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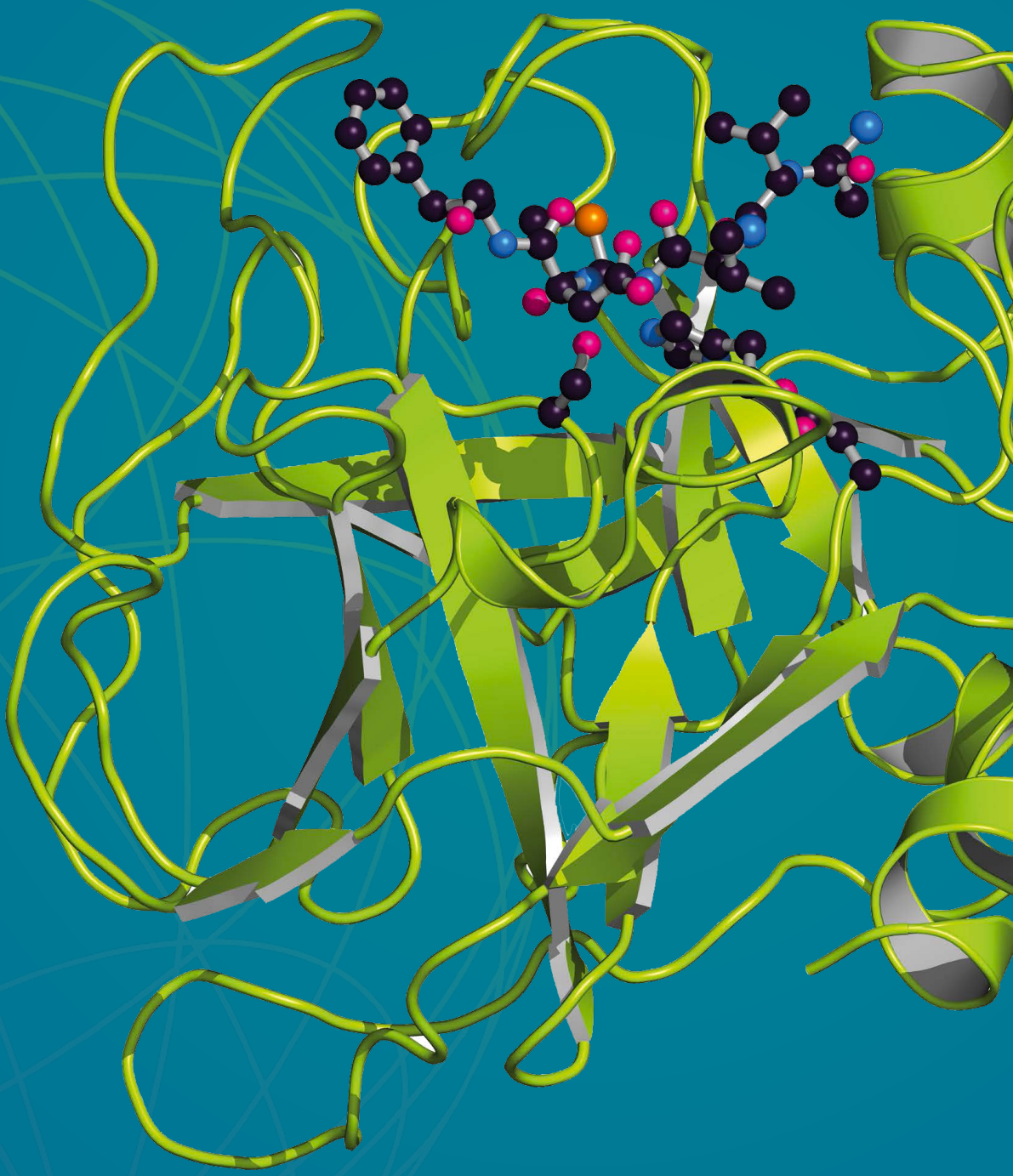
# Guidance for the Risk Assessment of Enzyme-Containing Products for Professional Cleaning



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# List of authors

**Merete Simonsen**
Novonesis A/S

**Anthony Panepinto**
Procter & Gamble Company

**Ilse Tuinman**
IFF

**Beth Concoby**
IFF

**Kimberly Harber**
Ecolab

**Mark Fieldsend**
Occupational Health, Safety and Sustainability Consultant

**Carrie Brown**
Household & Commercial Products Association (HCPA)

**Francis Kruszewski**
American Cleaning Institute (ACI)

**Julie Janssis**
International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.)

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# Chapter 1

## INTRODUCTION

Professional cleaning products are used in a wide variety of situations, from cleaning of private and public facilities such as hospitals, offices, hotels, restaurants, care homes and public transport, to cleaning of workplaces and industrial process involved in the manufacturing of foods, beverages, pharmaceuticals, etc. Essentially, any facility where people work or play, or any process where product hygiene is required, requires the use of professional cleaning products to maintain cleanliness, hygiene, and product safety.
Professional cleaning products undergo a safety risk assessment to ensure that they are safe to use by the end users applying them. This assessment also covers safety for the application they are intended for, up to and including material compatibility and the risk to people and products from residual surface or product contamination
.
The purpose of this document is to provide the manufacturers of professional cleaning products guidance on how to make appropriate risk assessment and risk management of such products, in connection with the specific risk of respiratory sensitisation related to enzymes. The focus in the risk assessment process will be on understanding fully the use situation and the potential risk of generating airborne particles. In the risk management process, the focus will be on avoiding the generation of airborne particles by optimal product design (formulation as well as design of product device) and on communication of how to avoid any potential risk to the end-user.

For many years, enzymes have been used safely as a functional ingredient in consumer products such as laundry detergents. They are also widely used in professional products for bulk laundering of fabrics and clothing from the manufacturing, healthcare, and service industries. Enzymes have also been used in niche professional products such as solutions for cleaning endoscopes, and specialised drain and surface cleaners. Due to their versatility and positive benefits, enzymes are more frequently incorporated into professional cleaning products. They can contribute to the sustainability and environmental safety profile of formulations that use them. Enzymes do this through reducing the temperature at which the product needs to be used, replacing the function of harsher ingredients, being fully and quickly biodegradable, and virtually nontoxic to people **(1)**, flora, and fauna.

Nevertheless, despite their low order of toxicity, it is well documented that enzyme proteins correspond to respiratory sensitisers and exposure by inhalation may lead to the development of occupational allergy (2). To have the potential to cause respiratory sensitisation, the enzyme must become airborne as a dust particle or

mist droplet. An additional challenge is that the concentrations required to cause respiratory sensitisation are extremely low. This means that not only do enzymes as raw materials present a risk, but also the finished products that contain them may present a risk. However, it is important to note that only a minor fraction of those individuals exposed to sufficiently high doses of airborne enzyme will at some point be at risk for developing a sensitisation towards the specific enzyme. The health risk is similar to the one associated with exposure to other proteins found in the natural environment such as pollen or house dust mite **(1)**.

Fortunately, under the stewardship of A.I.S.E., AMFEP, ACI, HCPA and the major enzyme suppliers, it has been proven over many years that the health risks from enzymes can be successfully and effectively controlled. This is achieved by use of engineering controls to contain and remove dust and aerosol during product manufacture, and the design of finished products to eliminate or minimise inhalable enzyme dust and aerosol to safe levels during application and use. Additionally, through a program of information and training, including a best practice guidance for safe handling and incorporation of enzymes into products during manufacturing, and the safe design of enzyme products for the end users, the cleaning products industry has effectively managed the health risks associated with exposure to enzymes by inhalation in the detergent industry, and in the home of the consumers using their products. For workers' safety when enzymes containing reagents are formulated, please see A.I.S.E. Guidelines for the Safe Handling of Enzymes in Detergent Manufacturing (3).

Professional products may be similar in formulation to consumer products, but they differ from consumer products in the variety of applications that they may be designed for, in addition to the frequency and duration of use. Therefore, the risk assessment to support professional products, whilst similar in format to consumer products, may be very different in calculation. In addition, based on the frequency of use of professional products, the risk of "foreseeable unintended use" could be higher. Given the variation in very different or very difficult cleaning scenarios that an end user in this industry may encounter, the "trial" application of products for which they were not designed is highly likely to occur. Therefore, foreseeable unintended use must be factored into the risk assessment and detailed instructions for application and use must be clearly documented on the product, along with clear warnings against product unintended use.

# Chapter 2

## APPLICATIONS FOR PROFESSIONAL CLEANING PRODUCTS

As briefly described above, professional cleaning products are used across a wide variety of different scenarios, but broadly can fit into one of three classes.

- Used in a fully closed system e.g.
  - Cleaning in place (CIP)
  - In a closed machine such as an industrial dishwasher / washing machine

- Used in a partially closed system or process, e.g.
  - Cleaning out of places (COP)
  - Manually dosed into a closed system or process (including drains)
  - Cleaning of equipment items by manual immersion in a closed container, for example medical device cleaning

- Used in the open for cleaning of surfaces and/or equipment, e.g.
  - Food preparation surfaces
  - Floors and walls
  - Sanitary equipment
  - Exterior surfaces of plant and equipment
  - Cleaning of equipment items by manual immersion in an open container
  - Cleaning of coffee machines
  - Hand dishwashing
  - Degreaser

Each scenario imparts a different level of risk for the end user, and that information allows product development and design to reflect the risk in each scenario. The higher level of containment and control for the end use application, the lower the risk of exposure. Likewise, the elimination and/or minimisation of inhalable dust or aerosol through product design will also reduce the risk of exposure. Combining containment, exposure control and safe product design is the obvious target to achieve.

Of the three main scenarios, the use of enzyme products for open cleaning of surfaces carries the highest risk of exposure and therefore these products require very careful design and use instruction. Training is also an important part of the safety management program for use of any professional cleaning product regardless of the formulation. But for enzyme containing products, very specific training is required. Examples of such open cleaning of surfaces could be the cleaning of walls, ceiling, and floors after working hours in slaughterhouses or fisheries. In these examples, enzyme containing cleaning products are often applied via a foam gun with subsequent rinsing. Exposure to enzyme aerosols is unavoidable in these situations, hence, specific training and the use of personal

protective equipment including respiratory protection must be mandatory.

In all scenarios, the risk from residual product contamination on surfaces, including the one coming from the enzymes used in the product, must also be considered. Residual enzymes on surfaces may become airborne from subsequent process steps or conditions resulting in uncontrolled exposure.

Since there is little difference in how consumer and professional products are manufactured and packed, it can be inferred the experiences, mistakes, best practices, and historical data from detergent manufacturing are entirely relevant. However, as previously stated, the end use can be different. This makes the need for exposure data or benchmarks for every type of application extremely important, if not critical, for the completion of an effective end user risk assessment.

This document aims to inform and guide companies developing and marketing professional cleaning products containing enzymes to ensure through the process of risk assessment, the design of professional products are safe for the intended application, and safe for the end users and others in the vicinity of their use.

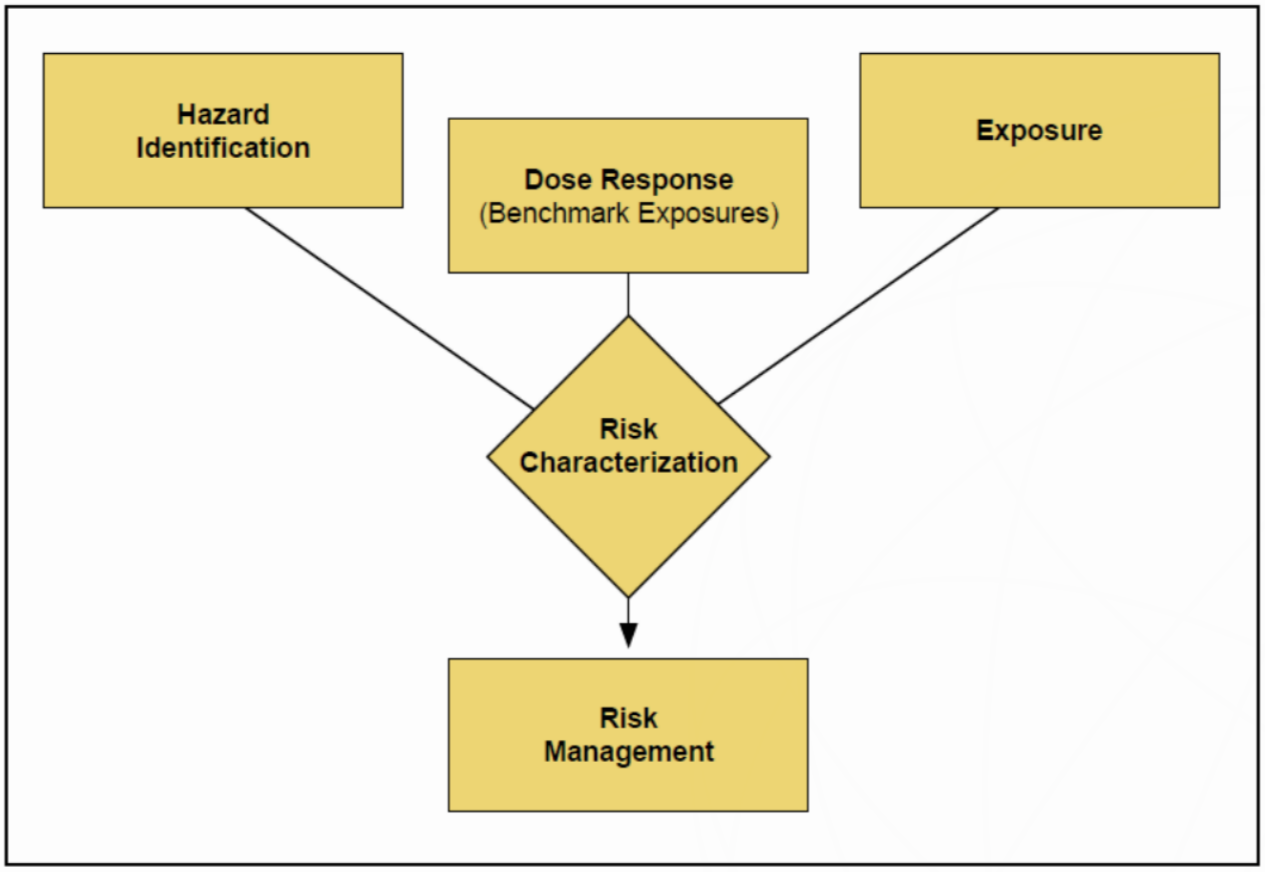
# Chapter 3

## RISK ASSESSMENT AND RISK MANAGEMENT

Enzymes can bring significant benefits to professional cleaning products, including improved efficiencies and product benefits. However, prior to introducing an enzyme-containing cleaning product onto the market, a risk assessment is conducted to ensure the safe use by the professional.

Enzymes have a very good safety profile but, as with many other proteins, they can act as respiratory sensitizers and so may lead to allergy symptoms, such as rhinitis. This potential risk is the primary focus for risk assessments for enzymes and must be managed carefully. Experience in the cleaning products industry demonstrates that the potential risk of adverse effects can be successfully managed by the strategies outlined in this guidance.

The combined risk assessment and risk management process can be illustrated as below:



## RISK ASSESSMENT

Risk assessment can be divided into four areas:

- hazard identification
- dose-response or benchmark identification
- exposure assessment
- risk characterization

The risk assessment process for enzymes follows this general approach. Benchmark doses to define adverse effect and no-adverse effect thresholds are used instead of classic dose-response curves.

### Hazard identification

Hazard identification is the characterization of the fundamental physical, chemical and biological effects of a material. The toxicology of enzymes poses no significant hazard. Acute and sub-chronic toxicity is not of concern for industrial enzymes. Enzymes have a low order of toxicity. In the context of this guidance document, the safety concern being addressed is a potential induction of respiratory allergies. Skin and eye irritation may be an issue in case of long-term exposure to high concentrations of an enzyme from the class of proteases. This hazard is not related to enzymes outside the class of proteases, and it is not discussed in this guideline.

### Dose-response or benchmark identification

Generally, in this step of the risk assessment process, the relationship between the level of exposure and the specific biological effect is characterized. However, since the dose-response relationship for enzyme allergy is not fully understood and there are gaps in our understanding of the relationship between exposure, sensitisation and symptoms, benchmarks are generally used to support decisions in enzyme risk assessments. Such benchmarks are based on studies in which measured or estimated exposure levels are associated with a demonstrated specific biological effect (such as whether an allergen-specific antibody is produced) in those exposed. A clear benefit of this strategy is that it can be based entirely on human data.

In EU countries, there is a requirement by the REACH legislation to define a Derived No Effect Level (DNEL) for registered substances. Where a DNEL cannot be established, e.g. for sensitizing substances like enzymes, then a Derived Minimal Effect Level (DMEL) is recommended. For enzymes, a DMEL has been established by thorough retrospective review of occupational and consumer experience (4). The DMEL for occupational exposure has been set as 60 ng/m³ thereby following the Threshold Limit Value (TLV) set for Subtilisin by the American Conference of Governmental Industrial Hygienists (ACGIH) in 1971. For consumer exposure, the DMEL is 15 ng/m³. Professional uses should apply one of these DMELs depending on the character of the use. If the professional receives training in the safe handling of an enzyme containing product and have access to personal protective equipment like correct respiratory protection equipment, then the DMEL of 60 ng/m³ may apply. For other professional users, the DMEL of 15 ng/m³ should apply.

### Exposure Assessment

Exposure assessment establishes the amount of airborne enzyme to which the user may be exposed during intended use or foreseeable unintended use. This value is then compared to the benchmark exposure (in most cases the DMEL) in order to make risk decisions. Measuring, or even estimating, exposure to enzymes is not necessarily a simple process. However, the determination of exposure values is needed for carrying out these risk assessments. In the absence of good quality exposure data, reasonable worst-case assumptions and uncertainty factors are employed, which lead to an overestimation of exposure levels and thereby limit unnecessarily the amount and type of enzyme that can be used in a professional cleaning product. It is therefore important that the exposure assessment be conducted thoroughly, in order to enable the optimum use of enzymes in the products. The most important step of an exposure assessment is trying to define the factors that may influence the exposure.

### Risk Characterization

Risk characterization is the examination of the relationship between human exposure (calculated or measured) and the inherent toxicity of a substance, in order that the likely incidence and severity of any effect can be assessed. This step is important because it integrates information regarding the hazard identification and exposure assessment associated with use and foreseeable unintended use of a product. It should be recognized that risk assessment is a continuously evolving discipline. The quality and reliability of a risk assessment is dependent on and is only as good as the data used to conduct the assessment. Uncertainties exist in dose-response relationships, exposure data and estimates from exposure models. Assumptions and estimations need to be stated clearly as they can affect the reliability and quality of the risk assessment. It is important to consider these points when evaluating information from the risk assessment in determining whether the risk is considered acceptable.

## RISK MANAGEMENT

The objectives of the risk management process are to determine the significance of risks to human health to ensure that the product use remains within the acceptable risk levels, and to communicate risks, or lack thereof, to appropriate audiences, in an effective manner.

### Risk Control and Communication

In general terms, the risk control step of the risk management process should strive to reduce the risk by limiting airborne exposure to enzymes included in the product formulation. Risk reduction options may include product modification, only allowing certain uses or a decision not to market the enzyme containing product. Modification options may include changing the matrix or delivery system of the enzyme containing product, reducing the enzyme concentration in the product, substituting other ingredients that may be affecting the potency of the enzyme, or a combination of these approaches. In the detergent industry, great steps have been taken to minimize risk through product modification. For example, in granular products, enzymes are encapsulated in order to limit exposure to users.

An integral part of the risk management process is to communicate enzyme product benefits, as well as potential risks, to appropriate audiences in an effective way. There are important audiences to target in designing a risk communication program:

- Users of the company's products
- Employers of professional users, e.g. cleaning companies
- Persons responsible for worker safety in the companies employing professional users
- Other stakeholders, such as the general public, governmental and non-governmental organizations, or industry partners.

Product labels are the primary means of communication with consumers and professionals. For enzyme-containing products, as with all consumer products, product labels are a mechanism for communicating composition of the product, first aid information, appropriate warning statements, and use and handling guidelines with detailed examples of correct use and concrete recommendations to steer towards proper use. For professionals, SDSs are available but these are often only suitable, i.e. understandable, for people with the proper training to interpret the SDSs. Hence, it is important for products used by professionals in small companies to provide all significant information in the same way as for consumers.

For more detailed information on the risk assessment and risk management process of enzyme containing products, please consult ACI's Guidance for the Risk Assessment of Enzyme-Containing Consumer Products (5).

# Chapter 4

## RISK COMMUNICATION ON INTENDED AND NON-INTENDED USES

As stated in the introduction, professional cleaning products undergo a safety risk assessment to ensure that they are safe to use by the end users applying them. This safety risk assessment includes the intended uses as well as the foreseeable non-intended uses.

In some instances, this safety risk assessment shows that specific safety precautions during use are needed or that a certain foreseeable unintended use situation should be warned against.

An example of this is the use of enzymes in professional products for cleaning endoscopes. Enzyme containing products for this use have been on the market for more than a decade and they have proven to be highly efficient in cleaning of this kind of surgical instrument. However, it was also shown that, in some cases, hospitals would apply the enzyme containing products into an open ultrasonic bath, which would result in aerosolization of the enzyme, thereby leading to risk of respiratory enzyme exposure of the staff doing the cleaning. This risk could easily be mitigated by doing ultrasonic cleaning only in a closed environment and by ensuring that the staff allows for a few minutes to pass after the ultrasonic step before opening the system. The realization of this issue made AMFEP and A.I.S.E. generate a guidance on safety in the use safety in the use of enzyme containing reagents for medical device cleaning (6). In this guidance document, the simple but necessary precautions for ensuring a safe working environment for professionals using enzyme containing products for cleaning of surgical instruments are described.

Using enzyme containing cleaning products in a non-authorized spray device corresponds to another example of non-intended use. Enzyme containing spray products can be used safely when a proper risk assessment has been made according to the protocol "Exposure measurements of enzymes for risk assessment of household cleaning spray products."(7) It is clear from this protocol that important parameters regarding the level of airborne enzyme exposure during use of such products will depend on the combination of: viscosity of the cleaning product, the nozzle device, and the enzyme concentration. Hence, the safety approval of these products will cover the exact combination of these parameters. In case one of these parameters is changed or substituted, a new risk assessment is needed. If guidelines are not followed, a foreseeable non-intended use in a random spray-device could lead to exposures higher than acceptable.

The examples above show the importance of conveying proper safety risk communication to the end-user. It is the enzyme suppliers' obligation to communicate safety for workers and professional users to their customers, and this safety information must be communicated further along the supply chain. The safety guidance and appropriate risk management for end-users (professionals) must be conveyed by the manufacturers of the products for professional use and this guidance should be ensured by the employers of the professionals.

# Chapter 5

## APPENDICES

**Examples of risk assessments made on professional cleaning products using enzymes**

### EXAMPLE 1

**Risk assessment and risk management of enzyme containing reagents for medical device cleaning**

Background: Enzymatic detergents are widely used for medical device cleaning of e.g. fiberoptic instruments/ endoscopes because of their excellent washing performance. In many cases, ultrasonic cleaning is used during the process to increase cleaning efficiency. However, studies have shown that ultrasonic cleaning in an open setting may generate enzyme containing aerosols exceeding the acceptable exposure level. The studies have also shown that the enzyme exposure can be controlled below the acceptable exposure level when performing ultrasonic cleaning in a bath that is closed by a lid, and by keeping the lid closed for 5 minutes after cessation of the ultrasonic treatment before opening. As the cleaning is performed by professionals at hospitals, it is important that these users are informed and instructed thoroughly in the correct handling of the cleaning products to avoid occupational incidents.

#### RISK ASSESSMENT

Analysis of habits and practices in the use of products for medical device cleaning: Analysing the habits and practices among end-users of products for medical device cleaning showed that a significant portion of the end-users would add the enzyme containing medical device cleaning product to an open ultrasonic bath. As this is not the intended use of these products and thereby not part of the general risk assessment covering these products, a need for making a risk assessment focusing on this non-intended use was identified.

Hazard Identification: Enzymes can act as respiratory sensitizers. At high concentrations, proteolytic enzymes can also irritate skin and eyes.

Dose Response: The acceptable exposure limit for professionals at hospitals will be 15 ng/m³ as stated in (4).

Exposure: Exposure studies using commercial enzyme containing detergent for medical device cleaning have been conducted. Study conditions included realistic enzyme concentration in the detergent product, as well as double dose of enzyme. Enzyme exposure was measured during intended use of ultrasonic cleaning, i.e. lid closed during and 5 min after the sonication process, and exposure was

also measured during non-intended use of ultrasonic cleaning, i.e. lid was open throughout the process.


The results from the studies show that having an open lid throughout the ultrasonic cleaning might provide exposures up to 80 ng/m³, whereas ultrasonic cleaning with lid closed and with 5 min rest before opening the lid resulted in enzyme exposures below the DMEL of 15 ng/m³.

Risk characterization: The data from the exposure studies show that the foreseeable non-intended use presents a risk of respiratory exposure to the professional, whereas the intended use presents exposure below the DMEL of 15 ng/m³ and therefore can be considered safe for professionals. This demonstrates a need for Risk Management.

## RISK MANAGEMENT

Risk Control: Modification options to reduce airborne enzyme exposure during foreseeable non-intended use would be: change of matrix or delivery system, reducing enzyme concentration, substituting ingredients that might affect the potency of the enzyme or a combination of these approaches. However, none of these options would result in reduced exposure during the non-intended use. The way to control the risk of enzyme exposure from this non-intended use is to prevent it from happening.

Risk Communication: According to the EU legislations including REACH, it is the enzyme suppliers' obligation to communicate safety for workers and professional users, and the safety information must be communicated further along the supply chain. As part of this, manufacturers of medical device cleaning agents must

convey the relevant safety information given in the exposure scenarios of each ingredient. The safety guidance and appropriate risk management for end-users (professionals) should be ensured by employers.

For enzyme ingredients, this means that the following safety precautions must be conveyed to the users:

- Apply good housekeeping and good personal hygiene.
- Ensure that enzyme aerosols are not created.
- Use closed systems whenever possible.
- Ensure exhaust ventilation if there is a risk of exposure (open handling, openings in closed systems)
- Do not splash or stir vigorously during dosing or mixing as aerosols may be created.
- Any spill must be cleaned up immediately without creating aerosols.
- For ultrasonic cleaning, the following specific safe handling procedures must be complied with:
  - The lid of the ultrasonic cleaning device must be closed during operation.
  - It is recommended to insert a lag time after cessation of the ultrasonic treatment (approx. 5 min) before opening the device.
- During rinsing or manual cleaning, care should be taken to avoid splashing and creation of aerosols and to keep instruments low in the sink.

### CONCLUSION

It is strongly recommended that manufacturers of medical device cleaning agents provide a clear and detailed use description, including the relevant safety precautions, to the users and include safety messages on labels as well. In case any changes are made to the product, a new risk assessment needs to be made and communicated if necessary.
Regarding safety for workers when enzyme containing reagents are formulated, please see A.I.S.E. Guidelines for the Safe Handling of Enzymes in Detergent Manufacturing (3).

## EXAMPLE 2

**Risk assessment and risk management of enzyme containing cleaning spray products**

Background: When enzyme containing spray products for household cleaning were launched, a concern was raised whether the aerosols generated when using the spray product would be able to sensitize the users. To meet this concern, a combined clinical- and exposure study was conducted in order to evaluate the potential risk of sensitisations (8).

The exposure study was simulated with a very heavy-use scenario in a controlled laboratory environment. The product was applied to a series of fabric targets held vertically over a standard washing machine. Eight replicates of the experiment were done, using 30 sprays for each replicate. Airborne particle distributions in the breathing zone were characterized using a TSI particle analyzer. Enzyme

concentrations in air were measured using ELISA for analysis of the PTFE membrane filters. Results indicated that aerosol concentrations returned to baseline within 10 min, during which the average enzyme concentration in air was 17 ± 1.6 and 12 ± 0.92 ng/m³ using low- and high-volume samplers, respectively.

The clinical study was a 6-month, controlled-use study involving approximately 100 subjects with confirmed atopic status by skin prick testing with common aeroallergens. Atopic persons will have a higher risk of developing sensitization than non-atopic persons, hence, as only atopic persons are included the study represents a worst-case scenario. The study involved daily exaggerated use of the pre-spotter product for 6 months, with prick testing for the enzyme carried out at baseline, 3 and 6 months. Results from the clinical study indicated that none of the subjects exhibited reactions that would indicate sensitisation to the enzyme by inhalation.
Because of the combination in this study of exposure levels and clinical outcome, the study provides a benchmark of 15 ng/m³ for acceptable exposure levels for enzyme containing spray products.

Within EU, enzyme containing spray products for household cleaning must meet various REACH requirements and suppliers of enzyme products, as well as downstream users, have obligations related to these requirements. A product specific exposure scenario must be generated documenting that the enzyme exposure during use will be below the acceptable exposure limit. Specific guidance how to provide data for such specific exposure scenario is described in (7).

## RISK ASSESSMENT

Analysis of habits and practices in the use of household cleaning spray products: The combined clinical- and exposure studies took into consideration a very exaggerated use of a spray product. If the analysis of habits and practices of a specific spray product does not show a different behavior, then the conditions used in the guideline (7) can be seen as worst-case condition covering most of the habits and practices within this use.

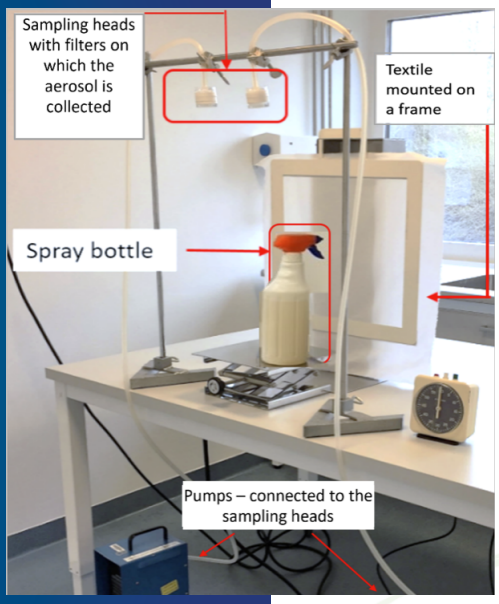
Hazard Identification: Enzymes can act as respiratory sensitizers. At high concentrations, proteolytic enzymes can also irritate skin and eyes.

Dose Response: The acceptable exposure limit for professionals will be 15 ng/m³ as stated in (4), (7) and (8).

Exposure: Enzymes exposure of professionals derived from use of household cleaning spray products must be evaluated to demonstrate safety prior to marketing. The level of exposure generated by a spray product is dependent on several parameters, e.g., formulation, enzyme concentration in product, temperature, nozzle design, habits and practices of the user, and the target surface and the distance from the spray to the target which will determine the impact velocity. The interaction of these parameters has been shown to play an important role influencing the levels of airborne enzyme during spray applications. Hence, a more concentrated product regarding enzyme inclusion in combination

with an appropriate delivery method and formulation may generate exposure well below the acceptable exposure limit, whereas a less concentrated product with an inappropriate delivery method may generate exposure several times above the acceptable exposure limit. Also, high viscosity formulations and foam-sprays would be expected to generate lower enzyme exposure than liquid formulations of low viscosity. However, each product and application of use will need an individual safety assessment based on actual exposure data, independent of such considerations. For more information on the exposure assessment the enzyme supplier should be contacted.

*A typical set-up for evaluating the exposure resulting from the use of a specific spray product will look like this :*




The A.I.S.E. guideline (7) describes how to assess enzyme exposure for a laundry pre-spotter spray product applied to fabric under normal use conditions. For other spray applications, the protocol will need to be modified as described above for the actual conditions of use, also considering habits and practices to determine the actual number of sprays and length of use employed by a typical user – which may differ from the use instructions. The test surfaces will also need to be modified to simulate the specific applications for each product under test. Due to the very low DMEL value, the analytical method for determination of enzyme protein accumulated on the air-sampling filters must be sufficiently sensitive.
Risk characterization: Exposure results are presented as an average exposure, calculated based on the performed experiments. Average exposure is compared to the DMEL for consumers: 15 ng/m³. If average exposure is < DMEL, the enzyme containing spray product will be considered safe to use.

### RISK MANAGEMENT

If average exposure is > DMEL, improvements of the enzyme containing spray product will have to be made before it can be considered safe, and after such improvements a new exposure assessment need to document the safety of the improved product.
Improvements could be:

- Reduce enzyme concentration
- Increase viscosity
- Replace spray nozzle

**IMPORTANT**: Each exposure assessment covers the exact product assessed: content, spray bottle and spray device. If any changes are made to any of these parameters, new exposure assessment must follow.

Risk Communication: According to the EU legislations including REACH, it is the enzyme suppliers' obligation to communicate safety for workers and professional users, and the safety information must be communicated further along the supply chain. As part of this, manufacturers of enzyme containing spray products for cleaning must convey the relevant safety information given in the exposure scenarios of each ingredient. The safety guidance and appropriate risk management for end-users (professionals) should be ensured by employers.

### CONCLUSION

Manufacturers of enzyme containing spray products for cleaning must ensure that a risk assessment according to the guidance provided in (7) is successfully conducted before launching the product. In case of any changes made to the product, a new risk assessment needs to be made. It is strongly recommended that manufacturers of spray products for cleaning provide a clear and detailed use description, including potential safety precautions, to the users and include such safety messages on labels as well.

## EXAMPLE 3

**Risk assessment and risk management of enzyme containing reagents for hard surface cleaning**

Background: Hard surface cleaning includes various applications like: floor cleaning, table cleaning, cleaning of walls, etc. It also encompasses various uses of enzyme containing cleaning agents ranging from the professional rinsing of walls in a slaughterhouse to the wiping of a small table. In any case, there is a need for a very specific risk assessment of the specific product and the specific application. This risk assessment needs to take into consideration all possible habits and practices and all potential exposure scenarios. Every application and every product are unique and therefore the risk assessment will have to be designed specifically in each case.

An example of risk assessment of a floor cleaning product is given below.

## RISK ASSESSMENT

Analysis of habits and practices in the use of enzyme containing floor cleaning product: According to the use directions, the floor cleaning product was diluted in water, applied to the floor, scrubbed while still wet, dried and swept.
Several variables were identified regarding type of floor material, brush used for sweeping, inter-personal variation, etc. These variables were to the extent possible taken into consideration in the study design.

Hazard Identification: Enzymes can act as respiratory sensitizers. At high concentrations, proteolytic enzymes can also irritate skin and eyes. .

Dose Response: The acceptable exposure limit for professionals will be 15 ng/m³ as stated in (4).

Exposure: A down-scaled laboratory exposure study was designed and conducted to address potential airborne exposure to enzyme during use of the product.
The product was to be primarily used on tile floors; hence, two types of tile floors were included in the study: un-glazed ceramic surface and un-glazed anti-skid ceramic surface. To account for over-dosing of the product during the dilution phase, additional enzyme was added to the product to target double dose of enzyme in the study.
The tiles were wetted with the weighed-out amount of the diluted product (added additional enzyme), and scrubbing with a very stiff brush was conducted for 30 min. After this, the tiles were left overnight to dry. Next step was the brushing of the now dried tiles for 30 min using a medium soft brush. See **Fig. 1**.

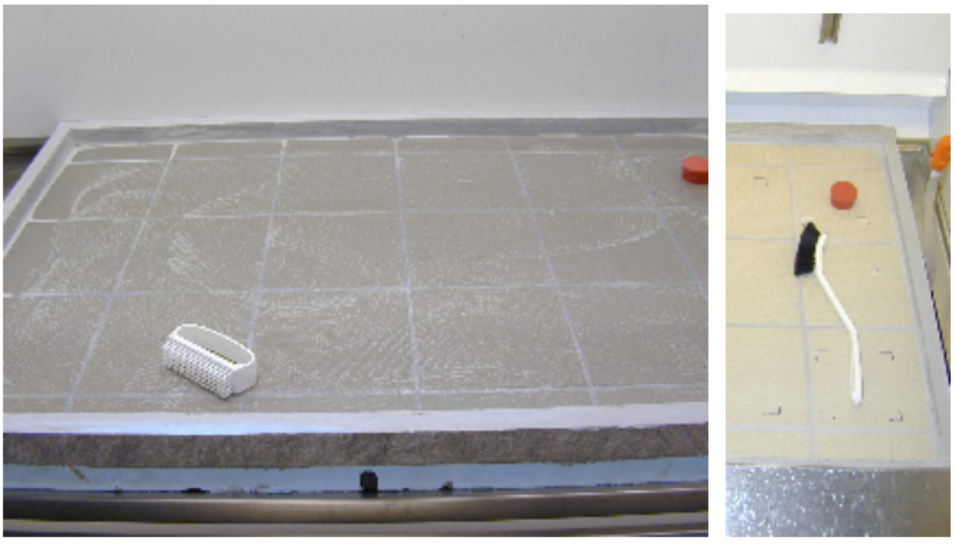


*Fig. 1. Two types of tiles were used : a stiff brush for wet scrubbing of the tiles and a medium soft brush for brushing the dried tiles.*

During the 30 min of either scrubbing or brushing the tiles air sampling was conducted by four samplers collecting potential airborne enzyme on Teflon filters 75 cm above the tiles. See **Fig. 2**.

*Fig. 2. Four sampler heads collecting air 75 cm above the tiles being either scrubbed or brushed.*

To account for potential accumulation of enzyme material on the tiles, each round of applying diluted product, scrubbing, drying, and brushing was repeated 14 times, and a background measurement was conducted in between each round to ensure that no background exposure would interfere with the results obtained. All filters were analysed using ELISA technique.
Exact enzyme concentration in each diluted sample was determined using ELISA technique.

Risk characterization: Measured exposures were all well below the limit of 15 ng/m³. No difference in exposure was seen between the two types of tiles, and no build-up of enzymes during the 14 repeated rounds of applying product, scrubbing, drying and brushing was registered.

## RISK MANAGEMENT

Risk Control: The measured enzyme exposure levels in the study described above were all below the DMEL limit of 15 ng/m³. However, as this study was a downscaled laboratory study, the results obtained would have to be confirmed by a real scale study in which various use scenarios are included.
In case the exposure results had shown exposures above the limit of 15 ng/m³, product adjustments would have been needed, e.g. reducing enzyme concentration, changing product formulation, etc. Again, the effect of such product adjustments would have to be tested in a new exposure study.

Risk Communication: According to the EU legislations including REACH, it is the enzyme suppliers' obligation to communicate safety for workers and professional

users and the safety information must be communicated further along the supply chain. As part of this, manufacturers of enzyme containing hard surface cleaning products must convey the relevant safety information given in the exposure scenarios of each ingredient. The safety guidance and appropriate risk management for end-users (professionals) should be ensured by employers.

### CONCLUSION

Manufacturers of enzyme containing hard surface cleaning products must ensure that a thorough risk assessment is successfully conducted before launching the product. In case of any changes made to the product, a new risk assessment needs to be made and communicated if necessary. It is strongly recommended that manufacturers of hard surface cleaning agents provide a clear and detailed use description, including the relevant safety precautions, to the users and include safety messages on labels as well. In case personal protective equipment like respiratory protection is needed during use, this information needs to be clearly communicated to the user.

## EXAMPLE 4A

**Risk assessment and risk management of enzyme containing reagents for manual dishwashing**

Background: Professionals will do manual dishwashing in various situations. This can take place in restaurant kitchens or hospital kitchens where relative harsh conditions, like flushing of the dishes, occur or the dishwashing situation can be very similar to the situation in a private home. Therefore, there is a need to investigate the exact conditions for which the manual dishwashing product is recommended for use and to take this into consideration when designing the risk assessment. Also, potential non-intended uses need to be considered in the risk assessment. One particular non-intended use is the use of manual dishwashing agents for bubble blowing by children. This particular non-intended use is mostly relevant for consumer (i.e., non-professional) use of manual dishwashing agents. However, in case this is a foreseeable non-intended use of the specific product, the enzyme concentration in the products should not exceed 0.015% active enzyme protein (AEP) a label should explain that the product is not to be used for bubble blowing (9).

An example of risk assessment of a manual dishwashing product for professional use is given below.

### RISK ASSESSMENT

Analysis of habits and practices in the use of enzyme containing manual dishwashing product: The directions of use of the product were studied and a laboratory scale exposure study mimicking the use conditions was designed. It appeared that a number of potential exposure scenarios had to be addressed: various enzyme concentrations in the dishwashing product, different water pressures during use, dishwashing under running tap water, scrubbing, and potential enzyme residuals in dried-in pot scourer. These variables were, to the extent possible, taken into consideration in the study design.

Hazard Identification: Enzymes can act as respiratory sensitizers. At high concentrations, proteolytic enzymes can also irritate skin and eyes.

Dose Response: The acceptable exposure limit for professionals will be 15 ng/m³ as stated in (4).

Exposure: A down-scaled laboratory exposure study was designed and conducted to address potential airborne exposure to enzyme during use of the product.

*Filling a dishwashing bowl:*
Dishwashing product and enzyme was dosed into a small container and placed in the dishwashing bowl. Three enzyme doses (normal, 5x, and 10x normal) were investigated to account for potential overdosing. 8 liters of water were added where the water pressure during adding was either 0.3, 0.5 or 0.7 bar, where 0.3 bar is considered the general choice by the user. After filling the bowl, the bowl was emptied. The filling and emptying of the bowl were repeated 8 times which took approx. 20 min and, during this period, exposure was measured above the bowl using two samplers of 25 L/min, followed by ELISA analysis of the sampling filters. See **Fig. 3**.

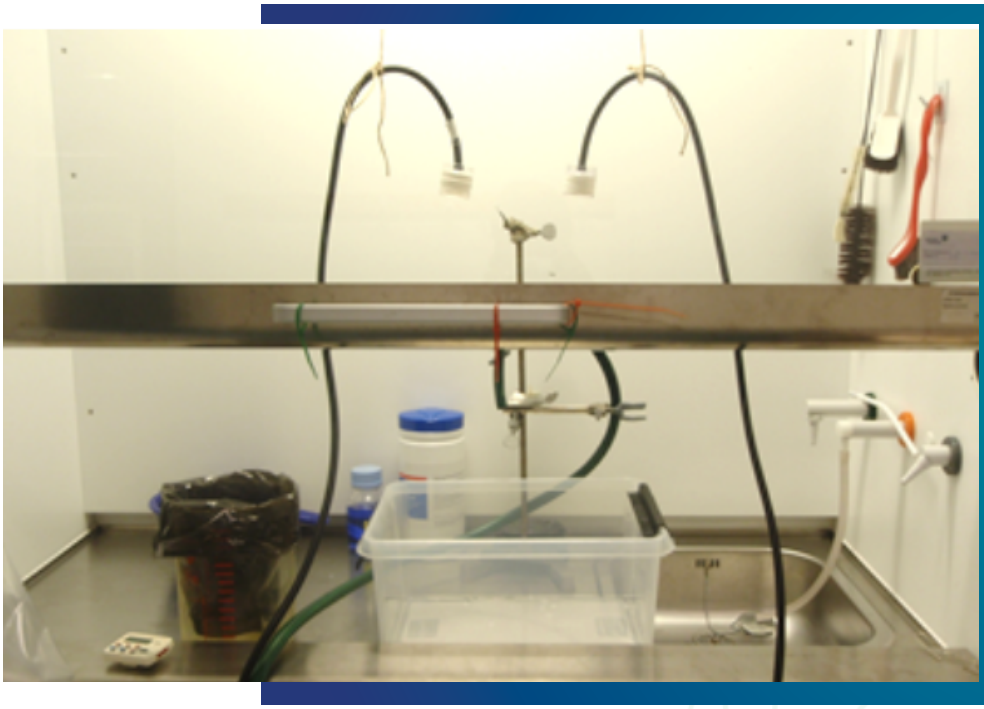


*Fig. 3.*

Risk characterization: Measured exposures were in all cases well below the limit of 15 ng/m³.

*Dishwashing under running tap water:*
Dishwashing product and enzyme was dosed into a small container and placed in the sink. 5x and 10x normal enzyme dose were investigated. Tap water (0.3 bar) was added. A stiff dishwashing brush was dipped into the enzyme containing dishwashing product and rubbed vigorously against the walls of the sink. The

procedure was repeated 8 times in a period of 20 min. Exposure was measured above the sink using two samplers of 25 L/min followed by ELISA analysis of the sampling filters. See **Fig. 4**.

*Fig. 4*

Risk characterization: Measured exposures were in all cases well below the limit of 15 ng/m³.

*Scrubbing Cutlery:*

Dishwashing product and enzyme was dosed into a small container and placed in the dishwashing bowl. Normal, 2x, 5 x and 10x normal enzyme dose were investigated. 3 L tap water (moderate to high pressure) was added to the bowl as well as various cutlery and plates. A stiff dishwashing brush was used for scrubbing the cutlery and plates which took place over a period of 20 min during which exposure was measured. Exposure was measured above the bowl using two samplers of 25 L/min followed by ELISA analysis of the sampling filters. See **Fig. 5**.

*Fig. 5*

Risk characterization: Measured exposures were in all cases well below the limit of 15 ng/m³.

*Dried-in pot scourer:*
A solution of dishwashing product with >100x normal enzyme dose was prepared. Ten pot scourers were soaked into the dishwashing solution and left to dry overnight. On the next day, the pot scourers were vigorously compressed every 5 second for 15 minutes, while measuring exposure. Exposure was measured above the pot scourers, using four samplers of 25 L/min, followed by ELISA analysis of the sampling filters.. See **Fig. 6**.

*Fig. 6.*

Risk characterization: Measured exposures were in all cases well below the limit of 15 ng/m³.

## RISK MANAGEMENT

Risk Control: The measured enzyme exposure level in the study described above were all below the DMEL limit of 15 ng/m³. In case the exposure results had shown exposures above the limit of 15 ng/m³, product adjustments would have been needed, e.g. reducing enzyme concentration, changing product formulation, etc. The effect of such product adjustments would have to be tested in a new exposure study.

Risk Communication: According to the EU legislations including REACH, it is the enzyme suppliers' obligation to communicate safety for workers and professional users and the safety information must be communicated further along the supply chain. As part of this, manufacturers of enzyme containing dishwashing products must convey the relevant safety information given in the exposure scenarios of each ingredient. The safety guidance and appropriate risk management for end-users (professionals) should be ensured by employers. users (professionals) should be ensured by employers.

### CONCLUSION

Manufacturers of enzyme containing dishwashing products for manual dishwashing must ensure that a thorough risk assessment is successfully conducted before launching the product. It should include habits and practices of use, as well as foreseeable non-intended uses. In case of any changes made to the product, a new risk assessment needs to be made and communicated if necessary. It is strongly recommended that manufacturers of manual dishwashing agents provide a clear and detailed use description, including the relevant safety precautions, to the users and include safety messages on labels as well. In case personal protective equipment like respiratory protection is needed during use, this information needs to be clearly communicated to the user.

## EXAMPLE 4B

**Risk assessment and risk management of enzyme containing reagents for professional dishwashing.**

Background: Professional dishwashers come in different sizes and can use different modes of operation. The single-tank dishwasher typically uses a batch-wise process, whereas the bigger multi-tank dishwasher operates in a continuous mode.

In single-tank machines, the rack containing the dishes stays in a fixed position during the entire washing cycle. A circulation pump is connected with the wash tank and flushes the detergent solution through rotating spray arms over the dishware. This circulation typically takes about 1 minute. At the end of this period, rinse aid containing fresh hot water is sprayed over the dishware. Single-tank machines are usually equipped with a hood, which can be opened at any time during the washing cycle. An automatic valve then switches off the water circulation and protects the operator from being sprayed by hot and usually alkaline solutions (10). However, the opening of the hood during a washing cycle may result in exposure to enzyme aerosols.

Another type of dishwasher using a batch-wise process is the CADW dishwasher (Commercial Automatic Dishwasher). This type of dishwasher is often found in smaller professional settings like cafés or minor restaurants. Like the dishwashers usually installed in private homes, the CADW is often placed underneath a kitchen table and opens to the outside with a door. Dosing is often done automatically via a pump. The special characteristic of the CADW compared to the household dishwasher corresponds to the very short washing cycle. The washing cycle of the CADW only lasts for a few minutes. This poses a risk of enzyme exposure from the vapor that is released when opening the door after a washing cycle.

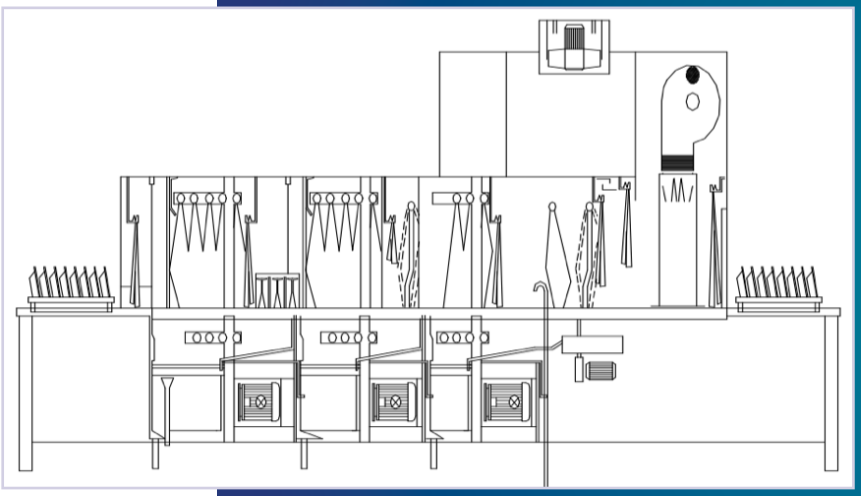


***Fig. 7*** *shows a typical multi-tank machine, containing different zones for washing, rinsing, and drying the dishware. Hot, rinse aid containing fresh water is introduced into the rinse zone and cascades into one or more wash zone(s) before it enters the drain. A conveyer belt transports the dishware in counter-current direction from (pre-)wash to drying zone. (10)*

The mechanical action in this type of dishwasher is usually very high. Flushes in the order of 1000 litres wash solution per hour over the dishware are not exceptional. This high mechanical action forms aerosols inside the machine. Since both the entrance and exit of the multi-tank machines are open during operation, aerosols may be released and inhaled by the kitchen personnel. Moreover, each tank can be accessed through a door at the side of the machine. For some machines, opening the door causes the machine to switch off immediately. For others, the machine needs to be switched off manually at a control panel. Upon opening a side-door, the machine operator can potentially be exposed to high levels of aerosol. Multi-tank dishwashers exist with either internal air circulation or ventilation of air from inside the machine to outside the building (10).

Some examples of risk assessments of the use of enzyme containing products for professional dishwashing are given below.

## RISK ASSESSMENT

Analysis of habits and practices and other factors influencing the potential level of enzyme exposure: As described in the background section, habits and practices are important parameters when assessing the risk of enzyme exposure during industrial dishwashing. The operators might open the hood of the single-tank dishwasher, or open the doors of a multi-tank dishwasher while running. This may result in potential enzyme exposure.
Other factors that will influence the level of potential enzyme exposure could be: if the dishwasher is not fully functional (e.g. the curtains at the end of a multi-tank dishwasher are missing), whether the room holding the dishwasher is ventilated or not, whether the dosing of the dishwashing product is controlled or not, and whether the operators are well trained.

Hazard Identification: Enzymes can act as respiratory sensitizers. At high concentrations, proteolytic enzymes can also irritate skin and eyes.

Dose Response: The acceptable exposure limit for professionals will be 15 ng/m³ as stated in (4).

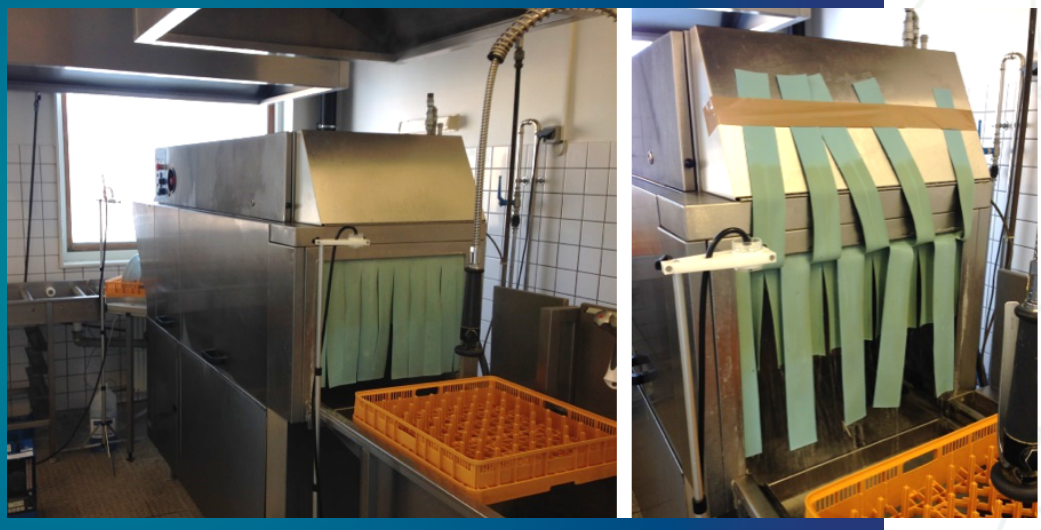
Exposure: Several exposure studies have been conducted on both multi-tank dishwashers, as well as single-tank dishwashers and CADWs. Some of these studies will be summarized below.

*Multi-tank dishwasher normal use and opening of doors:*
This study was conducted by an A.I.S.E. task force and it is published in the HERA document (10).
In this study, enzyme exposure close to the dishwasher was measured during normal use and during non-intended use where a side was opened 10 times. The sampling time was either 30 or 60 min, enzyme dose ranged from 10 to 200 mg/L Termamyl 300L, and filters were analyzed using ELISA technique

Risk characterization: Measured exposures were in all cases well below the limit of 15 ng/m³.

*Multi-tank dishwasher normal use with intact and broken curtains:*
Exposure was measured at a multi-tank dishwasher placed in a canteen kitchen. The room was ventilated. To mimic a situation where part of the curtains at the end of the dishwasher is missing, half of the curtain strips were removed by fixing them to the upper part of the dishwasher. See Fig. 8. Enzyme dose was fixed and below 0.44 ppm active enzyme protein in the final wash solution. Exposure was measured at the entrance and at the exit of the dishwasher using two samplers of 25 L/min, followed by ELISA analysis of the sampling filters.

*Fig. 8.*



Risk characterization: Measured exposures were in both cases well below the limit of 15 ng/m³.

*Single-tank dishwasher normal use and opening of the hood, with and without ventilation:*

Enzyme exposure was investigated, using increasing enzyme concentration with ventilation turned on, and during worst case with no ventilation on.

Dosage of detergent, enzyme products and ballast (a blend of food soils) was done manually to the "sump" prior to start-up. After each round of cleaning, 2.5 L water were drained from the "sump" and replaced with 2.5 L fresh water. Extra detergent, enzyme product and ballast were added to the "sump" after each cleaning cycle to keep a steady state concentration in the dishwasher. Three levels of enzyme concentration were used where the ventilation was turned on: low, medium, and high. The ventilation would ensure changing the air in the room approx. 16 times per hour. In the part of the study where the ventilation was turned off, only the low enzyme concentration was used. In this part, the hood was also opened twice during washing.

Air-sampling took place on each side of the dishwasher simulating operator exposure, and at the outlet of air from the inside of the hood. Air-samplers of 25 L/min were used followed by ELISA analysis of the sampling filters. See **Fig. 9**.

*Fig. 9.*

Risk characterization: For that part of the study where ventilation was turned on, a clear impact of the increasing enzyme concentration was observed. Raising the enzyme concentration from low to high increased the exposure from below detection limit to 10 ng/m³. Worst case conditions using the low enzyme concentration, opening the hood twice during the washing cycle, and no ventilation showed enzyme exposures of 2 ng/m³.

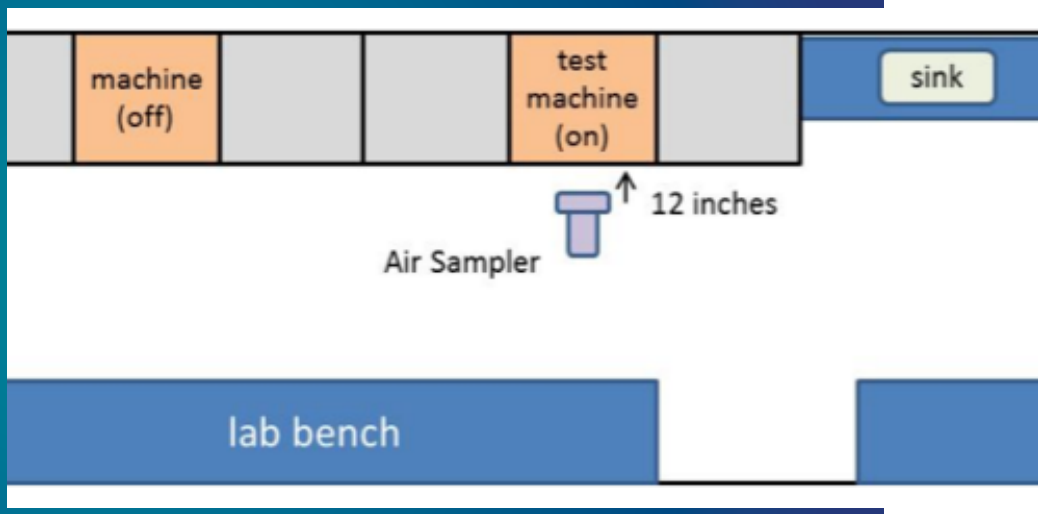
*Single-tank dishwasher operated at high temperature:*

In this high temperature exposure study, the sampler was positioned 12 inches (30.5 cm) from the machine door and at five feet (152,4 cm) height to be at an operator's breathing zone. See **Fig. 10**.

Each test consisted of 8 cycles, during which air samples were collected. Each

cycle consisted of a wash with dishwashing product at 3ppm enzyme wash level, followed by a rinse. The wash and the rinse lasted in total for 50 seconds, after which the door was opened and a 15 second pause took place. The pause was to simulate time between removing dish racks and adding new dishes for cleaning. Each test was preceded by a room air cleanout with high powered fans and a room background check with air collection for 10 minutes without the machine running. Three tests of each 8 cycles were made. No information is available regarding room ventilation during the 8 cycles.

*Fig. 10.*




Risk characterization: Overall, airborne enzyme was detected in all the three tests. Measured levels for each test range from 20 – 31 ng/m³ and are therefore above the DMEL of 15 ng/m³. Observation of the machine in use showed aerosol emissions at all times during operation. The largest volume of emissions is seen when the door is opened. Due to the very short cycle time, these door opening emissions occur 8 times in 10.5 minutes. The data and the observations clearly indicate the need for further product development to reduce the exposure before launching this high temperature dishwashing product.

*CADW operated at low temperature:*

Exposure was measured during operating a CADW machine at low temperature. In this exposure study, slightly extended rinse & washing cycles were used (120-300 seconds) when compared to the previous high temperature single-tank dishwasher study. Additional sanitizers were also added. The sampler was positioned 1 ft (30.5 cm) from the intercept between the door of the CADW and the service table, and at five ft (152,4 cm) height to be at an operator's breathing zone. See **Fig. 11**.

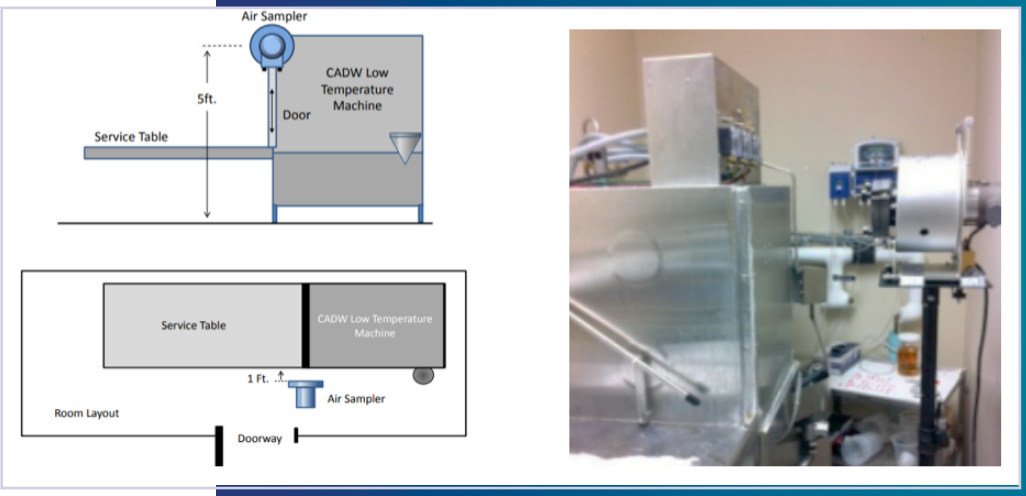



*Fig. 11.*

Exposure was measured in three tests, where each test consisted of 7 washing cycles using 3 ppm enzyme wash concentration. A room background air measurement (no machine running) was also done prior to each test to ensure that no background exposure could be detected at the start of a test. No information is available regarding room ventilation during the 7 cycles.

Risk characterization: The range of enzyme exposure level measured for the three tests was found to be 3.0 – 4.3 ng/m³ and are thus well below the DMEL of 15 ng/m³.

## RISK MANAGEMENT

Risk Control: The measured enzyme exposure level in the studies described above were all below the DMEL limit of 15 ng/m³, except for one study.
From a collection of independent studies (internal references), more than 100 air-samples show that the absolutely most important parameters for achieving an exposure below the DMEL are the enzyme concentration in the final washing solution and the presence of ventilation.
When efficient ventilation is applied, no exposure above the DMEL was observed among the 75 data points representing exposure where ventilation has been applied. Among these data points, the highest enzyme concentration in the final washing solution was 5.5 ppm active enzyme protein. In the absence of ventilation, it appears that the enzyme exposure will be highly dependent on the enzyme concentration in the final washing solution. In 16 out the 26 data points representing exposure where ventilation has not been applied, the DMEL is exceeded where the used enzyme concentration is high. **From these data, it appears that the enzyme concentration in the final washing solution should be below 1.5 ppm active enzyme protein to achieve an exposure below the DMEL.**
However, more specific exposure studies will be required for each enzyme containing product for professional dishwashing to establish the safe inclusion level for the specific product.

Risk Communication: According to the EU legislations including REACH, it is the enzyme suppliers' obligation to communicate safety for workers and professional users and this safety information must be communicated further along the supply chain. As part of this, manufacturers of enzyme containing products for industrial dishwashing must convey the relevant safety information given in the exposure scenarios of each ingredient. The safety guidance and appropriate risk management for end-users (professionals) should be ensured by employers.

## CONCLUSION

Manufacturers of enzyme containing products for professional dishwashing must ensure that a thorough risk assessment is successfully conducted before launching a product. This includes assessing habits and practices of use, as well as foreseeable non-intended uses. In case of any changes made to the product, a new risk assessment needs to be made. In case personal protective equipment like respiratory protection is needed during use, this information must be clearly communicated to the user. It is strongly recommended that manufacturers of professional dishwashing products provide a clear and detailed use description, including the relevant safety precautions to the users, and include safety messages on labels as well.

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# Chapter 6

## GLOSSARY

**Aerosol** — Small airborne solid or liquid particles suspended in air, i.e. dust or mist.

**Allergen** — A substance that specifically induces the production of allergic antibodies.

**Amylase** — A class of enzymes that speed up the breakdown of the chemical bonds between the connecting sugar molecules in starch.

**Antibody** - Molecule which mediates an immune response following induction by an antigen.

**Antigen** – Molecule that can bind to a specific antibody and which may trigger an immune response.

**Atopic** – Showing a predisposition to respond immunologically to diverse antigens/ allergens.

**CoRAP**— Community Rolling Action plan – evaluation established by the European Commission as part of the REACH Regulation.

**DMEL**— Derived Minimal Effect Level – An exposure limit used by ECHA in REACH registration.

**Detergent** — A mixture of surfactants, builders, bleach and other chemicals used to facilitate cleaning.

**ECHA** — European Chemical Agency, based in Helsinki, Finland.

**ELISA** — A sensitive laboratory immunoassay for detection of antibodies or quantitation of antigen, widely used in biology and medicine.

**Encapsulation** — A chemical coating applied to an enzyme granule to reduce the potential for dust generation.

**Enzyme** — Molecule that helps speeding up chemical reactions.

**Protease** — A class of enzymes that speed up the breakdown of the chemical bonds between connecting amino acids in proteins.

**Protein** — A class of chemical compounds found in plant and animal cells. Proteins are made up of long chains of amino acids.

**Proteolytic** – Able to break down proteins.

**RAST (Radio Allergo-Sorbent Test)** — A sensitive laboratory test used for detecting and measuring antibodies in the blood of persons exposed to allergens, widely used in allergy clinical work.

**REACH** — Registration, Evaluation, and Authorisation of Chemicals – principal legislation on chemicals in the European Union (EU).

**Respiratory allergy** — An immunological condition acquired through exposure to a substance (allergen) that results in an enhanced, adverse reaction to the substance upon re-exposure. Allergies to enzymes, as with other proteins, are mediated by allergic IgE antibodies. Symptoms of enzyme allergies may include any or a combination of the following: sneezing; nasal or sinus congestion; coughing; watery and itchy eyes or nose; hoarseness or shortness of breath; and asthma. Symptoms not typically observed with allergy to enzymes include digestive upset, urticaria and atopic dermatitis.

**Rhinitis** — An inflammation of the nasal mucosal membrane that can be caused by irritation or by an allergic response. Rhinitis is characterized by runny nose with or without itching, watery eyes, sneezing and congestion.

**Sensitisation** — The stimulation of the immune system by an allergen that leads to the development of allergic antibodies to the allergen. This is not a disease. See induction of allergy, which is defined as the development of allergen specific IgE antibodies that specifically bind to the allergen when it is present.

**Skin Prick Test** — An in vivo technique for detecting allergic antibodies in persons exposed to specifAn in vivo technique for detecting allergic antibodies in persons exposed to specific allergens. The test consists of pricking the superficial layer of the skin with a solution of the allergen. In an individual with allergic antibody, the allergen binds to the allergic antibodies on the mast cell leading to the release of mediators such as histamine. A raised reddened area with surrounding erythema (wheal and flare) will appear on the skin.

**Subtilisin** — A protease derived from Bacillus subtilis or closely related species.


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