identified as a starting point for safety assessment of detergent enzymes with a warning that co-exposure to surfactants

may enhance the allergenic effects of enzymes. It also recommends that overall dust levels in detergents manufacture should be maintained below 1 mg/m$^3$ to avoid respiratory irritation from other detergent ingredients.

Providing that these measures are implemented effectively, the guiding principles document states that the rate of induction (newly identified workers with raised enzyme specific IgE) should be no more than 3% per annum, with no workers (including those with enzyme specific IgE) progressing to develop airway symptoms.

The study published by Basketter et al, covered around 100 manufacturing facilities in the EU and reviewed air monitoring and health surveillance data for the period 2006 - 2010. Air monitoring is performed using high volume static samplers which operate for between 1-4 hours at a time, with flow rates in the region of 600 L/min for powders and 300 L/min for liquids. For the years 2006 - 2010, air monitoring results were available for between 82 and 95 manufacturing facilities (mean 88). The mean number of enzyme measurements across all sites per year was 296681. Most results were close to the limit of detection. The mean number of results above the action level was 1919 per year, representing 0.65% of readings (no information was provided about which enzymes were measured). The action levels that have been set at each site depend on the enzyme and process and may lie between 6 – 15 ng/m$^3$ or 60 ng/m$^3$). The lower standards are generally adopted where surfactants are present.

The review of the implementation of health surveillance programmes found that both the spirit and letter of the industry guidance on health surveillance was followed at all participating companies. All workers undergo pre-employment screening. Workers with pre-existing enzyme specific antibodies (IgE) or identified as atopic (thought to have a greater susceptibility to develop sensitisation to high molecular weight allergens) are not screened out. There is a high degree of worker participation in health surveillance programmes which typically exceeds 95% of the workforce. The yearly rate of induction was found to be below 1% with 1 in 10 of those going on to develop symptoms of rhinitis or asthma. Where asthma is confirmed, workers are reassigned away from enzyme exposure.

Brant et al, 2009 reported monitoring data from one UK detergent manufacturer collected between 1985 and 2002. Static monitoring was performed using high volume samplers according to industry guidelines. Initially, samples were analysed using a method based on enzyme activity. In later samples this was replaced by ELISA. Personal inhalable total dust measurements are also available from this site. A total of 2054 samples were collected from 688 different workers between 1989 and 2002 using either 7 hole or IOM sampling heads. Since it was not possible to analyse personal dust samples using analytical techniques based on enzyme activity, the assumption was made that the protease content in personal dust samples would be the same as that in static total dust measurements. The mean annual protease levels in static samples ranged from 2 – 5 ng/m$^3$. Values calculated for personal samples ranged from 2 - 6 ng/m$^3$. The very limited number of personal samples collected by HSE (see tables 14 and 15) suggests that the relationship between the relative protease vs total dust content in personal samples may not be as straightforward as the relationship assumed by Brand et al, particularly for activities leading to short term peak exposure and hence conclusions about personal enzyme exposures based on total dust measurements may be inaccurate.

#### *7.12.1.1.2.4 Conclusions about formulation at downstream user sites and industrial use as a processing aid*

These uses have been assessed on the basis of measured data. The eMSCA has identified uncertainties in the data relating to personal exposures under the OCs and RMMs described in the exposure scenario. However, the eMSCA understands the challenges to accurately measure personal exposures to very low concentrations of enzymes and places a greater emphasis on the identification of suitable technical, operational and behavioural measures to secure a high level of control. As a result of discussions between the eMSCA and the

Registrants during the evaluation, additional advice has been included in the exposure scenarios on the need for regular cleaning using low dust techniques and worker training and the Registrants have provided links to training materials for workers. The eMSCA does not identify a need for more information and considers that the RMMs described in the CSRs supported by the additional housekeeping and worker training advice will be suitable and adequate providing that they are implemented correctly. Information provided to the eMSCA relating to the use of enzymes in textile processing suggests that there are areas where this sector could improve handling practices. The eMSCA will discuss with the Registrants whether additional sector specific guidance could be developed.

#### 7.12.1.1.3 Processing aid to manufacture pulp and paper products

Amylase, α- is supplied for this use as a concentrated liquid product containing up to 15% aep. The process described in the CSR uses automated pumping of enzymes and physical enclosures around the pumping system to avoid release of enzymes into the working area. Measurements for this process were collected in December 2012. All data points were below the DMEL.

Where this process is operated as described in the CSR with automated pumping of enzymes and physical enclosures around the pumping system the eMSCA does not identify a concern. The eMSCA notes that a potential for dusts to form has been identified during the pulp settling process and these dusts may contain traces of enzymes. Given that the concentration of aep in the pulp is very low, the eMSCA expects the risks of becoming sensitised and developing respiratory symptoms due to enzymes during this stage of the process will be low. The eMSCA will discuss the provision of additional good practice advice for this sector with the Registrants.

#### 7.12.1.1.4 Processing aid used by professionals

Scenarios falling under this general heading cover professional use of cleaning products containing amylase, α-. The exposure assessments rely on simulation studies with exaggerated use conditions and some personal monitoring data.

The eMSCA notes that there are limitations in the exposure data for this scenario. The numbers of products/situations that have been assessed is small and the number of measurements is also small. However, the eMSCA does not think that it is necessary to gather additional measured data for this evaluation. The eMSCA considers that the tasks that have been assessed are representative for the types of cleaning activities that are covered by the exposure scenarios described in the CSR. The Registrants have taken a precautionary approach when selecting exposure values for the risk characterisation and have used the least sensitive LOQ for each product.

During the evaluation, the eMSCA noted products intended for application by hose gun or sprayed as a foam may be supplied with advice to wear a mask with a P3 filter during the product application and rinse off phases and discussed with the Registrants the provision of additional good practice advice in the exposure scenarios for activities where aerosol formation may be relevant. As a result, the Registrants have added instructions on the need for task specific assessments to determine if RPE will be necessary. The eMSCA considers that decisions on whether or not to use RPE should be made on a case-by-case basis taking into account the specific circumstances of use and as such it would be inappropriate for the Registrants to specify mandatory use of RPE for these types of cleaning activities. The eMSCA notes that article 4 of the Chemical Agents Directive (98/24/EC) (CAD) places a duty on employers to assess the risks of hazardous chemicals that are used in the workplace taking into account the circumstances of use. Providing it is made clear to the person carrying out this assessment that the products they are

assessing contain enzymes[6] and there is a potential risk for respiratory sensitisation if the product is used in a way that could lead to aerosol formation, the eMSCA considers that the instructions provided in the exposure scenario backed up by duties under the CAD should enable suitable risk management measures to be identified for cleaners. In the light of recent research by HSE which found that the transfer of safety information on cleaning products was not always performed in an efficient or productive manner (HSE report to be published), the eMSCA will discuss with the Registrants what additional actions could be taken by suppliers to ensure suitable advice is provided to end users on how to handle enzyme containing cleaning products safely.

#### 7.12.1.1.5 Use at a professional industrial and institutional laundry

This scenario covers professional use of enzyme containing laundry detergents in large scale industrial and institutional (I&I) laundries.

##### *7.12.1.1.5.1 Information from the registration*

The Registrants have performed simulation studies intended to mimic heavy use to estimate exposure during dosing of enzyme containing detergents into a washing machine. The detergent used for this study was prepared specifically for the study and contained the maximum quantity of enzyme (in this case protease only was used) covered by the exposure scenario. With two exceptions, all measurements were below the LOQ which was just over 1 ng/$m^3$. Where samples contained quantifiable levels of protease, the levels were close to the LOQ and the duplicate sample collected at the same time from the alternate sampling head was below the LOQ.

To supplement this information, in June 2014, a small scale monitoring study was performed at a commercial I&I laundry using a tunnel washer. All samples were below the LOQ of 0.6 ng/$m^3$.

##### *7.12.1.1.5.1 Published exposure data relevant for the professional use of laundry detergents.*

Other simulation studies (which are included in the registration) have been reported in HERA (2007) and Sarlo et al (2010). Both studies measured protease levels. The studies reported in HERA (2007) were carried out in the early 1970's to demonstrate the reduction in enzyme exposure to consumers achievable with granulated enzyme compared with the powdered enzyme products that were marketed in the 1960's. The exposure value quoted for granulated enzyme in the HERA report (1.01 ng/$m^3$) is lower than the LOQ for the more recent simulation studies and was not used for the risk characterisation.

The studies reported by Sarlo et al (2010) used products containing between 1 and 100 times the enzyme levels present in commercial products and conditions consistent with habits and practices information for North America and EU consumers. High volume samplers were used, sampling on to glass fibre filters and analysed by ELISA. The detection range for the assay was reported as 1.9 - 190 + 0.5 ng/$m^3$ at an air sampling flow rate of 0.67 $m^3$/min. Further details of the experimental protocols were not provided. The paper reports the following values:

- Pour liquid detergent into top-loader wash machine, 0.012 ng protein/$m^3$

[6] The Detergent Regulation (EC) No 648/2004 sets a requirement that enzymes should be listed on products labels irrespective of their concentrations. Cleaning products covered by this scenario are assumed to be covered by the definitions of 'detergent' and 'cleaning' laid out in Article 2 of this regulation.

- Pour granule detergent into top-loader wash machine, 0.00022 ng protein/$m^3$
- Addition of water to liquid or granule detergent in top loader wash machine, 0.7 – 2.9 ng protein/$m^3$
- Addition of detergent to front-loader wash machine, 0 ng protein/$m^3$
- Detergent refill (pour granule from 6 kg sack), 0.5 ng protein/$m^3$
- Dryer vent (indoors), < 0.5 ng protein/$m^3$
- Clean dryer lint trap, 0.04 – 1.2 ng protein/$m^3$
- Spray pre-treat laundry items, 14.5 ng protein/$m^3$

The eMSCA notes that the majority of these values are below the stated limit of detection for the assay raising questions about how these values were arrived at. No further information is provided in the Sarlo paper to help resolve this uncertainty.

#### *7.12.1.1.5.3 Conclusions about use at a professional I&I laundry*

The eMSCA considers that the exposure measurements provided by the Registrants reflect worst case conditions. The eMSCA does not identify a concern for this use of amylase, α- providing it is carried out in accordance with the exposure scenario.

### 7.12.1.1.6 Professional laundry pre-spotter spray

#### *7.12.1.1.6.1 Information from the registration*

The Registrants state that products covered by this scenario are tested individually to ensure each product formulation and its associated delivery system do not generate exposures above the DMEL. In this case the consumer DMEL is used as the benchmark to provide additional protection for professionals. According to Weeks et al, 2011, pre-treatment products may be supplied as liquids, gels or aerosols with liquid trigger spray products being the most common. For the registration, two hand-held trigger sprays were tested. Other product types will undergo similar testing and product specific exposure scenarios will be prepared. The information in the registration is intended to illustrate the procedure used to confirm that use of spray products does not lead to unacceptably high exposure.

The testing protocol that has been established to test enzyme containing spray products is described in an AISE guidance document (AISE, 2013) and by Weeks et al, (2011). The product is situated 15 cm away from a vertical fabric (prewashed polycotton) panel approx. 40 $cm^2$ in area. The sampling head is situated 60 cm away from the panel approx. 1.5 m from the floor and tilted at a 30 – 45 degree angle to mimic the breathing zone of the consumer. The flow rate for the air pump is set to give an air velocity through the sampling head of around 1.25 m/s to simulate nasal inhalation. One minute after the pump is switched on, the spray is operated 5 times with a frequency of 1 spray per second. The textile panel is then changed during a 10 second rest period. This is repeated 5 times resulting in a total of 6 cycles with the final panel left in place and the sampling pump allowed to run until 10 minutes has elapsed after the first spray, giving a total sampling time of 11 minutes. This experiment is then repeated at least 4 times or until the data are sufficient to demonstrate statistical significance. Exposures are expressed as the average of all experiments. For the products included in the registration, the value used for the risk characterisation is the arithmetic mean of protease measurements for the product giving the highest measurements.

#### *7.12.1.1.6.2 Published exposure data for laundry pre-spotter sprays*

Weeks et al, (2011) published data for a pre-treatment trigger-spray product supplied by a North American company obtained using the above protocol. The experimental chamber used by Weeks et al was constructed to mimic a small laundry room 14.5 $m^3$ and was

unventilated during the trial. The experiment was repeated 8 times and both high (200 L/min) and low volume (18 L/min) sampling pumps were used. Protease concentrations of 12 ± 0.92 ng/$m^3$ were measured using the high volume sampler and 17 ± 1.6 ng/$m^3$ using the low volume sampler. This paper also reported some real time particle monitoring data obtained during a preliminary trial. The tested product generated particles with mean aerodynamic diameters less than 1 µm (i.e. potentially respirable). The particle number concentration peaked between 1-4 minutes into the trial and returned to baseline within 10 minutes indicating that the sampling duration used in the spray product testing protocol appears to capture the period when there is the greatest potential for exposure.

Sarlo et al (2010) reported an exposure value of 14.5 ng protein/$m^3$ for a pre-spotter spray product.

#### *7.12.1.1.6.3 Conclusions for professional use of laundry pre-spotter sprays*

The eMSCA is satisfied that the Registrants are taking sufficient measures to ensure that the enzymes they supply for use in laundry pre-spotter sprays can be used safely. Taking into account the absence of sensitisation in volunteers using a trigger spray product intensively each day for 6 months (see section 7.9.9 of this report for details), the eMSCA does not identify concerns for this use.

### 7.12.1.1.7 Use at professional industrial and insititutional ware washer

#### *7.12.1.1.7.1 Information from the registration*

Enzymes including amylase, α- are used in products intended for professional dishwashing to help remove contamination. Amylase, α- is used during the final stage of the washing process to remove any build-up of starches that have not been removed by the highly alkaline detergents. The use of enzymes also allows less highly alkaline (and therefore less corrosive) detergents to be used without a loss of cleaning performance.

Between 2012 and 2014, the Registrants conducted simulation studies with single tank automatic dish washing machines to supplement earlier studies on multitank dishwashers that were published in the HERA report (2007). These include in-use studies with two machines (one fixed rack and one conveyor) and laboratory tests with one fixed rack machine. Machines were operated under normal conditions using typical, medium (5x) and high (10x) dosages of enzyme and with typical enzyme dosages and the air ventilation switched off. In each case, static sampling heads were placed at a position to approximate the head height of a worker operating the machine. Samples were collected over a 30-35 minute period using a flow rate of 25 L/min and were analysed for amylase, α- and/or protease using ELISA.

Under normal operating conditions and using typical dosing rates, levels of enzymes were below the LOQ which was between 0.5-1 ng/$m^3$. With medium and high dosage rates and with the room ventilation in operation, measurable concentrations were detected and also when the machine was operated with typical dosage rates and the room ventilation switched off. In all cases, measurements were below the DMEL.

#### *7.12.1.1.7.2 Published data covering use at professional I&I ware washers*

The data reported in HERA (2007) come from simulation studies performed by 2 suppliers of industrial multitank dishwashing equipment conducted during the summer/autumn of 1997. These studies are also described in an internal AISE report from 1998 "*Enzyme exposure in industrial dishwashing*". Static sampling heads were placed at the entrance and exit points and side door of the machines at a position to approximate the head height of a worker. Samples were collected over a 30 or 60 minute period using a flow rate of 300 L/min and were analysed using ELISA. Under normal use conditions, with amylase, α- dosed at a rate of 100 mg/l, airborne levels did not exceed 0.1 ng/$m^3$. Under worst case operating conditions (side door opened during operation) with amylase, α- dosed at a rate

of 50 mg/l, airborne levels did not exceed 2 ng/m$^3$. The highest concentration recorded was 4.7 ng/m$^3$ at the entrance of the machine running with doors closed and dosed with 200 mg/l amylase, α- .

These levels are similar to those found in the in-use and laboratory studies with single tank machines with the exception of the laboratory studies using a high dose rate and with no ventilation. The exposure scenario stipulates that LEV should be in use and that measures to prevent the washer being opened during the wash cycle should be in place.

#### *7.12.1.1.7.3 Conclusions for professional use at I&I ware washers*

The eMSCA does not identify a concern for this use of amylase, α- providing it is carried out in accordance with the exposure scenario.

### 7.12.1.1.8 Use of manual dishwashing products by professional workers

Simulation studies have been performed by the Registrant to assess exposure to amylase, α- during use of manual dishwashing products. Some of these studies have also been described briefly by Basketter et al, 2012.

The simulation used detergents formulated specifically for this trial containing protease at up to 20 times the levels of enzymes that are found in commercially available products. In the first simulation, detergents containing up to 0.75% aep were dosed to a weighing boat and placed in the washing up bowl. The bowl was then filled with tap water at a pressure of 0.3 bar (considered by the registrants to represent normal pressure for sink filling but may be lower than the operating pressure for domestic water supply[7]), 0.5 or 0.7 bar (identified in preliminary tests by the registrants to be the maximum pressure which did not produce unacceptable splashing), the water discarded and the filling procedure carried out a further 7 times. In the second simulation, a weighing boat with detergent containing up to 0.4% aep was placed in the corner of the sink, a stiff washing up brush was dipped into the concentrated detergent and scrubbed against the bottom of the sink under running water at a pressure of 0.3 bar. This procedure was also repeated a further 7 times. Duplicate sampling heads were positioned approximately 50 cm above the washing up bowl to mimic the breathing zone of the operator. Samples were collected over a 20 minute period using a flow rate of 25 L/min and were analysed using ELISA. All samples were below the LOQ of 2 ng/m$^3$.

During the evaluation, the eMSCA raised a concern with the Registrants that this study may not accurately reflect the dish scrubbing phase as it is performed in the workplace. In the simulation, the washing up brush was scrubbed against the flat surface of the bottom of the sink which will tend not to generate aerosols and will tend to contain any splashes. In a professional kitchen, the articles that are being scrubbed will have awkward shapes and are likely to be raised above the surface of the water and potentially above the level of the sink during scrubbing. This will create a much greater potential for aerosols to form from droplets flicked from the scrubbing brush as it is moved around the object being cleaned, and there will be a much greater opportunity for these droplets to spread beyond the sink.

[7] In the UK, water companies are required to supply water with a minimum pressure of 1 bar at the boundary of the property (http://www.bristolwater.co.uk/your-home/water-pressure/). However, information from anecdotal sources suggests that in practice, domestic water in the UK may typically be supplied at a pressure of 2-4 bar (https://uk.answers.yahoo.com/question/index?qid=20070822075231AAS6LV4).

To address this concern, the Registrants conducted an additional simulation study to assess exposures during scrubbing of dishes and cutlery. All measurements were below the limit of detection (LOD) of 2 ng/m$^3$. Further details are provided in the confidential annex.

The eMSCA also discussed with the Registrants the situation where a dishwashing product may be used undiluted to aid removal of heavy contamination. In this case, the viscosity of the product is likely to protect against aerosol formation. In response to a question about whether enzyme dusts could form if droplets containing enzyme are allowed to dry, the Registrants noted that they commonly find enzyme residues on the benches in their laboratories and that these residues are difficult to remove suggesting that dusts are unlikely to be generated from any surface contamination that may be left to dry during dishwashing.

In the light of the new information that has been provided, the eMSCA does not identify a concern for the use of amylase, α- in professional dishwashing products.

#### 7.12.1.1.9 Use for cleaning medical devices

This scenario covers the use of amylase, α- in products used to clean medical devices such as endoscopes after they have been used to examine a patient and to prepare the devices prior to sterilization. Such products may be supplied as solutions and hand-held sprays. In addition to the simulation studies conducted by the Registrants, data is available from monitoring studies carried out by HSE at a small number of hospitals in the UK. The HSE data was published in 2013 and has been summarised below (Evans et al, 2013). Since it was not included in the registration, the eMSCA provided the Registrants with the report to ensure that they are aware of this study.

##### *7.12.1.1.9.1 Information from the registration*

The simulation studies conducted by the Registrants investigated potential exposure to amylase, α- during sonication and the subsequent rinsing phase. Assorted objects were placed into a sonicator containing the cleaning solution. The sonicator was left to operate for 2 hours with the lid on (normal conditions) and also with the lid off (worst case conditions). This duration is substantially longer than the 5 minutes used for cleaning medical devices but was necessary in order to collect sufficient sample for analysis. Rinsing was performed in a sink using low pressure tap water. A sampling duration of 10 minutes was used for this phase. Duplicate sampling heads were positioned approximately 20 cm above the sonication device/sink and air was sampled using a flow rate of 25 L/min. Samples were analysed using ELISA (protease was analysed as a surrogate for amylase). Measureable levels of protease were reported for all situations but were well below the DMEL for sonication with the lid on and rinsing. The LOQ in this case was 0.4 ng/m$^3$. When sonication was performed with the lid off, levels in excess of the DMEL were measured, indicating the need to keep sonication baths closed during operation. The exposure scenario includes advice to close lids during sonication and leave baths closed for 5 minutes after the sonication process has ended to allow aerosols to clear.

At the time the registration was submitted, the Registrants had not been able to gain access to endoscopes or similar medical devices to use in simulation studies. They therefore referred to the simulation studies used for the manual dishwashing scenario as a suitable analogous situation. The eMSCA concluded that this simulation did not reflect a typical or worst case situation for the dishwashing task given that the behaviours assumed for the simulation do not match those expected in real workplaces and in the light of the behaviours that have been reported in the published studies for endoscope cleaning, the same conclusions apply for this scenario. The eMSCA discussed this with the Registrants during the evaluation and the points raised in the published studies about inconsistent communication of safe use information to end users. Following these discussions, the Registrants conducted new simulation studies for ultrasonic and manual cleaning using

medical devices (details are reported in confidential annex). Further advice has been added to the exposure scenario on the need for regular cleaning of work areas using low dust techniques and for worker training. Instructions for formulators of medical device cleaning products have also been added specifying that the presence of enzymes where they are used in products should be identified on the label along with instructions on methods to suppress enzyme aerosol formation during use.

A separate scenario has been included for spray products. These are tested using a modification of the protocol that is used to test hand-held laundry pre-treatment sprays with the piece of fabric being replaced with a tray. The sampling pumps were switched on and after one minute, the product was sprayed at the tray 20 times from a distance of 25 cm and an angle of 45 degrees over a 30 second period. The tray was changed and the spraying protocol repeated two more times. The sampling pumps were left running for a further 7.5 minutes after the final spray giving a total sampling time of 11 minutes. Duplicate sampling heads were situated 60 cm from the tray to mimic the breathing zone. Air was sampled continuously at a rate of 25 L/min during the study and in the study seen by the eMSCA, samples were analysed for multiple enzymes including amylase using ELISA. The test was repeated 8 times for the study used for the registration. All samples were below the LOQ which was slightly below 2.5 ng/$m^3$ for amylase, α-.

#### *7.12.1.1.9.2 Published studies of exposure during cleaning of medical devices*

Some additional information on exposure to protease during endoscope cleaning is available from a small scale survey of exposure to enzymes carried out by HSE in 7 endoscope cleaning units at 3 UK hospitals (Evans et al, 2013).

Six different types of enzyme cleaning solution were used across the three hospitals and a variety of cleaning methods were used. Some units wiped endoscopes with an enzyme cleaning solution immediately after the patient examination. Others immediately soaked the endoscopes in a bucket containing enzyme cleaner, or transferred the endoscopes to a washing facility before cleaning. Some units used small brushes to clean the outer surface, holding the endoscope at eye level. This generated fine sprays in close proximity to the operators head. Other units only carried out surface cleaning with the instrument submerged. Procedures for cleaning inner surfaces also varied with some units using syringes to flush concentrated enzyme solution through under pressure while the endoscope was held flat on a work surface. Elsewhere this was performed with the endoscope submerged. Splashing was observed where the enzyme concentrate was poured into the sink and spray droplets were released when the taps were turned on. Where pumps and fluid delivery lines were provided to enable the enzyme product to be added beneath the water, spray droplets were not observed. These observations support the view of the eMSCA that the behaviours used in the dishwashing simulation study do not accurately reflect real world behaviours.

Time spent working with enzyme cleaning solutions varied from a few minutes to several hours. Most staff involved in manual cleaning were provided with disposable gloves, aprons and overalls and only some were provided with (or wore) protective visors and longer length gloves (or covers) to protect the skin on the lower arms. Where RPE was provided, it was for the specific purpose of changing stocks of cleaning agents. The types of RPE provided included fluid resistant surgical masks with face shields, half masks and full face masks, FFP2D particulate disposable respirators and organic vapour particulate respirators. Face fit testing was not routinely undertaken hence it is not clear if the RPE that was provided was fully effective.

Potential exposure to airborne enzyme was assessed using personal and static sampling and surface wipes. Personal and static samples were collected using IOM samplers with flow rates set at 2 L/min. Wipe samples were collected from surfaces adjacent to the endoscope washing activity (in both manual and machine cleaning areas), on the washing machines, on floors and at sites away from the main cleaning activity at the beginning and end of cleaning. A uniform 100 $cm^2$ grid was sampled on all flat surfaces. On other surfaces,

a uniform length was wiped (e.g. length of an endoscope tube). At one site, additional wipe sampling of personal clothing was undertaken before and at the end of the work period. Samples were only analysed for protease. The level of proteolytic activity in air and wipe samples was quantified using an enzyme substrate activity assay. Bulk samples of the cleaning solutions were also analysed for their proteolytic activity and revealed a 10 fold variation in proteolytic activity across the products with the product containing highest activity having second to lowest protein concentration. This finding indicates that the stated enzyme concentration in a product will not provide useful information about the potential allergenicity of that product. The assay for proteolytic activity that was used for this study is the one used to determine compliance with the UK Workplace Exposure Limit (WEL) for subtilisin and was calibrated against a subtilisin standard. Since proteases differ in the rate at which they degrade the substrate used for this assay, the results of this study can be used to compare relative levels of contamination for individual products but not to compare levels of contamination between different products. Also the results will not be directly comparable to the results obtained in the simulation studies performed by the registrants.

Of 14 personal samples collected, only 4 contained detectable levels of enzyme (8.9, 14.5, 17.4 and 66.7 ng/$m^3$ expressed as an 8-hr TWA). These were all taken during manual cleaning in sinks (wet wiping, scrubbing and injecting enzyme cleaner). Six out of the 11 static samples also contained detectable levels of enzyme (0.6, 7.0, 9.3, 10.2, 14.4 and 45.1 ng/$m^3$ expressed as an 8-hr TWA). These were also taken during manual cleaning in sinks. These results highlight a potential concern that exposures under real use conditions may significantly exceed the levels assumed in the CSR.

Wipe samples revealed surface contamination in 6 of the 7 units with surface contamination generally higher in the busier units. High levels were measured on the floor closest to the sinks and where pre-cleaned endoscopes were carried to the automatic wash machines. In some cases the enzyme activity in the wipe sample was of the same order of magnitude as that found for the undiluted stock solution. Lower levels of contamination were also found away from the washing areas and in some cases traces were also found on the outside of boxes used to transport cleaned endoscopes and on clothing and hands. Surface contamination presents an indirect risk for inhalation because it presents an opportunity for enzyme containing dusts to form as the contamination dries. This dust may then be lifted into the air adding to the background concentration. The report also noted a potential for exposure to dried enzyme solution during servicing of automated machines. This has not been considered in the registration. The unit with the lowest levels of surface contamination performed wet surface cleaning throughout the day whereas other units only cleaned once per day. This illustrates the importance of regular cleaning in managing exposure to enzymes.

Evans et al, 2013 commented on the advice being disseminated to end users via safety data sheets (SDSs). No SDS contained information about a potential respiratory sensitisation hazard. The eMSCA notes that CLP rules may prohibit hazard warnings to be provided if this contradicts the legally required hazard information. One supplier had advised use of disposable absorbent pads around the sink to limit the spread of contamination and in some cases, suppliers had advised users to keep the endoscope under water whilst scrubbing off surface contamination. The extent to which this inconsistent provision of good practice advice contributed to the variations in housekeeping standards between endoscope cleaning units is not clear.

Prior to this study, high levels of enzyme contamination and evidence for poor working practices were reported by Adisesh et al, (2011) following visits to 2 healthcare sites. Poor practices observed during these visits included staff wiping down surfaces with enzyme detergents, spillages on floors during the transfer of pre-cleaned endoscopes to the automated washing equipment, drips of enzyme product from tubes inside washing equipment being allowed to dry to form a crust, spillages left uncleaned, enzyme products being decanted into pump bottles and atomisers used to spray enzyme onto the tips of endoscopes. These authors also noted that the manufacturers' SDSs for the products in use did not identify respiratory sensitisation as a potential hazard.

Measurements were only collected at one site. Samples were analysed for protease activity using the same method used by Evans et al 2013. At this site, two liquid products were in use for soaking/washing and a foam spray was used for manual cleaning. It was also reportedly used for hard surface (e.g. work bench) cleaning. In the sterilisation unit, the floor where the enzyme dosing containers were located was heavily contaminated. Wipe samples revealed a protease level of 7475 ng/100cm$^2$. Airborne levels measured using personal samplers (sampling flow rate 2 L/min) were found to be below 10 ng/m$^3$. Further wipe samples were taken during a follow-up visit and revealed levels of 18 ng/100 cm$^2$ on prewashed cystoscopes, 71 ng/100 cm$^2$ on the cystoscope transport box, 2.98 ng/100m$^2$ on the hands of workers handling the pre-washed cystoscopes and 4.75 ng/100 cm$^2$ on several surfaces (not specified). No airborne protease was detected during the second visit.

Measurements were also made in the urology theatre at this hospital. Wipe samples taken around a sink used to clean cystoscopes before they are transferred to a specialised sterilisation unit revealed 2083 ng protease/100 cm$^2$ on the taps, 1581 ng/100m$^2$ at the sink edge, 131 ng/100 m$^2$ on the floor and 199 403 ng/100 cm$^2$ on a shelf. Static air sampling was also performed in the sink area with a level of 10.22 ng/m$^3$ reported (details of the flowrate and sampling duration were not reported).

These studies provide a useful additional perspective on the exposure assessment for endoscope cleaning. Both studies demonstrate that good practices are not necessarily followed in relation to the use of enzyme containing products. This may be in part due to inconsistent communication of good practice advice from suppliers. Although airborne enzyme levels were generally found to be below the DMEL, this is not always the case. If poor working practices are adopted by someone working in a busy cleaning unit, they could experience many peak exposures that exceed the DMEL during their working day. This clearly represents a risk to their health. The extent to which surface contamination adds to the risk is not clear. The eMSCA notes that surface contamination with enzymes is commonly found in the Registrants' laboratories but is difficult to remove suggesting that enzyme solutions may not tend to dry and form dusts that can then contribute to airborne enzyme levels. It is also not clear to what extent enzymes retain their allergenicity in the situation where they are present as surface residues. The use of good working practices such as regular cleaning of surfaces to minimise contamination should limit any potential exposure via this route.

#### *7.12.1.1.9.3 Conclusions for cleaning of medical devices*

Taking all of the available evidence into account, it seems reasonable to conclude that when medical device cleaning is performed carefully, in accordance with the guidance on safe use identified in the exposure scenario, exposures will be maintained below the DMEL. However, high exposures can occur if these procedures are not followed. This applies to both manual cleaning and ultrasonication. The eMSCA is satisfied that the Registrants have identified suitable and adequate measures to ensure safe use and are taking reasonable steps to support the dissemination of good practice advice through the supply chain to end users. At this time, the eMSCA does not identify further specific actions that the Registrants need to take.

### 7.12.1.2. **Consumer**

Amylase, α- is not supplied to consumers as the substance itself but may be present in products supplied for consumer use. The consumer exposure assessment is based on data from the same simulation studies that have been used for equivalent professional use scenarios.

#### 7.12.1.2.1 Laundry and dishwashing products including pre-spotter stain treatment sprays

Consumer exposure to amylase, α- in enzyme containing laundry detergents and automatic dishwashing products mainly arises during dosing of the product to the washing machine. The exposure value used for the risk characterisation is derived from the simulation studies reported by Sarlo et al (2010) which have been described in section 7.12.1.1.5, use at a professional I&I laundry.

In response to a concern raised during the evaluation by the eMSCA that the assessment did not consider possible consumer use of enzyme containing laundry detergents for handwashing, the Registrants provided additional information including a new simulation study which mimicked the addition of water to a bowl containing laundry detergent. The new simulation study demonstrated that exposures remained below the DMEL for this activity.

Earlier work conducted by Procter and Gamble to investigate enzyme exposure during hand washing has been published (SDA, 2005). Studies covered use of laundry bar and laundry granules according to Philippine hand laundering conditions which were assessed as worst case based on a comparison of habits and practices information for different global regions. A total of eight hand-laundering trials were performed using five volunteers. The trials took place in a poorly ventilated square shaped room with an approximate volume of 23$m^3$. Hand washing was conducted in a wash basin while squatting or sitting on a stool. Air sampling began when the volunteer started the wash and continued through the 10-minute laundering process. Static samplers were situated at breathing zone height and samples were collected onto glass fibre filters at a flow rate of 0.67 $m^3$/min. Partially open slatted blinds were placed in front of the sampler to deflect splashes without interrupting the air flow. Samples were analysed using ELISA. For the laundry bar, levels of 0.004 – 0.026 ng/$m^3$ were reported. For laundry granules, levels of 0.06 – 0.18 ng/$m^3$ were reported. The LOQ for this trial was not reported

In the case of products intended for use in spray applications, individual products are tested by the Registrants to determine whether the product and packaging design are sufficient to ensure that the levels of airborne enzyme generated during use will remain below the DMEL. The exposure value used for the risk characterisation is the same as the value used for professional use of laundry pre-wash sprays.

The eMSCA does not have concerns relating to the use of amylase, α- in consumer laundry products.

#### 7.12.1.2.2 Use of manual dishwashing products

Consumer exposure to amylase, α- in products supplied for manual dishwashing has been assessed with the same data used to assess professional manual dishwashing products. Further data was provided during the evaluation to address a concern raised by the eMSCA that the dish scrubbing phase was not accurately reflected in the simulation study (see section 7.12.1.1.8). The Registrants also provided clarification about potential exposure in the situation where dishwashing products are used undiluted to aid removal of heavy contamination.

Based on current information, the eMSCA does not have concerns relating to the use of enzymes in manual dishwashing products where these are used as intended by the manufacturer or where they may also be used for general household cleaning activities.

It is possible that dishwashing liquids may be used by consumers to make bubble blowing liquids for children or for other children's activities. It is not possible for the eMSCA to reach conclusions about the potential risks to consumers from these unintended but foreseeable uses. The eMSCA discussed this use with the Registrants. The Registrants commented that their information on the use of enzymes in consumer dishwashing products suggests

enzyme containing products have a small share of the dishwashing product market. The eMSCA also notes that detergent bubbles generally travel away from the breathing zone of the person blowing the bubbles. Based on currently available information, the eMSCA concludes that the risks to consumers from these foreseeable uses of enzyme containing detergents are uncertain but probably low. The eMSCA will discuss with the Registrants additional actions that could be taken to avoid the situation arising where enzyme containing manual dishwashing products are used for different activities.

#### 7.12.1.2.3 Use of cleaning products (drain cleaners)

Consumer exposure to amylase, α- in drain cleaners has been assessed using the same simulation study that was used to assess exposure for professionals. The eMSCA considers that the study replicates worst case conditions for consumer use and does not identify a concern.

#### 7.12.1.2.4 Conclusions for consumer use

The eMSCA considers that the exposure information provided in the registration provides a suitable basis to assess the risks for consumer use of products containing amylase, α- and with the exception of the possible use of enzyme containing manual dishwashing products for different activities, the eMSCA does not identify concerns for these consumer uses.

### 7.12.2. Environment

This evaluation was targeted to human health concerns and did not consider environmental exposure.

#### 7.12.2.1. Aquatic compartment (incl. sediment)

#### 7.12.2.2. Terrestrial compartment

#### 7.12.2.3. Atmospheric compartment

### 7.12.3. Combined exposure assessment

The registrants have not tried to quantify combined exposures but have considered combined exposure in their risk characterisation. The eMSCA agrees that it is not necessary to quantify combined exposures for amylase, α-.

## 7.13. Risk characterisation

### 7.13.1 Human health

The only health concern for amylase, α- is respiratory sensitisation. The Registrants have identified a DMEL of 60 ng/m$^3$ for workers and 15 ng/m$^3$ for the general population. The general population DMEL has also been used as a benchmark to assess professional use in cases such as laundry and dishwashing use because of the similarities with consumer use in terms of the risk management measures that are applied.

#### 7.13.1.1 Workers

The exposure assessment for workers relies on information obtained using static sampling methods because of the difficulties to accurately measure personal exposures to small quantities of enzymes. Although this creates uncertainty about the levels of short term peak and full shift exposures each worker will experience, the eMSCA considers that the

exposure values that have been used in the Registrants' risk characterisation are likely to overestimate full shift exposure in most cases.

Given the uncertainties about the level of effects expected with exposures at or below the DMEL and about the levels of exposure that individual workers may experience in practice, the eMSCA considers it is more relevant to assess the suitability of the risk management measures in a qualitative way. The eMSCA considers that the risk management measures identified by the Registrants for worker scenarios are suitable and adequate providing they are implemented correctly. The eMSCA has discussed concerns about the way safe use information is communicated to end users with the Registrants and as a result, additional instructions have been included in the exposure scenarios for medical device cleaning about the information that should be provided with enzyme containing products. The Registrants have also included links to training materials that can be used by downstream users to train their workforce. The training materials that the eMSCA has seen so far tend to be aimed at formulators rather than end users. The eMSCA will discuss with the Registrants what additional guidance could be provided to improve the way safe use information is being communicated to end users.

#### 7.13.1.2 Consumers

The consumer exposure assessments rely on the same data that was used to assess equivalent professional uses and therefore the values taken forward for the risk characterisation are expected to overestimate consumer exposures where products are used as intended. The only concerns identified by the eMSCA relation to the case where enzyme containing dishwashing detergents are used to make bubble blowing liquids or for other purposes that are not intended by the product manufacturers. The eMSCA will discuss with the Registrants additional actions that could be taken to avoid the situation arising where enzyme containing manual dishwashing products are used for different activities.

#### 7.13.1.3 Indirect exposure via the environment

The eMSCA does not expect there will be any meaningful exposure to the forms of amylase, α- covered in the registration from sources other than thorough direct use in the workplace or from use of products containing enzymes. The eMSCA therefore considers indirect exposure via the environment to be irrelevant for this assessment.

#### 7.13.1.4 Combined

The eMSCA does not identify concerns for combined exposure to amylase, α-.

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## 7.15. Abbreviations

| | |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| AISE | International Association for Soaps, Detergents and Maintenance Products |
| aep | active enzyme protein |
| $CaCO_3$ | Calcium carbonate |
| CAD | Chemical Agents Directive |
| CI | Confidence Interval |
| CIP | Clean in Place |
| cm | centimetres |
| CSR | Chemical Safety Report |
| DECOS | Dutch Expert Committee on Occupational Safety |
| DMEL | Derived Minimal Effect Level |
| DNEL | Derived No Effect Level |
| ECHA | European Chemicals Agency |
| ELISA | Enzyme Linked Immunosorbent Assay |

| | |
|---|---|
| eMSCA | evaluating Member State Competent Authority |
| FAA | Fungal Alpha Amylase |
| HEPA | High Efficiency Particulate Air |
| HSE | Health and Safety Executive |
| I&I | Industrial and Institutional |
| IBC | Intermediate Bulk Container |
| IgE | Immunoglobulin E |
| I&I | Industrial and institutional |
| IR and CSA | Information Requirements and Chemical Safety Assessment |
| IOM | Institute of Occupational Medicine |
| kg | kilogrammes |
| L | litres |
| L/min | litres per minute |
| LEV | Local Exhaust Ventilation |
| LOQ | Limit of Quantification |
| m | metres |
| mg/l | milligrams per litre |
| µm | micrometre |
| m/s | metres per second |
| ng/$m^3$ | nanograms per metre cubed |
| ng/cm3 | nanograms per centimetre cubed |
| OCs | Operating Conditions |
| OEL | Occupational Exposure Limit |
| PROC | Process code |
| RCR | Risk Characterisation Ratio |
| RPE | Respiratory Protective Equipment |
| RMMs | Risk Management Measures |
| SDS | Safety Data Sheet |
| THOR | The Health and Occupation Research Network |
| TWA | Time Weighted Average |

UK United Kingdom

WEL Workplace Exposure Limit