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Enzymes in consumer products

An inventory of non-food products, regulatory frameworks, hazards and considerations for risk assessment

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J. Kettelarij et al.

Colophon

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Synopsis

Enzymes in consumer products

An inventory of non-food products, regulatory frameworks, hazards and considerations for risk assessment

Enzymes are increasingly being added to consumer products such as cleaning products and personal care products. This is done to dissolve dirt or stains, for example. The Netherlands Food and Consumer Product Safety Authority (NVWA) wants to know whether the use of enzymes is safe, especially in new types of products. RIVM has therefore made an overview of the products containing them and which regulatory frameworks apply to them. It was also examined whether it is possible to assess whether the use is safe.

The overview makes it clear that there is insufficient information available about the amount of enzymes in certain products and about the amount that is safe for specific enzymes. It is also not clear to what extent consumers are exposed to enzymes. As a result, there is not enough information to assess whether the use of products containing enzymes is safe. This research helps to make recommendations for future evaluation of enzymes in consumer products.

The inventory identified 184 cleaning products, 46 personal care products, 12 veterinary hygiene products and 2 pet care products containing enzymes. The products are subject to various regulatory frameworks, such as for personal care products or for cleaning products. The type of enzymes used and the amount were usually not stated on the packaging. In addition, a survey among manufacturers showed that most would not share their product enzyme concentrations. They do expect to use more enzymes in the future.

The main health effect that enzymes can cause is respiratory sensitisation. This effect can arise, for example, if consumers are exposed to enzymes through air when using sprays.

RIVM conducted this study on behalf of the Netherlands Food and Consumer Product Safety Authority (NVWA). It is a follow-up to the earlier study into microbial cleaning products.

Keywords: enzymes, consumer products, respiratory sensitisation, consumer exposure, detergents, cosmetics

Publiekssamenvatting

Enzymen in consumentenproducten

Een inventarisatie van non-food producten, wetgeving, gevaarseigenschappen en overwegingen voor risicobeoordeling

Enzymen worden steeds vaker toegevoegd aan producten voor consumenten, zoals schoonmaakmiddelen en persoonlijke verzorgingsproducten. Dit wordt gedaan om bijvoorbeeld vuil of vlekken op te lossen. De Nederlandse Voedsel- en Warenautoriteit (NVWA) wil weten of het gebruik van enzymen veilig is, vooral in nieuwe soorten producten. Het RIVM heeft daarom een overzicht gemaakt van de producten waar ze in zitten en welke wetten daarvoor gelden. Ook is gekeken of het mogelijk is om te bepalen of het gebruik veilig is.

Het overzicht maakt duidelijk dat er onvoldoende informatie beschikbaar is over de hoeveelheid enzymen in bepaalde producten en welke hoeveelheid voor specifieke enzymen veilig is. Ook is niet duidelijk in welke mate consumenten aan enzymen blootstaan. Daardoor is er ook niet genoeg informatie om te kunnen beoordelen of het gebruik van producten met enzymen veilig is. Dit onderzoek helpt om aanbevelingen te doen voor een toekomstige evaluatie van enzymen in producten voor consumenten.

In ons marktonderzoek zijn er 184 schoonmaakproducten met enzymen gevonden, 46 producten voor persoonlijke verzorging, 12 veterinaire hygiëneproducten en 2 huisdierverzorgingsproducten. De producten vallen onder verschillende wetten, zoals voor persoonlijke verzorgingsproducten of voor schoonmaakmiddelen. Het gebruikte type enzymen en de hoeveelheid stonden meestal niet op de verpakking vermeld. Ook bleek uit een enquête onder producenten dat de meeste hun enzymenconcentraties niet konden delen. Wel verwachten ze in de toekomst meer enzymen te gaan gebruiken.

Het belangrijkste effect op de gezondheid dat enzymen kunnen veroorzaken is sensibilisatie van de luchtwegen. Dit effect kan bijvoorbeeld ontstaan als consumenten bij het gebruik van sprays via de lucht aan enzymen worden blootgesteld.

Het RIVM heeft dit onderzoek gedaan in opdracht van de NVWA. Het is een vervolg op de eerdere studie naar microbiële reinigingsmiddelen.

Kernwoorden: enzymen, consumentenproducten, sensibilisatie van de luchtwegen, consumentenblootstelling, detergenten, cosmetica

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Introduction

Enzymes are, according to the Collins dictionary, "any of a group of complex proteins or conjugated proteins that are produced by living cells and act as catalysts in specific biochemical reactions". In detergents, they are for example used to help dissolving stains of proteins, fat, or starch. Enzymes are the key to an efficient and good washing or cleaning product. They have been used in some household products since 1913, when the first washing powders containing enzymes for soaking were introduced to the market (Maurer, 2004). The popularity of these washing powders slowly increased over time. With a growing consumer demand on reduction of chemical substances, the range of enzymes in consumer products broadened, as well as their area of application, including cleaning products and personal care products. Thanks to the increasing knowledge of adverse effects of substances in general, it became clear in the 1970's that enzymes, specifically proteases, could cause respiratory sensitisation, as well as irritant effects (Basketter et al., 2012a; Vanhanen et al., 2000). Regulations were adapted in response to this knowledge, to minimize potential exposure of consumers and workers. However, use of enzymes and consumer's habits have changed the past decades, and little information is available on the concentrations in products, exposure routes and potential risks. The question arises whether the use of enzymes, especially when introduced in new applications, is safe.

Since the industrialisation of household products, products that were initially intended for industry started to be used for household tasks and stains. Formulation of these products evolved with the knowledge, the regulations, and the expectations of the consumers. Since the beginning of the 21st century, there is a trend towards introducing enzymes into new uses and product types. This trend is driven by development of the sustainability agenda and progress in biotechnology (Nicholson, 2022). The formulation of so-called "green" household products has evolved from synthetic to more natural based products. Within this natural based formulation, enzymes are increasingly used since the first production of washing products (Novozymes, 2020).

In 2020, RIVM conducted a study on the use and risks of microbial cleaning products (Razenberg et al., 2020) on behalf of the Office for Risk Assessment & research of the Netherlands Food and Consumer Product Safety Authority (NVWA-BuRO). The use of enzymes in those products was outside the scope of that project, but was indicated as point of attention for further research. It has become the focus of the current project, which is performed upon a successive request by NVWA-BuRO. Based on information from the project on microbial cleaning products, the focus in the current project is on cleaning products and personal care products. The project aims at:

- providing an overview of current regulations / relevant legal frameworks and existing evaluations related to the safe use of enzymes in non-food consumer products;

- providing an explorative overview of non-food consumer products that contain enzymes, and assessing the types of enzymes used in these products;
- determining the potential hazard and evaluating exposure of consumers to enzymes; and
- understanding potential risks for consumers.

Chapter 2 of this report provides an introduction on enzymes. It also contains a summary of the overview of current regulations and relevant legal frameworks. A detailed overview of the regulatory frameworks is given in Annex 1. An explorative overview of non-food consumer products containing enzymes is given in Chapter 3 (through market research) and 4 (through a questionnaire). Chapter 5 gives information on hazard, chapter 6 on exposure. Considerations for risk assessment can be found in chapter 7. Chapter 8 provides summarizing information and recommendations.

In this project, the phrase "products that contain enzymes" covers products that (claim to) contain single enzymes or enzyme preparations, or products in which enzymes are produced *in situ* by added micro-organisms. Other ingredients of these products are not assessed as these are outside the scope of the project.

1. Enzymes

1.1. What are enzymes?

Enzymes are large proteins composed of polypeptide chains. These chains are composed of amino acids and their sequence determines the folding pattern of the protein structure, which give the enzyme its specific properties (Britannica, 2022). Enzymes act as a catalyst: they regulate the rate of chemical reactions without being altered in the process (see Figure 1). Enzymes can increase the speed of chemical reactions (NIH, 2022). Within these reactions, enzymes digest substrates such as carbohydrates, fats and proteins and transform them into smaller molecules. These smaller molecules might, in the case of detergents, be easily whipped or dissolved in water. Enzymes are very sensitive to fluctuation of temperatures and pH. A change can denature them and make them lose their ability to transform a substrate in a product (Britannica, 2022).

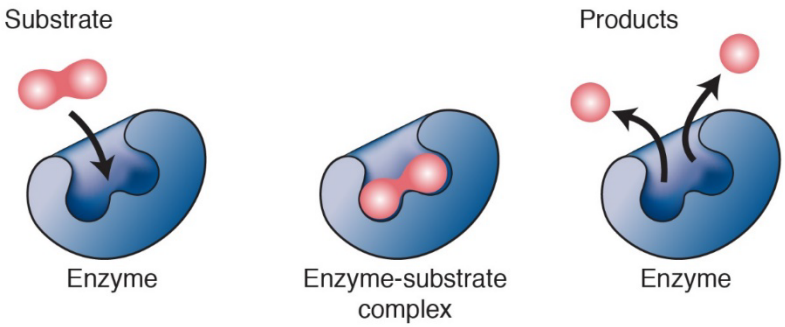

Figure 1 mechanism of enzyme activity. Source: NIH (2022).

1.2. In which context are enzymes used?

The majority of enzymes in non-food consumer products are used in cleaning products such as laundry products, dishwashing products, all-purpose cleaners, kitchen and sanitary cleaners, floor and furniture cleaners, septic tank biodegraders, stain removers, etc. Enzymes are also used in personal care products. Examples are hair care products, products for bathing, showering, skin care, sun care and oral hygiene. Additionally, enzymes can be used in products for veterinary hygiene purposes (e.g. odour control) or pet care (e.g. ointments). Different enzyme types are used for different purposes. The most commonly used enzymes used in cleaning products and personal care products are described in the following paragraphs.

1.2.1. Enzymes in cleaning products

In cleaning products, there are six major enzyme types currently used (Creative Enzymes, 2022; Gürkök, 2019; Malgas et al., 2015; Olsen & Falholt, 1998; Zheng et al., 2021):

- *Proteases:*
  This type of enzyme is used for its capacity to degrade proteins in stains in textiles (e.g. blood, sweat, egg yolk, grass) through proteolysis. It can also be used for example to remove a

proteinaceous food film, which may occur on glassware and cutlery. Proteases are the most widely used enzymes. Examples of proteases are: subtilisin, trypsin, papain, bromelain (the latter two are used in personal care products, not cleaning products).

- *Amylases*
  This type of enzyme facilitates the removal of starch-containing stains (e.g. pasta, potato, gravy, chocolate, baby food). When starch is cooked, it undergoes gelatinization and swelling with the addition of heat and water. These processes make the starch more sensitive to the enzymatic breakdown by amylases. Amylases also prevent swollen starch from adhering to the surface of laundry and dishes that may otherwise act as a glue for particulate soiling. Examples of amylases are alpha-amylase and amyloglucosidase.
- *Lipases*
  This type of enzyme is able to hydrolyse triglycerides (fats and oils) to more hydrophilic mono- and diglycerides, free fatty acids, and glycerol at low temperatures. Several wash cycles are needed to notice the effect of lipases, due to increased activity of the enzyme when laundry dries. Over the years, lipases have been developed that work directly at the first wash cycle.
- *Cellulases*
  This type of enzyme acts against microfibrilles (fibres made of glycoprotein and cellulose) and gives a white effect on fabrics. To achieve this purpose it cleaves β-1,4-glucosidic bonds in cellulose and shaves off the fuzz and pills of cotton fibrils that are generated on fabric by normal wear and washing. In cleaning products, they make cotton fabrics regain and maintain clear colours, a smooth surface, and softness. This effect applies to the natural cotton fibres or cotton/flax blends and on the cellulose portion in synthetic fibres. Endoglucanases and exocellulases are examples of types of cellulases.
- *Mannanases and mannosidases*
  These types of enzymes break down mannans by hydrolysis. Mannans are polysaccharides that bind cellulose in plants. They can have different compositions, structures and complexities. Different enzyme types exist to degrade mannan; e.g. mannanases and mannosidases. They may interfere with each other, and should therefore not be used at the same time. An example of mannanases is beta-mannanase, an example of mannosidase is beta-mannosidase.
- *Pectinases*
  This type of enzyme hydrolyses pectin, a polysaccharide found in fruits and vegetables. The enzymes can therefore be used to remove stains caused by for example fruits, vegetables, sauces and jams. *Examples of pectinases are*: pectate lyase, pectin lyase (pectolyase).

1.2.2. Enzymes in personal care products

In personal care products, enzymes extracted from fruits are often used, for example from pineapple (bromelain) and papaya (papain). According to the CosIng database of the European Commission (https://ec.europa.eu/growth/tools-databases/cosing/), bromelain is used as a keratolytic and skin conditioning agent, and papain as an

antistatic, hair conditioning and skin conditioning agent (EC, 2022a). Enzymes in personal care products are more difficult to identify as the enzymes are not always applied in a pure form. Instead, an extract of the fruit is used directly in a product. Enzymes that are known to be used in personal care products are amyloglucosidase, bromelain, glucose oxidase, lactoperoxidase, lipase, lysozyme, papain, subtilisin and superoxide dismutase. As said, enzymes from natural sources like fruits or honey are often said to be used, which is only apparent from claims on the product label. In those cases, the ingredient list only declares the use of some type of fruit extract, and not the actual enzyme in that fruit extract.

1.3. Production

Due to trade secrets, little is known in detail about how enzymes are produced at an industrial level. However, the major processes are known. There are 3 different processes to produce enzymes: (a) extraction and separation, (b) chemical synthesis, and (c) biosynthesis. During the extraction and separation process, enzymes are extracted from animal or plant tissue. They are separated through grinding, then extracted, filtered, concentrated and purified (Creative Enzymes, 2022). During chemical synthesis enzymes are produced by execution of chemical reactions. Chemical synthesis is a costly process that is not used anymore since biosynthesis has been discovered (Sanchez & Demain, 2011).

Biosynthesis is the most used process to produce enzymes on an industrial level (Novozymes, 2022). The enzymes are produced by microorganisms under controlled conditions. To produce specifically desired enzymes, recombinant DNA techniques can be used (Hasan et al., 2010). Microorganisms can produce abundant quantities of enzymes and they are the most convenient source of commercial enzymes. The types of microorganisms used to produce enzymes are fungi, yeast, bacteria, and actinomycetes , examples of them are described below (Table 1). The production takes place with a process called microbial fermentation. Two different types of microbial fermentation exist: submerged fermentation in liquid broth and solid-state fermentation. After the fermentation, supernatant (for extracellular products) and cell biomass (for intracellular products) are separated. The product is then precipitated, filtered, dried and ready for packaging (Creative Enzymes, 2022). It is not known whether the product can contain residues from the production organisms.

Table 1 Examples of types of organisms used for production of enzymes.

Microorganisms	Organism type	Enzymes produced
Aspergillus oryzae	Fungi	Amylases, Cellulases, Lipases
Saccharomyces cerevisiae	Yeast	Amylases, Invertases
Bacillus subtilis	Bacteria	Cellulases, Pectinases, Phytases, Proteases
Streptomyces sp.	Actinomycetes	Amylases, Isomerases

1.4. Regulatory frameworks

Several regulatory frameworks within the European Union and the Netherlands are relevant for non-food consumer products which can contain enzymes. These include REACH, CLP, the Biocidal Product Regulation (BPR), the Cosmetic Product Regulation (CPR), the Regulation on Detergents, the Genetically Modified Organisms (GMO) legislation, and the General Product Safety Directive and Dutch Commodities Act (Warenwet algemene productveiligheid). In addition, the Chemical Strategy for Sustainability (CSS) strategy is of relevance. A detailed overview of these regulatory frameworks is provided in Annex 1, with special attention to the aim of the framework, the requirements for access to the market, possible requirements with regard to the safety evaluation and quality of the product or substance (i.e. the enzyme) falling under that regulation. Also possible specific requirements for enzymes or microbes as well as possible labelling requirements of the product under the respective regulation are described.

While each regulation has a defined scope, there may be borderline cases where it is not immediately clear to which regulation or legislation a particular product may fall. In such cases, there are several possibilities to elucidate which regulatory framework applies, for example with the use of guidelines, such as the Borderline Manual by the sub-group on borderline products of the working group on cosmetic products (Working Group on Cosmetic Products, 2020) with regard to the CPR, or the guidance on the borderline between the legislation for cosmetics and biocides (EC, 2013). Ultimately, a court ruling can be used in order to determine which regulatory framework and requirements are applicable.

Important conclusions that can be drawn from the overview of regulatory frameworks (Annex 1) are that enzymes are substances that have to be registered under REACH, and are often (self)classified for respiratory sensitisation. Presently, no enzymes have been approved as active substances under the BPR, however, there are cleaning products containing enzymes that could be considered biocidal products, and there are intentions to bring such enzymatic cleaners under the BPR. Under the CPR, there are no provisions for enzymes specifically. Ingredients (including specific enzymes or substances containing enzymes, i.e. extracts) have to be allowed to be used in cosmetic products, and should be indicated on the label. The Regulation on Detergents allows the use of enzymes, and they need to be mentioned on the ingredient list. As enzymes are no organisms, they are not regulated by the GMO legislation, but GMOs that might be used for the production of enzymes are subject of the GMO legislation. When enzymes are produced by GMOs, the final product, being the enzyme preparation, must be free of any residual living GMOs.
Consumer products to which none of the above described regulatory frameworks are applicable fall within the scope of the General Product Safety Directive, which is implemented in the Dutch Commodities Act, providing general rules on public health, product safety, fair trading and adequate information.

2. Market research

This chapter provides an explorative overview of consumer products that contain enzymes, categorized by type of product. In addition, the type of enzymes used in those consumer products is provided. This overview is non-exhaustive and gives an indication of the enzyme-containing consumer products available on the Dutch market. Sources with information from products on other markets, such as the Skin Deep Cosmetics Database (EWG, 2022) or the Consumer Product Information Database (CPID, 2022), were therefore not taken into account.

The overview in this chapter was made by visiting several shops and performing an online search in web shops that deliver products in the Netherlands. The search strategy is described in Annex 2. The main focus was on cleaning products, detergents, washing powders, biocidal products and personal care products. In addition, other relevant products were included if identified during the search (e.g. animal care products). In regard of this list, 'contain enzymes' means that a product has the claim of containing enzymes on the package or the website of the producer or distributor, or states the use of enzymes on their ingredient list (as 'enzymes' or the name of specified enzyme(s)). As a wide range of enzymes is available for use in products, it is possible that some enzymes have gone unnoticed in this search.
Many products lacked the name(s) of the enzyme(s) on the ingredient list. For some products, additional information (e.g. specification of the enzyme(s) in the product) could be found when purposely searched for on other websites or in safety data sheets. It should be noted that advertising websites do not always provide the same information as that declared on the label or packaging of the product.

2.1. Overview of products and enzymes

Table 2 lists the explorative overview of products that were found that contain enzymes. A total of 244 products were identified: 184 cleaning products, 46 personal care products, 12 veterinary hygiene products, and 2 pet care products. If possible, products were categorized according to product types used in the ConsExpo factsheets (Bremmer et al., 2006; Meesters et al., 2018). Laundry products include laundry detergents, stain removers and washing machine cleaners. Miscellaneous cleaning products include drain cleaners, kitchen cleaners, septic tank biodegraders, outdoor cleaners, a few odour control products, a toy cleaner and an air conditioner cleaner. No ConsExpo product type is available for veterinary hygiene and pet care products; these categories are therefore listed under 'other' (Table 2). The veterinary hygiene products include animal housing cleaners and odour control products.

Table 2 Overview of products found that contain enzymes and/or have the claim of use of enzymes.

Product type	Total no. of products containing enzymes[1]	No. of products with details on type of enzyme(s)[2]	No. of enzyme types per product (min-max)[3]	Type of enzymes on ingredient list
Category: cleaning products (detergents)				
Laundry products	99	77	1-6	(Alpha-)amylase Cellulase Lipase Mannanase Pectate lyase Pectinase (pectin lyase) Protease / Subtilisin Xanthan lyase
Dishwashing products	22	20	1-4	(Alpha-)amylase Cellulase Lipase Protease / Subtilisin
All-purpose cleaners	12	1	3	(Alpha-)amylase Lipase Protease / Subtilisin
Sanitary products	4	0	N/A	N/A
Floor and furniture cleaning products	9	1	2	(Alpha-)amylase Protease / Subtilisin
Miscellaneous cleaning products	38	11	1-4	(Alpha-)amylase Cellulase Lipase Protease / Subtilisin
Category: personal care products (cosmetics)				
Hair care	4	0	N/A	N/A
Bathing, showering	2	0	N/A	N/A
Skin care	15	9	1-2	Bromelain Lipase Papain Protease / Subtilisin Superoxide dismutase
Make-up and nail care	5	3	1-2	Glucose oxidase Lactoperoxidase Papain
Sun care cosmetics	3	1	1	Superoxide Dismutase
Oral hygiene	17	16	2-4	Amyloglucosidase Bromelain Glucose oxidase Lactoperoxidase Lysozyme Papain
Category: other				
Veterinary hygiene	12	0	N/A	N/A
Pet care	2	0	N/A	N/A

[1] Combination of the number of products in which the word "enzymes" is on the ingredients list and the number of products on which the type of enzyme is noted
[2] Number of products for which the type of enzyme was provided on the ingredient list

[3] Minimum and maximum number of enzymes found in 1 product; only for products for which the type of enzyme was provided on the ingredient list
N/A: not available

2.2. Chapter summary

The ingredient list of many of the products in the overview did not declare specific information about the enzymes used in the product. For 110 of the cleaning products and 29 of the personal care products the type of enzyme(s) was declared on the ingredient list. For the other cleaning products and personal care products, and for all disinfectants and pet care products, none of the ingredient lists included information on the type of enzymes. No specific information means that the ingredient list either only stated "enzymes" (no other specification), only the claim on the product label stated the use of enzymes, or no ingredient list could be obtained.

The explorative overview showed that some types of products contain only 1-2 different types of enzymes, e.g. floor and furniture cleaning products, dishwashing detergents, stain removers, skin care and sun care products, and make-up and nail care products.
On the other hand, some product types contain at least 2 or more types of enzymes, e.g. septic tank biodegraders, and products for oral hygiene.
The number of enzymes found in laundry products was diverse, as some contained only one type of enzyme, whereas others contained up to 6 different types of enzymes.

Several cosmetic products (mainly hair care and bathing/showering products) claim to contain enzymes when reading product descriptions on the label or website. However, when reading ingredient labels, the enzymes are often not stated specifically. They are usually claimed to originate from fruit or plant extracts or honey. In those cases, the product label does state the fruit/plant extract or honey, but not the possible enzymes.

3. Questionnaire

The market research (Chapter 3) revealed a lack of information on the type and concentration of enzyme(s) used in consumer products. To gain insight into the composition and production of enzyme-containing consumer products, a survey was performed among the companies that were identified as producer or distributor of products in the explorative overview. The final goal was to use the collected information as input for evaluation of potential hazards, consumer exposure and possible risks involved in the use of consumer products with enzymes.

3.1. Strategy

A questionnaire (see Annex 3) was set up by the RIVM, and carried out by Kantar Public on behalf of the RIVM. Producers or distributors of products with enzymes were selected based on the products in the explorative overview (Chapter 3). A total of 72 producers or distributors were identified and contacted. The survey was performed between July 4th and August 16th 2022.
If a phone number was available, contact with companies was attempted by phone. If possible, the questionnaire was conducted by interview over the phone. Otherwise, the questionnaire was sent to the company by e-mail. If no phone number was available, contact was attempted by e-mail or contact form on the company website.

3.2. Response

- The questionnaire was completed by 19 respondents; all by interview over the phone
- Four of these respondents were producers of enzymes, two were producers of products with enzymes; 13 were distributors.
- The respondents that completed the questionnaire were mainly small companies. Large multinationals were not reached, despite multiple attempts.
- The persons that completed the questionnaire were mainly owners, managers and/or employees of R&D departments.
- 25 contacts refused to participate over the phone; but requested to receive the questionnaire by e-mail.
- 20 contacts were not reached, 5 contacts refused to participate, 3 contacts did not speak English or Dutch and could therefore not participate.

3.3. Key results

The four producers of enzymes that completed the questionnaire did not produce products, and did therefore not answer questions about enzyme content of products.
The two respondents that produced products with enzymes were asked detailed questions about the type and concentration of enzymes in a product of his/her choice. However, they did not provide information on the enzyme concentration in these products. One of these respondents indicated that they produce 1-10 disinfectants and biocidal products with enzymes; it can however not be traced from the questionnaire which products these are.

Two of the 13 responding distributors were able to tell something about the type of enzyme used in the product they distribute, because it was declared on the ingredient list. However, they did not have information on the enzyme concentration in the product. None of the other distributors had information regarding type and concentration of enzymes in the products they distributed.

Respondents were asked about future use of enzymes. The majority producers of enzymes answered that they foresee an increased use of enzymes in the future, in a wider range of products. However, the opinion of other respondents ranged between all possible answers being the same or a decreased number of products and a same to a narrower range of products.

Eight contacts that received the questionnaire by e-mail did start filling in the questionnaire. However, none of them completed the questionnaire, even after sending a reminder. One contact indicated that the questionnaire was too detailed, which is why he/she did not complete the full questionnaire.

3.4. Chapter summary

Unfortunately, no information on enzyme types or concentrations were retrieved from the questionnaire. One respondent told the interviewer that he/she would have preferred direct contact with RIVM, instead of being interviewed by Kantar Public. A more personalized approach may have resulted in higher response rates and more relevant information.

One possibility for future references is to contact producers through the NVZ (Dutch trade association for importers and manufacturers of washing, cleaning, maintenance and disinfection agents and cleaning machines) or AMFEP (Association of Manufacturers & Formulators of Enzyme Products). After finishing the questionnaire, RIVM approached the NVZ to explore if they could retrieve information on enzyme type and concentration for a few specific spray application detergents. It turned out that this way, producers were more willing to share that information, since it could be done anonymized. Two producers shared information on enzyme type and concentration in trigger spray products, when specifically asked about that certain product. The results of this exploration are described in Chapter 6.

4. Hazard

4.1. Chapter outline

This chapter focuses on hazards related to enzymes in non-food consumer products; more specifically the type of products found in the product search in Chapter 3.
When consumers apply products containing enzymes, they may be exposed to the enzymes but also to other ingredients of the product. Although it is recognized that there are hazardous ingredients found in cleaning products and personal care products, e.g. isothiazolinones and perfumes, the hazard identification of other ingredients falls outside the scope of this report. As there is no information available whether residues from the production organisms of enzymes are present in the products concerned, these are also not taken into account in the present report.

4.2. Hazard identification of enzymes

Enzymes are considered relative safe substances. Enzymes, like other proteins, are readily biodegraded in the gastrointestinal tract and due to their large molecular weight, enzymes do not easily penetrate the skin or mucous membranes (Basketter et al., 2012c; HERA, 2005). As therefore no significant oral, respiratory or dermal absorption can be expected, bioavailability of enzymes is low and systemic exposure and toxicity is not considered an issue. According to the available information, there is no concern with regard to genotoxicity, carcinogenicity, repeated dose-toxicity and reproductive toxicity (Basketter et al., 2012bc). According to available studies, in general, also the acute toxicity of enzymes is low. An exception is the ability of some proteases to produce irritating effects to the eye and skin at high concentrations, and more importantly, to act as respiratory sensitisers (Basketter et al., 2012c; HERA, 2005). Enzymes, like other proteins, do not generally pose a risk of allergic contact dermatitis (Basketter & Kimber, 2022).

That many enzymes can act as respiratory sensitisers is known since the introduction of enzymes into laundry detergents in the 1960s (HERA, 2005; HERA, 2007; Pepys et al., 1969). Over the years, research performed on the occupational hazard of enzymes has confirmed this hazard, as demonstrated in Table 4 below. Respiratory sensitisation expresses as asthma and allergic rhinitis in humans. This type 1 hypersensitivity reaction does not originate from the enzymatic activity, but from the fact that the protein structure may cause the development of allergen-specific IgE antibodies (SDA, 2005).
Substance information retrieved from the REACH C&L inventory and registration dossiers shows that many of the enzymes found in our product overview are classified as respiratory sensitisers (Table 4). In addition, as mentioned above, at high concentrations proteolytic enzymes (proteases) can irritate skin and eyes. Animal studies have shown the irritant effect of subtilisin on skin. The studies showed a range of effects; from non-irritant to severe irritant, depending on the concentration of enzyme and the conditions under which enzymes were

applied. Studies in human volunteers and data from workers also show the irritant effect of subtilisin. This effect is attributed to the proteolytic activity of subtilisin (HERA, 2007). Animal studies and an investigation among factory workers confirm the irritant effect of subtilisin to the eyes (HERA, 2007). On the other hand, no irritant effects were found for (alpha-)amylase, cellulase or lipase (HERA, 2005). Studies in animals, and among workers and consumers have shown no evidence for skin sensitising effects of either of these enzymes (Basketter, 2012b; HERA, 2007; HERA, 2005).

4.3. Coating of enzymes

In order to reduce exposure to enzymes of workers and consumers, producers started to look for risk reduction measures. One solution was to coat enzymes, which increased their weight and made them less volatile, therefore reducing inhalation exposure. This is especially used for solid enzymes, as for example used in powder laundry detergents. Coating is also used for example for stabilization of enzymes against abrasive forces during manufacturing and use of a product, for protection against other chemical agents in the product, and for improvement of appearance of the product (Herman et al., 1997).
The type of coating used depends on the producer and the purpose. Examples of substances used as coatings are sodium chloride, calcium chloride, sodium sulfate, cellulose gum (enzyme stabiliser), and manganese-II-oxalate dehydrate (enzyme controller). As the coating is not dissolved in the formulation it is therefore still present in the final product.
Furthermore, titanium dioxide (TiO_2) may be used as pigment in coatings of enzyme granules. This substance is classified as possibly carcinogenic to humans by IARC (Group 2B) (IARC, 2010), classified as category 2 suspected carcinogen by inhalation in 2019 (EC, 2019), and not considered safe as a food additive as concerns for genotoxicity after oral exposure cannot be ruled out (EFSA, 2021).

Table 3 Selection of studies on the effects of enzymes in workers.

Reference	Study design (type of study, number of workers)	Types of enzymes	Effects observed	Remarks regarding quality of study
Brant et al., 2009	Case–referent analysis of a retrospective cohort Employees working in a European detergent factory between 1989 and 2002. Cases with new lower or upper respiratory disease were ascertained by examination of occupational health records and matched to referents on date of first employment. Personal exposures were estimated from 12 000 measurements taken in the factory during the period of study.	Airborne detergent protease	Results: A total of 221 employees developed chest disease (3.5 per 100 person-years) and 214 employees developed eye/nose disease (3.3 per 100 person-years). A total of 135 employees developed both eye/nose and chest disease. Of these, 77 (57%) were identified with chest disease first and 38 (28%) with eye/nose disease first, 20 (15%) identified with both at the same time point. 107 employees (79%) developed both chest and eye/nose disease while in the same job.	Not based directly on personal enzyme exposure measurements. It was assumed that the enzyme content in personal dust measurements would be the same as that in the static area samples.
Van Rooy et al., 2009	Cross-sectional study 108 workers of a detergent products plant interviewed for respiratory and allergic symptoms 106 blood samples to examine sensitisation to enzymes. Those sensitised to >1 enzymes were referred for clinical evaluation. Characterisation of exposure qualitatively and estimate exposure semi quantitatively. Workers classified into three exposure groups with varying exposure profiles to enzymes, based on frequency, duration, and level of exposure.	proteases, α-amylase, lipase, cellulase	Results: Highest exposure in mixing area. Exposure of workers via skin (splashes) and inhalation (aerosols). Symptoms observed (of 108 workers): 5 (5%) constant problems breathing, 14 (13%) wheezing, 27 (25%) allergy including hay fever, 15 (14%) work-related itching nose, and 17 (16%) work-related sneezing. Of 106 workers, 15 (14%) were sensitised to >1 enzymes, mainly to bacterial α-amylases and proteases. Within these 15 workers, 4 were sensitised also to Lipase and/or cellulase. Thirty-eight workers (36%) were atopic. Of workers who were sensitised, 11 (73%) were atopic and 8 (53.3%) were current smokers. The median of years of employment was 8.0 years (range 1–20) which was not associated with	The study population was small, and this limited statistical power in internal comparisons, especially after adjusting for confounding variables in multiple regression modelling.

Reference	Study design (type of study, number of workers)	Types of enzymes	Effects observed	Remarks regarding quality of study
			sensitisation (PR=0.96, 95% CI 0.89 to 1.04). Atopics were 4.9 times more likely to be sensitised to detergent enzymes than non-atopics (PR=4.92, 95% CI 1.68 to 14.39). Sensitisation was not associated with smoking (PR=0.99, 95% CI 0.95 to 1.03).	
Budnik et al., 2017	Cross-sectional study Specific IgE antibodies against workplace specific individual enzymes were measured in 813 exposed workers seen in cross-sectional surveys. Men: 66%, aged 20–60 years and women: 34%, aged 20–50 years	α-amylase stainzyme pancreatinin savinase papain ovozyme phytase trypsin lipase	Results: Twenty-three per cent of all exposed workers (n=187) showed type I sensitisation with IgE antibodies: α-amylase (44%), stainzyme (41%), pancreatinin (35%), savinase (31%), papain (31%), ovozyme (28%), phytase (16%), trypsin (15%) and lipase (4%). In a clinical data subgroup of 134 workers, questionnaire data were available to correlate specific IgE with symptoms: 64% asymptomatic, 19% work-related rhinitis and/or conjunctivitis, and 17% work-related wheezing and/or asthmatic dyspnoea. Pearson's correlation analysis showed a significant correlation between symptoms and specific IgE (r=0.75, 95% CI 0.61 to 0.84, p<0.0001).	Limited access to data and authority to identify specific bioengineer enzyme formulae. The clinical data subgroup was not randomly selected from all workers tested, thus possible selection bias could not be excluded.

Table 4 Enzyme hazard classification and information from the REACH registration dossier (note that only classifications relevant for human health are included). Data collected in September 2022.

Name of enzyme	**CAS-nr**	**REACH CLP harmonized classification (Annex VI of EC Regulation (EC) No 1272/2008), *or self-classification (-)*[1]**	**Derived No- or Minimal Effect Level (DNEL/DMEL) for the general population from REACH registration dossier[2]**
(Alpha-)amylase	9000-90-2	Resp. Sens. 1 (H334)	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Bromelain, juice	9001-00-7	Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Resp. Sens. 1 (H334) STOT SE 3 (H335)	N/A
Cellulase	9012-54-8	Resp. Sens. 1 (H334)	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Lipase	9001-62-1	*- (1561 out of 1753 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Lysozyme	9001-63-2	*- (43 out of 46 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	N/A
Mannanase (endo-1,4-β-)	37288-54-3	*- (206 out of 208 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Pectate lyase	9015-75-2	*- (All 73 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Pectinase (pectin lyase)	9033-35-6	*- (9 out of 10 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Subtilisin	9014-01-1	Skin Irrit. 2 (H315) Eye Dam. 1 (H318) Resp. Sens. 1 (H334) STOT SE 3 (H335)	Respiratory: DMEL: 15 ng/m³ (sensitisation (respiratory tract)) Oral: DNEL: 1.8 mg/kg bw/day (repeated dose toxicity)
Xanthan lyase	113573-69-6	*- (All 73 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Papain	9001-73-4	Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Resp. Sens. 1 (H334) STOT SE 3 (H335)	N/A
Superoxide dismutase	9054-89-1	-	N/A
Glucose oxidase	9001-37-0	*- (183 out of 279 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	N/A

Name of enzyme	**CAS-nr**	**REACH CLP harmonized classification (Annex VI of EC Regulation (EC) No 1272/2008), *or self-classification (-)*[1]**	**Derived No- or Minimal Effect Level (DNEL/DMEL) for the general population from REACH registration dossier[2]**
(Lacto-) peroxidase	9003-99-0	*- (8 out of 15 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	DMEL: 15 ng/m³ (sensitisation (respiratory tract))
Amyloglucosidase	9032-08-0	*- (90 out of 91 notifiers self-classify the substance as Resp. Sens. 1 (H334))*	DMEL: 15 ng/m³ (sensitisation (respiratory tract))

[1] H315 (Causes skin irritation); H318 (Causes serious eye damage); H319 (Causes serious eye irritation); H334 (May cause allergy or asthma symptoms or breathing difficulties if inhaled); H335 (May cause respiratory irritation);
'-': no harmonised classification.
[2] DMEL: Derived minimal effect level; DNEL: Derived no effect level; information retrieved from REACH registration dossier; provided for long-term effects.
N/A: no registration dossier available / no DMEL present in registration dossier.

4.4. Hazard characterization: health based limits

DMEL derivation by industry associations
Respiratory sensitisation is the main hazard when considering the hazards of enzymes. For respiratory allergens (and thus for enzymes), no animal model is available to set a dose-response relationship. Hazard characterization is therefore based on benchmark values from human studies. These values are derived from studies in which exposures are measured or estimated in an experimental setting, and associated with an effect or lack of effect in an exposed population (SDA, 2005). The knowledge acquired through occupational studies is often used as a starting point to also establish exposure limits for consumers.
As part of the chemical safety assessment (CSA) under REACH, a Derived No Effect Level (DNEL) should be derived for substances with threshold endpoints. For substances with non-threshold effects, like sensitisers or carcinogens, a DNEL cannot be established. Therefore, a Derived Minimal Effect Level (DMEL) should be determined for these substances under REACH. As defined in REACH Guidance documents, a DMEL "*expresses an exposure level corresponding to a low, possibly theoretical, risk, which should be seen as a tolerable risk*" (ECHA, 2012).

As shown in Table 4, for most of the enzymes relevant to this report, the DMEL for the general population based on the development of respiratory sensitisation is set at 15 ng/m^3 (concentration of airborne enzyme protein). This is lower than the occupational DMEL of 60 ng/m^3. Basketter et al. (2010) proposed a more cautious level because of the lack of control and monitoring of consumer exposure. This consumer DMEL of 15 ng/m^3 was set mainly due to industry efforts. This DMEL is based on a study performed by Weeks et al. (2011). This was a human volunteer study in which a laundry stain-remover product with trigger spray containing a protease was used daily for six months and 30 sprays per day. That resulted in no adverse effects in a carefully monitored atopic population (96 subjects) tested by a skin prick test. Enzyme concentrations in air were measured with low- and high-volume samplers (representing an adult performing light and heavy activity, respectively). Measured values were up to an average of 12 (standard

deviation 4.4) ng/m^3 for high-volume sampling and 17 (standard deviation 2.6) ng/m^3 for low-volume sampling. This data was the basis for the estimation of the DMEL by Basketter et al. (2010) as acceptable exposure limit for consumers and the vast majority of professional users.

REACH registration dossiers of enzymes shown in Table 4 state the following in reference to the declared DMEL:
"Industry has documented that respiratory irritation or toxicity due to enzyme preparations is a very rare phenomenon which will not occur at the low concentrations of enzymes found in consumer products as for example detergents. The risk to consumers is considered very low and regarded as toxicologically insignificant [(Basketter et al., 2010; Basketter et al., 2012a; Basketter et al., 2012b)]*. This is supported by the positive safety outcome of a clinical study of the highest reported consumer exposure level, 15 ng/m^3, with spot cleaning by spray* [(SDA, 2005; Weeks et al., 2011)]. *Consumer DMEL has been discussed among the enzyme allergy specialists from enzyme and detergent manufacturers and it was concluded by the involved industry partners in a recent publication and the limit of 15 ng/m^3 was suggested* [(Basketter et al., 2010)]*. With a LC-50 value of 0.1 g active enzyme protein/m^3, the actual exposure is more than a factor of 10^6 less than this LC50 value."*

OEL derivation by government organisations
In the Netherlands, the public Occupational Exposure Limit (OEL) for alpha-amylase is 0.000010 mg/m^3 (TGG – 8hr); this limit is valid from 1 January 2024. The validation for this value is based on the respiratory sensitisation property of alpha-amylase (SER, 2022a). There is no public OEL for any of the other enzymes shown in Table 4, however, a private OEL is available for subtilisin (60 ng/m^3 8h limit value), and proteinase (0.06 mg/m^3 (TGG – 8hr), 0.015 mg/m^3 (TGG – 15min). based on Finish limit values (Heederik, 2019; SER, 2022b).

In other countries, including Australia, Belgium, Canada, Denmark, Ireland, New Zealand, Norway, China, Singapore, South Africa, Spain, Sweden, Switzerland, USA and the UK, only OELs for subtilisin were found. Values are comparable to the private OEL of 60 ng/m^3, although most countries apply this value as short term (15 minute or 60 minute average, or ceiling limit) value. Short term limit values ranged from 60 to 120 ng/m^3 between countries. In China, the 8h limit value is 15 ng/m^3, whereas in the UK this value is 40 ng/m^3.
One OEL was found for lipase in Latvia, which is 1 mg/m^3. For all enzymes in Table 4 that have a DMEL for consumers for respiratory sensitisation, the REACH Registration dossiers show a DMEL for workers of 60 ng/m^3 for this effect. However, companies claim to use more stringent occupational exposure limits (Basketter et al., 2010, 2021; Kelling et al., 1998; AISE, 2015)

4.5. Chapter summary

Enzymes are considered relative safe substances with no significant oral, respiratory or dermal absorption to be expected on their chemical characteristics. As a result, their bioavailability is low and systemic

exposure and toxicity not considered an issue. According to the available information, there is no concern with regard to genotoxicity, carcinogenicity, repeated dose-toxicity and reproductive toxicity and also, in general, the acute toxicity of enzymes is low. An exception and therefore the most important hazard characteristic of enzymes is that they can act as respiratory sensitisers, which is confirmed by occupational hazard studies. This effect does not originate from enzymatic activity, but from the protein structure inducing allergen-specific IgE antibody formation. At higher concentrations (i.e. higher than their use in personal care products products), enzymes can irritate the skin and the eye. Coating of enzymes can reduce their volatility and therefore reduce exposure, and thus maybe prevent inhalation and respiratory sensitisation.
As no threshold can be set for respiratory sensitisers, a DNEL cannot be established, and therefore a DMEL should be established for enzymes. For most of the enzymes, the DMEL for the development of respiratory sensitisation is set at 15 ng/m^3. This DMEL is established by a study by Basketter et al. (2010), based on a study by Weeks et al. (2011). The value is determined for protease, a class of enzymes considered worst-case with regard to respiratory sensitisation properties, under specific circumstances. At a national level, some OEL values are reported, such as a public OEL for alpha-amylase (10 ng/m^3) in the Netherlands, or private OELs for subtilisin usually of 60 ng/m^3. The latter value is, as the DMELs for workers, based on the study by Weeks et al. (2011) with protease.

5. Exposure

This chapter focuses on exposure of consumers to enzymes via non-food consumer products (as described in Chapter 3). Worker exposure is also described, as much on consumer exposure can be learned from studies of workers. Environmental exposure falls outside the scope of this report.

5.1. Concentration data in consumer products

The questionnaire did not yield information on the concentration of specific enzymes in consumer products (Chapter 4). However, two producers were willing to share information on enzyme type and concentration in two specific trigger spray products, by personal communication via the NVZ. One producer declared to use 0.014% subtilisin in a trigger spray stain remover. Another producer declared using a final concentration of 0.02% subtilisin, 0.05% cellulase and 0.03% alpha-amylase in a trigger spray odour control product. Note these concentrations reflect the Active Enzyme Protein (AEP), the concentration as proteolytically active enzyme, corresponding to a higher concentration of total enzyme protein. Although not taken into account with regard to the Dutch market, it is worthwhile mentioning that the CPID provides some concentrations (usually ranges) of enzymes in certain American and Canadian products (CPID, 2022).

5.2. Exposure routes

Consumers may be exposed to enzymes in non-food consumer products by three possible routes: inhalation, oral and dermal exposure.

5.2.1. Inhalation route

Inhalation is one of the possible routes of exposure to enzymes for consumers and workers. For workers, exposure through inhalation may start during the preparation of the enzymes after a drying process. During the production process, other steps like moving bags of powder enzymes can make enzymes volatile and become airborne. Mixing for the formulation of cleaning products or any product containing enzymes is also a crucial step where inhalation exposure can occur.
A study by Budnik et al. (2017) shows that the majority of workers exposed are men (66%) between 20-60 years old and women (34%) between 20-50 years old (Table 3). Median working time is 8 years according to Van Rooy et al. (2009).
The exposure of workers is mostly evaluated by reconstruction as seen in Table 3. For consumers, inhalation exposure may also occur for consumers when using products containing enzymes, especially if the product is sprayed. Evaluation of consumer exposure has been performed by the International Association for Soaps, Detergents and Maintenance products (AISE) to estimate the potential risks for consumers and develop a protocol to measure consumer exposure (AISE, 2020). This protocol is explained in section 5.2.

5.2.2. *Oral route*

Oral exposure to enzymes in non-food consumer products may occur while using cosmetic products or products that have been washed with enzymes, or in case of an accidental ingestion or hand-mouth contact after using products with enzymes.
The types of personal care products that most likely give rise to oral exposure are face creams/serums, toothpastes, and mouthwash.
After the use of cleaning products for items that come in contact with the mouth such as cutlery, glass or plates, ingestion of enzymes may occur.
Another type of oral exposure to enzymes may also occur in case of an accidental ingestion (i.e. misuse). This may happen with any product that contains enzymes. Accidental ingestion is more likely to happen with young children because of their behaviour, which may be of particular concern because of their physiological characteristics, such as their lower body weight compared to adults (OECD, 2019). The actual exposure levels are very difficult to assess.

5.2.3. *Dermal route*

Dermal exposure is a major route of exposure to enzymes for consumers. Skin exposure, mainly of hands and arms, may occur during the use of products for cleaning purposes: cleaning a surface and/or diluting the product in water. Additionally, the use of cosmetic products containing enzymes leads to skin exposure for the parts of the body that the product is applied on. The use of items or surfaces that have been cleaned with enzymes such has clothes, chairs or tables may also lead to skin exposure to enzymes. Exposure to multiple products with enzymes increases the amount of skin exposure.

5.3. Consumer exposure assessment

5.3.1. *General considerations*

Several factors should be considered during exposure assessment of enzymes from use of enzyme-containing consumer products. These factors include (ACI, 2019):

- formulation and delivery mechanism of the product;
- conditions of use, misuse and accidental exposure (e.g. amount, duration, frequency);
- physical environment in which the product will be used;
- exposure routes.

Depending on the available data, exposure can be estimated or measured. Initial estimations are usually made with worst-case assumptions regarding the exposure scenario, which are then compared with available exposure limits (i.e. the Derived Minimal Effect Level (DMEL)) for risk assessment. If this indicates a risk for adverse health effects, it may be possible to use less conservative assumptions, for example considering normal consumer uses. If existing data are insufficient, exposure measurements should be performed (ACI, 2019).

Exposure to enzymes is not expected to lead to significant oral, respiratory or dermal absorption as a result of their chemical characteristics (Chapter 5). Exposure assessments for enzymes often focus on inhalation exposure, since respiratory allergy is considered to

be the main concern. Consumers are usually exposed to products which can potentially lead to inhalation exposure, for short periods of time. It is difficult to assess short term exposure to enzymes, due to the fact that enzymes are used in very low concentrations, and therefore air concentrations are low. To be able to measure low air concentrations, prolonged sampling time and/or sensitive analytical methods are required. Methods to measure enzymes in air are sensitive up to an air sampling collection time of about 11 minutes (Weeks et al., 2011). Shorter sampling times lead to concentrations below detection limits. Inhalation exposure for consumers is therefore often assessed by performing laboratory experiments in which use conditions from consumer surveys are mimicked, or by estimations under certain assumptions.
Some estimations were for example presented in a HERA[1] report from 2007 on protease (subtilisins) in detergents. According to their estimations, consumer exposure to subtilisins can occur via the respiratory route during the dispensing of detergent products in the washing machine (exposure up to 0.16 ng subtilisin/m^3) or during handwash of laundry (0.01 ng/m^3), or by suddenly opening the dish washer during the cleaning step (<1.9 ng/m^3) (HERA, 2007).

5.3.2. Spray applications

Spray products with enzymes are relatively new applications, and are recognized by the industry to be applications that need thorough risk assessment before a spray product is put to the market (AMFEP, 2013). To evaluate potential inhalation exposure for consumers to spray applications, companies use a protocol written by the International Association for Soaps, Detergents and Maintenance Products (AISE), with the example of the pre-spotter spray (see Figure 2). During exposure assessment, the exact final product is assessed: content, spray bottle, and spray nozzle. If any changes are made to any of these parameters, a new exposure assessment must follow. Air-sampling equipment is used to collect airborne enzymes during the application of the spray on a surface corresponding to the specific use. After air sampling, the filters are analysed for enzyme protein collected during air sampling.

5.4. Exposure estimates based on enzyme concentrations in products

As mentioned in Chapter 4, two producers were willing to share information on enzyme type and concentration in trigger spray products, when specifically asked about that certain product by the NVZ. One producer declared to use 0.014% subtilisin in a trigger spray stain remover. Another producer declared using a final concentration of 0.02% subtilisin, 0.05% cellulase and 0.03% alpha-amylase in a trigger spray odour control product. This information was used to perform an exposure assessment with ConsExpo, using default values from ConsExpo factsheets. For the stain remover, the Cleaning product factsheet was used (Meesters et al., 2018), with values from the spot treatment with spot remover spray. Exposure frequency was 128 times per year, and exposure duration was set at 10 minutes with a spray

[1] HERA (Human and Environmental Risk Assessments on ingredients of household cleaning products) is a voluntary industry partnership program between the makers of household cleaning products (A.I.S.E.) and the chemical industry (Cefic) who supplies the raw materials.

duration of 0.05 minutes (3 seconds). Values from the Air fresheners factsheet (Meesters et al., 2022) were used for the exposure assessment of the odour control product, with the furniture spray as default. Exposure frequency was 52 times per year (once a week), and exposure duration was set at 240 minutes with a spray duration of 0.167 minutes (10 seconds). Results of this exposure assessment are described in Table 5 below (details can be found in Annex 4). The exposure estimate displayed is the mean event concentration. As sensitisation is an event that may happen even after short term exposure, this was considered to be the most pragmatic value, instead of using a day or year average concentration. In addition, values from risk assessment studies are added to Table 5 as comparison.

Figure 2 Association for Soaps, detergents and Maintenance Products (AISE) protocol for inhalation exposure assessment of spray products for consumers

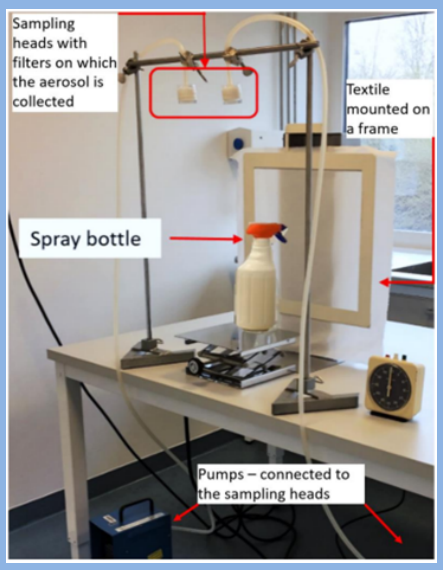

Figure 2a The set-up for spray exposure studies. Source: International Association for Soaps, detergents and Maintenance Products (AISE, 2020).

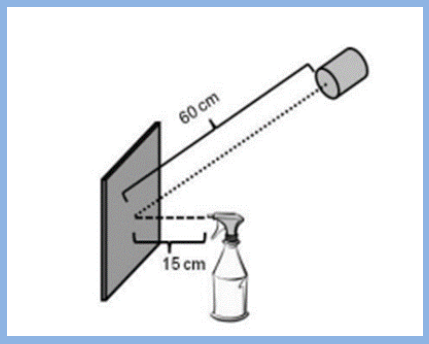

Figure 2b Schematic presentation of the distances between the spray bottle, textile target and the air filter. Source: International Association for Soaps, detergents and Maintenance Products (AISE, 2020).

The steps of this protocol are as follows:

- Starting the pumps;
- After 1 minute: spraying 5 times on the framed textile with a frequency of 1 spray per second;
- Waiting for 10 seconds, during this time the framed textile is changed;
- Repeating the spray cycle 5 times, resulting in a total of 6 cycles;
- The last framed textile is left on as the pump is still running
- The pumps are turned off after a total of 10 minutes from the first spray cycle

Table 5 Air exposure estimates based on enzyme concentrations in products, either modelled/calculated or measured under experimental conditions.

Exposure estimate (ng/m³)	Enzyme (concentration)	Product	Method	Remarks	Reference
Modelled/Calculated					
1.6	Subtilisin (0.014% AEP*)	Trigger spray stain remover	ConsExpo	Modelled using default values for exposure parameters from ConsExpo factsheet: Cleaning products	Data: personal communication (see Chapter 6.4); Method: Meesters et al. (2018)
350	Subtilisin (0.02% AEP*)	Trigger spray odour control	ConsExpo	Modelled using default values from ConsExpo factsheet: Air fresheners	Data: personal communication (see Chapter 6.4); Method: Meesters et al. (2022)
890	Cellulase (0.05% AEP*)	Trigger spray odour control	ConsExpo	Modelled using default values from ConsExpo factsheet: Air fresheners	Data: personal communication (see Chapter 6.4); Method: Meesters et al. (2022)
530	Alpha-amylase (0.03% AEP*)	Trigger spray odour control	ConsExpo	Modelled using default values from ConsExpo factsheet: Air fresheners	Data: personal communication (see Chapter 6.4); Method: Meesters et al. (2022)
0.16	Subtilisin (0.06%)	Powder laundry detergent	Calculation	Loading of washing machine	HERA (2007)[1]
0.01	Subtilisin (0.06%)	Powder laundry detergent	Calculation	Washing with detergent in sink	HERA (2007)[1]

Exposure estimate (ng/m³)	Enzyme (concentration)	Product	Method	Remarks	Reference
Modelled/Calculated					
0.11	Amylase	Powder laundry detergent	Calculation	Loading washing machine	HERA (2005)[1]
0.08	Cellulase	Powder laundry detergent	Calculation	Loading washing machine	HERA (2005)[1]
0.016	Lipase	Powder laundry detergent	Calculation	Loading washing machine	HERA (2005)[1]
0.006 (Amylase), 0.005 (Cellulase), 0.001 (Lipase)	Mixture of amylase, cellulase and lipase	Powder laundry detergent	Calculation	Loading washing machine	HERA (2005)[1]
1.9	Amylase	Dishwasher tablet	Calculation	Dish washing task; opened dishwasher door	HERA (2005)
Exposure estimate (ng/m³)	**Enzyme (concentration)**	**Product**	**Method**	**Remarks**	**Reference**
Measured					
12 ± 4.4 (high-volume sampling) 17 ± 2.6 (low-volume sampling	Savinase (81 µg endo-protease/g product)	Pre-spotter trigger spray product	Laboratory exposure study	5 sprays (1g of product per spray) onto a piece of fabric in a 14.5 m³ unventilated room.	Weeks et al. (2011)
183 ± 142 (high volume sampling)	Protease (1% AEP*)	Personal cleansing product	Experimental exposure study with test subjects	Experiment: One person uses a shower bar in shower for 2 min (mean use of 10.2 ± 5.1 gram of product per experiment). No. of experiments: 21	Kelling et al. (1998)
15.7 ± 8.4 (high volume sampling)	Protease (0.2% AEP*)	Personal cleansing product	Experimental exposure study with test subjects	Experiment: One person uses a shower bar in shower for 2 min (mean use of 6.0 ± 5.9 gram of product per experiment). No. of experiments: 14	Kelling et al. (1998)
11.4 ± 7.8 (high volume sampling)	Protease (0.1% AEP*)	Personal cleansing product	Experimental exposure study with test subjects	Experiment: One person uses a shower bar in shower for 2 min (mean use of 9.1 ± 4.4 gram of product per experiment). No. of experiments: 8	Kelling et al. (1998)

Exposure estimate (ng/m³)	Enzyme (concentration)	Product	Method	Remarks	Reference
Measured					
0.012 (protein)	Several enzymes (from products of Procter & Gamble)	Liquid detergent	Laboratory exposure study	Pour liquid detergent into top-loader wash machine; task duration <30s; task frequency 4-7x/week)	Sarlo et al. (2010)
0.00022 (protein)	Several enzymes (from products of Procter & Gamble)	Granule detergent	Laboratory exposure study	Pour granule detergent into top-loader wash machine; task duration <30s; task frequency 4-7x/week)	Sarlo et al. (2010)
0.7-2.9 (protein)	Several enzymes (from products of Procter & Gamble)	Liquid or granule detergent	Laboratory exposure study	Addition of water to liquid or granule detergent in top-loader wash machine; task duration <30s; task frequency 4-7x/week)	Sarlo et al. (2010)
0 (protein)	Several enzymes (from products of Procter & Gamble)	Detergent	Laboratory exposure study	Addition of detergent to front-loader wash machine; task duration <30s; task frequency 3-10x/week)	Sarlo et al. (2010)
0.5 (protein)	Several enzymes (from products of Procter & Gamble)	Granule detergent	Laboratory exposure study	Detergent refill (pour granule from 6 kg sack); task duration <1 min; task frequency once/month)	Sarlo et al. (2010)
<0.5 (protein)	Several enzymes (from products of Procter & Gamble)	N.A. (dryer venting task)	Laboratory exposure study	Dryer vent (indoors); task duration <30s to 1h; task frequency <4-7x/week)	Sarlo et al. (2010)
0.04-1.2 (protein)	Several enzymes (from products of Procter & Gamble)	N.A. (dryer cleaning task)	Laboratory exposure study	Clean dryer lint trap; task duration <30s; task frequency <4-7x/week)	Sarlo et al. (2010)
1-3 followed by <0.3 (protein)	Several enzymes (from products of Procter & Gamble)	Liquid dish soap	Laboratory exposure study	Hand wash dishes using liquid dish soap; task duration <30s followed by several minutes; task frequency daily	Sarlo et al. (2010)

*AEP: Active Enzyme Protein; concentration as proteolytically active enzyme, corresponding to a higher concentration of total enzyme protein.
[1] Please note that these estimations erroneously refer to a released mass of 0.27 µg powder per cup (200 g) of product used for machine washing instead of 'inhaled mass', as derived by van de Plassche et al. (1999) (Meesters et al., 2018).

5.5. Chapter summary

In this chapter, the exposure of consumers has been assessed.
Oral exposure to enzymes in non-food consumer products may occur while using cosmetic products or products that have been washed with enzymes, or in case of an accidental ingestion or hand-mouth contact after using products with enzymes.
Dermal exposure, mainly of hands and arms, may occur during the use of products for cleaning purposes: cleaning a surface and/or diluting the product in water, but also as a result of the use of personal care products.
Inhalation exposure of consumers is mostly based on the use of powder products and sprays.
Oral, dermal or inhalation exposure to enzymes is not expected to lead to significant absorption and subsequent systemic toxicological effects. Respiratory sensitisation is considered the main hazardous effect of enzymes. Therefore, exposure assessments for enzymes often focus on inhalation exposure, since respiratory allergy is considered to be the main concern. Inhalation exposure for consumers is consequently often assessed by performing laboratory experiments in which use conditions from consumer surveys are mimicked, or by estimations (modelling/calculation) under certain assumptions. In order to estimate the consumers' exposed concentration of enzymes after the use of a spray, a protocol created by AISE to measure the mimicked exposure (Figure 2).

The measured and estimated enzyme concentrations in the air through which humans can be exposed by inhalation demonstrated a wide concentration range in different studies (Table 5), (partly) dependent on the type of product (e.g. spraying application from which a higher exposures through the air can be expected), concentration of the enzymes present, and the method of estimating:

- Measured concentrations: from 0.00022 ng protein/m^3 up to 183 ng/m^3;
- Modelled/calculated concentrations: from 0.01 ng/m^3 up to 1.9 ng/m^3 by calculations of HERA (2005, 2007) and from 1.6 ng/m^3 up to 890 ng/m^3 by ConsExpo modelling. Note that the differences in outcome of the different methods of calculations is dependent of different product concentrations and assumption on usage and the exposure parameters and scenarios.

6. Considerations for risk assessment

In order to illustrate the potential risks related to enzymes in non-food consumer products, a risk assessment should be performed. However, performing a robust risk assessment for an enzyme or enzymes in a non-food consumer product is presently complicated due to several uncertainties, some related to the hazards of specific enzymes, others to the exposure assessment of products with enzymes, including the concentration of enzymes in consumer products. Unfortunately, a market research (Chapter 3), and a questionnaire among producers and distributors (Chapter 4) did not provide the essential input (concentrations of specific enzymes) for the performance of an exposure assessment. Only through contact with a trade association of detergents, the concentration of enzymes in two specific products on the Dutch market was retrieved.
Due to all uncertainties and incomplete information, it is not possible to perform a robust risk assessment for the present application of enzymes in non-food consumer products at this moment. The uncertainties will be outlined in this chapter, as well as a perspective on future work that is needed to carry out a risk assessment.

6.1. Hazard assessment

Enzymes are considered relative safe substances with no significant oral, respiratory or dermal absorption to be expected on their chemical characteristics. As a result, their bioavailability is low and systemic exposure and toxicity not considered an issue. Subsequently, there is no concern with regard to genotoxicity, carcinogenicity, repeated dose-toxicity and reproductive toxicity. Also the acute and systemic toxicity of enzymes is low. An exception is the ability of some proteases to be eye and skin irritants at higher concentrations (i.e. higher than their use in personal care products products). The critical toxicological effect, however, is respiratory allergy, which is found after inhalatory exposure studies. Enzymes, like other proteins, do not generally pose a risk of allergic contact dermatitis.
The generic DMEL of 15 ng/m^3, as proposed by Basketter et al. (2010), is based on a human volunteer study by Weeks et al. (2011), using spray application of a spot cleaning product containing protease (Weeks et al., 2011). Compared to other types of enzymes, proteases are considered to be the most potent respiratory sensitising enzymes (Basketter et al., 2010). However, several factors should be kept in mind considering this DMEL. Although a DMEL is not a DNEL, meaning that exposure to this level does in principle not exclude possible adverse health, in this case the DMEL has been derived on a study in which no effects were found, but contained uncertainties. First, there were several disadvantages with respect to the study design that the DMEL of 15 ng/m^3 is based on:

- The skin prick test performed by Weeks et al. (2011) in order to detect type I allergic reactions used a relatively small study population and short study duration (96 subjects completed a 6-month exposure period). The study has limited power to detect a positive effect (sensitisation) over such an amount of time. To

support their study design, Weeks et al. (2011) referred to a study by Kelling et al. (1998), in which a 6-month period was long enough to demonstrate development of respiratory allergy due to proteases. However, although similar enzyme concentrations and similar populations were used, exposure characteristics were different in the study by Kelling et al. (1998). The study was designed to evaluate enzyme exposure to a potential personal cleansing product while showering. The moist environment and possible exposure through other routes while applying the product may lead to a different exposure.

- The level of 15 ng/m^3 seems to be an approximation or average of exposure levels found in the study by Weeks et al. (2011) at which no subjects were sensitised. Enzyme concentrations in air were measured with low- and high-volume samplers (representing an adult performing light and heavy activity, respectively). Measured values were up to an average of 12 (standard deviation 4.4) ng/m^3 for high-volume sampling and 17 (standard deviation 2.6) ng/m^3 for low-volume sampling. Setting a level of 15 ng/m^3 as DMEL is therefore debatable.
- The concentration of 15 ng/m^3 stated in the study by Weeks et al. (2011) was based on measurements made during a laboratory exposure study. Consumer exposure was simulated in a clinical study, but actual exposure was not measured. Instead, subjects were instructed to perform a certain exposure scenario, which mimics the laboratory setting. Subjects were exposed to 30 sprays per day for 6 months. The authors do not describe if and how it was verified that subjects have complied with the instructions during the 6-month period. It is therefore not known if exposure during the clinical study is comparable to the laboratory exposure.

Further, the general DMEL of 15 ng/m^3 for protease does not distinguish between different types of proteases, which could have different, individual sensitizing properties. In addition, it may not cover sensitizing properties of other types of enzymes too. For instance, the Health Council of the Netherlands has derived a dose-response relationship on respiratory sensitising effects for fungal alpha-amylase (Health Council of the Netherlands, 2014). Based on two epidemiological studies in bakeries and flour mills, a reference value of 0.9 ng/m^3 was derived. This reference value is based on an additional respiratory sensitisation risk of 1 percent. It should be noted that this (occupational) limit value (SER, 2022a) is much lower compared to the generic DMEL of 15 ng/m^3. The information on which the derivation is based, however, seems to be more robust compared to the volunteer study of Weeks et al. (2012).

6.2. Exposure assessment

As outlined in Chapter 6, consumer exposure can be assessed in multiple ways, namely by modelling/calculation or by measuring exposure. Exposure modelling can be done using ConsExpo, which is a web-based software tool that is often used to estimate consumer exposure to substances in consumer products. It provides possibilities to perform an exposure estimation using default values for exposure

parameters such as amount of product, frequency, room volume, body weight (as published in ConsExpo Fact Sheets).

As shown in Table 5, consumer exposure to two spray trigger products was estimated with ConsExpo, as for these two products the concentration of the enzyme(s) in the product was available. One product consisted of a trigger spray odour control product containing three different enzymes (subtilisin, cellulase, and alpha-amylase). The exposure estimation for this product resulted in enzyme concentrations of 350, 890, and 530 ng/m^3 in the air, respectively. The exposure estimation of the other product, a trigger spray stain remover with subtilisin, resulted in an enzyme concentration of 1.6 ng/m^3 in the air. Differences between the outcome can be largely explained by other exposure parameters due to the use of the different ConsExpo scenario's, i.e. the Air freshener Fact Sheet for the odour control product and the Cleaning products Fact Sheet for the stain remover. In general, these enzyme concentrations are much higher than the exposure estimates for other product types, i.e. powder laundry detergents (0.01 ng/m^3 up to 0.16 ng/m^3) or a dishwasher tablet after opening the dishwasher door (1.9 ng/m^3), as calculated by HERA, according to a different methodology (Table 5).

Another way to perform exposure assessment is by measuring exposure. A standardized protocol developed by AISE (Figure 2) is available for this purpose. However, the AISE protocol is developed only for spray applications, whereas other exposure scenarios may also be of relevance. In literature, through laboratory exposure studies or experimental exposure studies with test subjects, the measured exposure estimates range from 0.00022 ng protein/m^3 up to 183 ng/m^3 (Table 5). Interestingly, the enzyme concentration of 183 ng/m^3 in the air resulted from the use of a personal cleansing product while showering.

The information gathered in the current project teaches us that, as a starting point, future exposure assessment should focus on products with a relevant exposure route for respiratory sensitisation, such as spray applications or (potential dusty) powders. Further, it was shown that the use of the cleansing shower product also resulted in relevant enzyme concentrations. It is known that the exposure to enzymes via "dusty" washing powder is decreased by using "internal" risk management measures such as coating of the washing powder to decrease the dustiness. Therefore, spray applications of products with enzymes are regarded as (the most) riskfull application, due to the combination of respiratory sensitising properties of enzymes and the formation of respirable particles by spraying. In addition, special attention should be paid to products that are not ready to use, but need to be diluted and/or transferred into a vessel by the consumer before use. For example, the market research uncovered a few garden cleaners that were concentrated mixtures that should to be diluted by the consumer before use. For such products, the actual exposure might not be correctly assessed as the materials for the application are not well described.

Important to note is that, as more common in consumer exposure estimation, there is a lack of data on concentrations of enzymes used in consumer products.

6.3. Risk assessment

As indicated above, the ConsExpo modelling demonstrated that the consumer exposure through the use of a trigger spray odour control product containing 3 different enzymes led to an exposure level above the DMEL. Results from measurements resulted in enzyme concentrations (far) below or around the DMEL of 15 ng/m^3. Concluding on the actual risk is difficult, taken into account the uncertainties regarding the DMEL, and a certain level of conservatism in the ConsExpo calculations. Therefore, the outcome does not automatically mean there is a risk associated with the use of this product.

Sarlo et al. (2010) provided an overview of data on respiratory sensitisation on consumer populations using enzyme-containing laundry and cleaning products. Based on 6 publications, in total more than 5000 people, there were only low numbers (n-15) with symptoms. All were from studies before 1977, after which cleaning products were improved. On the other hand, the exposure from new kinds of products with spraying applications and/or other enzymes might not be covered by these studies.

Altogether, this indicates that the exposure to the above mentioned trigger spray products, and similar products, could be assessed more closely, as it raises the question whether the exposure measurement studies are covering the actual exposure by this type of products.

The implemented test by AISE (see Figure 2) with the example of the pre-spotter spray to evaluate the concentration of enzymes in the air uses a tiered approach on the average exposure measured as compared to the DMEL:

- If the average exposure is < DMEL, the enzyme containing spray product is safe to be used according to the manufacturer;
- If average exposure is > DMEL, improvements of the enzyme containing spray product will have to be made by the manufacturer before it can be approved – based on data from a new exposure assessment. Possible improvements include:
  - Adjusting (lowering) the enzyme concentration(s)
  - Increase the viscosity
  - Replace the spray nozzle with a different type

6.4. Future needs for risk assessment

For subtilisin, alpha-amylase, cellulase, and lipase, REACH registration dossiers provide the following statement: "The ability of enzymes to elicit respiratory sensitisation is well known and the classification of enzymes as respiratory sensitisers is widely accepted. However, when exposure is controlled and limited, experience demonstrates that enzymes can be used safely." This statement is supported by comparing worker exposure measurements and sensitisation data in worker populations from before and after 1977 (Sarlo et al., 2010).

As explained in previous sections, several uncertainties and incomplete information concerning the DMEL and exposure assessment factors make it difficult to perform robust risk assessments for consumer products containing enzymes. When setting a DMEL, varying exposure scenarios (e.g. spraying, using dusty powder, using products with enzymes in liquids in the shower) and different enzymes should be taken into account. And in addition to respiratory sensitisation, other hazards should not be overlooked when performing risk assessment, including irritation of lungs, skin and eyes due to proteases, possible hazards of enzyme coatings, or possible new hazards of enzymes that have not been used in consumer products before.

As shown in Chapter 3, information on the type of enzyme(s) in the products is not always declared. This depends for instance on the relevant legal framework that the product falls under, the concentration of the ingredient, or hazard (classification) of the ingredient. Information of the NVZ shared with RIVM gave some insight in the type and concentration of enzymes in two spray trigger applications. It would be helpful also to retrieve the enzyme composition and concentrations of other trigger spray products present on the Dutch market in order to perform exposure assessments with ConsExpo. Also, personal care products with enzymes would be of interest. Such products could potentially not only lead to high enzyme concentration in the air, as shown by Kelling et al. (1998), but might also be of concern for skin irritation. The use of enzymes in personal care products is often aimed to enzymatically peel the skin, i.e. by exfoliation (Gonçalves, 2021), and skin care products with enzymes are present on the market. It should be noted that for the (prototype) cleansing bar product which resulted in the high enzyme concentration of 183 ng/m^3 in the air reported by Kelling et al. (1998), the further development was halted because of the induction of immunogenic responses among the study subjects, which might potential lead to clinical allergy symptoms in a larger population (Kelling et al., 1998).

As Basketter et al. (2010) suggest, the DMEL of 15 ng/m^3 should be used as starting point for the risk assessment of enzymes that have no other specific DMEL and for which there is no other data available that indicate a more appropriate level. That current, generally used, DMEL is however based on a limited amount of data, and results from epidemiology studies for other enzymes result in even lower health based limits. Ideally, studies should be used in which actual exposure is measured. There are, however, technical limitations in measuring personal exposure, as explained in Chapter 6. Overcoming these technical difficulties, it would be desirable to be able to perform personal exposure assessments.

7. Summarizing information and recommendations

This report provides an overview of current regulations related to the safe use of enzymes in consumer products and an explorative overview of types of consumer products with enzymes, as well as the types of enzymes used in those products through a market survey. It shows the use of varying types of enzymes in cleaning products for consumer use and personal care products, and a few products for pets.

The market survey also indicates that for a number of products, the type of enzyme used is not specified in the list of ingredients. Additionally, the final concentration of enzymes is not specified in any of the products. A more transparent listing of enzymes in the ingredients, possible including a range of concentrations like in detergent products, could help to provide relevant information regarding the type and content of the enzyme(s). The declaration of specific enzymes and especially their final concentrations on the product label is not warranted in every product legislation. In case CLP is applicable (the enzyme has a harmonised classification for respiratory sensibilisation), the concentration of enzymes in many products is often below the generic concentration limit triggering declaration. A further (limited) survey amongst users and producers of enzymes did not reveal more information on concentration and type of enzymes and products. However, this information should be available throughout the chain according to the downstream communication obligation under REACH, including an Exposure Scenario (ECHA, 2013; Wijnhoven & Affourtit, 2018). This could be examined during an inspection. The survey also indicated that users (formulators) expected an increased use of enzymes in the future.

For the majority of products it was clear under which regulations they belong. The enzyme ingredients are regulated as a substance under REACH. A number of restrictions or obligations are present under the REACH Regulation as well as in certain product specific legislations.

Knowledge on the effects of human exposure to enzymes was based on studies on workers and consumer populations. According to the available studies, in general, the acute and systemic toxicity of enzymes is low. However, they can cause eye and skin irritation at higher concentrations (i.e. higher than their use in personal care products products) and most importantly, cause respiratory sensitisation.
In addition, as observed in the literature research on the development of allergies in companies producing enzymes, the number of workers being sensitised increases with the exposure time. Therefore, monitoring the development of sensitisation among the general population through product surveillance or assessment of consumer incidences could help to identify potential allergy development as a result of the increased use of products containing enzymes. Hazard identification showed that respiratory sensitisation is the main effect when considering enzymes. Industry proposed a DMEL of 15 ng/m^3, based on protease, but proposed to be generally used for enzymes. This DMEL is associated with several uncertainties, as is explained in Chapter 7.

Evaluation of consumer exposure also revealed a lack of information, starting with the concentration of enzymes used in consumer products, as shown by the market research. Unfortunately, no additional information was obtained by the questionnaire among producers and distributors of products with enzymes. Information on two specific products was retrieved through personal contact with a trade association of detergents. ConsExpo was used for modelling exposure of these products. It demonstrated that the consumer exposure through the use of a trigger spray odour control product containing 3 different enzymes led to an exposure above the DMEL. Available measurements on different enzyme-product combinations showed enzyme concentrations in the air up to 183 ng/m^3. This indicates such products should be assessed more closely, to evaluate whether their safety is sufficiently covered.

More information is needed in order to estimate the exposure through other consumer products, and more insight is needed on the validity of the present DMEL. Respiratory sensitisation is a hazard for which no regulatory animal test is available. Possibly, new DMELs could be set for specific enzymes based on additional studies. As the number of products containing enzymes will continue to increase according to different manufacturers, there is a need to improve the current practise of assessing the risks of consumer exposure to enzymes through the use of non-food products containing enzymes. This should include the risk of sensitisation by repeated exposure to enzymes.

The overview in this report helps to set recommendations for future evaluations. Such recommendations could be to:

- Perform an inspection in enterprises formulating or distributing products containing enzymes and examine the available information, i.e. Safety Data Sheets and Exposure Scenario documents.
- Perform a market survey on spray (cleaning) products with enzymes, as well as personal care products with enzymes, obtaining concentration data, to be able to perform more informed exposure assessments;
- Perform product surveillance or assessment of consumer incidences on respiratory sensitisation (possibly) caused by exposure to enzymes;
- Setting health based limits for specific enzymes in order to replace the current, generic DMEL, also taking into account new or more robust study data.

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References

ACI (American Cleaning Institute). (2019). Guidance for the risk assessment of enzyme-containing consumer products. https://www.cleaninginstitute.org/sites/default/files/research-pdfs/ACIConsumerEnzymeProductRiskAssessmentGuide.pdf

AISE (International Association for Soaps, detergents and Maintenance Products). (2020). Exposure measurements of enzymes for risk assessment of household cleaning spray products. https://www.aise.eu/documents/document/20210401175430-a_i_s_e__enzyme_spray_protocol_revision_july_2020.pdf

AMFEP (Association of Manufacturers & Formulators of Enzyme Products). (2013). Guide to the safe handling of industrial enzyme preparations. https://amfep.org/_library/_files/amfep-guide-on-safe-handling-of-enzymes-updated-in-2013.pdf

Basketter, D., Moreno, N., Simonsen, M. (2021). Occupational exposure limits for enzymes: practical considerations. *International Journal of Pulmonary & Respiratory Sciences 5*(1): 555651. https://doi.org/10.19080/IJOPRS.2021.05.555651

Basketter, D., Berg, N., Kruszewski, F. H., Sarlo, K., Concoby, B. (2012a). Relevance of sensitization to occupational allergy and asthma in the detergent industry. *Journal of Immunotoxicology 9*(3): 314-319. https://doi.org/10.3109/1547691x.2012.656855

Basketter, D., Berg, N., Kruszewski, F. H., Sarlo, K., Concoby, B. (2012b). The toxicology and immunology of detergent enzymes. *Journal of Immunotoxicology 9*(3): 320-326. https://doi.org/10.3109/1547691X.2012.659358

Basketter, D., Berg, N., Broekhuizen, C., Fieldsend, M., Kirkwood, S., Kluin, C., Mathieu, S., Rodriguez, C. (2012c). Enzymes in cleaning products: an overview of toxicological properties and risk assessment/management. *Regulatory Toxicology and Pharmacology 64*(1): 117-123. https://doi.org/10.1016/j.yrtph.2012.06.016

Basketter, D. A., Broekhuizen, C., Fieldsend, M., Kirkwood, S., Mascarenhas, R., Maurer, K., Pedersen, C., Rodriguez, C., Schiff, H. E. (2010). Defining occupational and consumer exposure limits for enzyme protein respiratory allergens under REACH. *Toxicology 268*(3): 165-170. https://doi.org/10.1016/j.tox.2009.12.014

Basketter, D.A., Kimber, I. (2022). Enzymes and sensitization via skin exposure: a critical analysis. *Regulatory Toxicology and Pharmacology 129*: 105112. https://doi.org/10.1016/j.yrtph.2021.105112

Bremmer, H.J., Prud'homme de Lodder, L.C.H., van Engelen, J.G.M. (2006). Cosmetics Fact Sheet. To assess the risks for the consumer. Updated version for ConsExpo 4. RIVM report 320104001/2006. https://www.rivm.nl/bibliotheek/rapporten/320104001.pdf

Britannica. (2022). Enzyme (biochemistry). Retrieved 14 April from: https://www.britannica.com/science/enzyme

Budnik, L. T., Scheer, E., Burge, P. S., Baur, X. (2017). Sensitising effects of genetically modified enzymes used in flavour, fragrance, detergence and pharmaceutical production: cross-sectional study. *Occupational & Environmental Medicine 74*(1): 39-45. https://doi.org/10.1136/oemed-2015-103442

CJEU. (2019). Judgement of the Court (Ninth Chamber). 19 December 2019. In case C-592/18. https://curia.europa.eu/juris/document/document.jsf?text=&docid=221808&pageIndex=0&doclang=EN&mode=lst&dir=&occ=first&part=1&cid=32280

CPID. (2022). Consumer Product Information Database. https://www.whatsinproducts.com/

Creative Enzymes. (2022). Application of Enzymes in Detergent Industry. Retrieved 14 April from: https://www.creative-enzymes.com/resource/application-of-enzymes-in-detergent-industry_61.html

EC. (1998). Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. http://data.europa.eu/eli/dir/1998/8/oj

EC. (2001a). Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration. http://data.europa.eu/eli/dir/2001/18/oj

EC. (2001b). Directive 2001/95/EC of the European Parliament and of the Council of 3 December 2001 on general product safety. http://data.europa.eu/eli/dir/2001/95/oj

EC. (2003a). Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. http://data.europa.eu/eli/reg/2003/1829/oj

EC. (2003b). Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. http://data.europa.eu/eli/reg/2003/1830/oj

EC. (2003c). Regulation (EC) No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on transboundary movements of genetically modified organisms.
http://data.europa.eu/eli/reg/2003/1946/oj

EC. (2004). Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents.
http://data.europa.eu/eli/reg/2004/648/oj

EC. (2006). Regulation (EC) no 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency.
http://data.europa.eu/eli/reg/2006/1907/2014-04-10

EC. (2008). Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. http://data.europa.eu/eli/reg/2008/1272/oj

EC. (2009a). Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.
http://data.europa.eu/eli/reg/2009/1223/oj

EC. (2009b). Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. http://data.europa.eu/eli/dir/2009/41/oj

EC. (2012). Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products.
http://data.europa.eu/eli/reg/2012/528/oj

EC. (2013). Borderline between the legislation for cosmetics and biocides. CA-Jul13-Doc.5.1.h. https://english.ctgb.nl/binaries/ctgb-en/documenten/assessment-framework-biocides/2017/09/21/borderline-between-the-legislation-for-cosmetics-and-biocides-%E2%80%93-scope-bpr/CA_July13_Doc.5.1.h_borderline_cosmetics_biocides_.pdf

EC. (2017). Technical document on cosmetic claims. Agreed by the Sub-Working Group on Claims (version of 3 July 2017). Available at:
https://www.bing.com/ck/a?!&&p=249e42b48b88d60cJmltdHM9MTY2MzYzMjAwMCZpZ3VpZD0xODBlNjZhMy1lMzMyLTZjOWMtMzJiZi03NDgzZTJjNzZkY2MmaW5zaWQ9NTE2Nw&ptn=3&hsh=3&fclid=180e66a3-e332-6c9c-32bf-7483e2c76dcc&u=a1aHR0cHM6Ly9lYy5ldXJvcGEuZXUvZG9jc3Jvb20vZG9jdW1lbnRzLzI0ODQ3L2F0dGFjaG1lbnRzLzEvdHJhbnNsYXRpb25zL2VuL3JlbmRpdGlvbnMvbmF0aXZl&ntb=1

EC. (2019). Commission delegated Regulation (EU) 2020/217 of 4 October 2019 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures and correcting that Regulation. http://data.europa.eu/eli/reg_del/2020/217/oj

EC. (2022a). CosIng database. Cosmetic ingredient database [consulted September 2022]. https://single-market-economy.ec.europa.eu/sectors/cosmetics/cosmetic-ingredient-database_en

EC. (2022b). Commission Implementing Decision (EU) 2022/146 of 1 February 2022 determining whether a product containing Alkyl (C12-16) dimethylbenzyl ammonium chloride is a biocidal product, pursuant to Article 3(3) of Regulation (EU) No 528/2012 of the European Parliament and of the Council. http://data.europa.eu/eli/dec_impl/2022/146/oj

ECHA. (2012). Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health. Version 2.1. https://echa.europa.eu/documents/10162/17224/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258?t=1353935239897

ECHA. (2013). Forum REACH-EN-FORCE 2 Project Report. Obligation of downstream users - formulators of mixtures. ECHA-13-R-12-EN. https://echa.europa.eu/documents/10162/17088/forum_report_ref2_en.pdf/6ae12cf0-a24d-4263-a30f-3dabf9928aed?t=1387376070391

EFSA. (2021). EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Castle, L., Engel, K.-H., Fowler, P., Frutos Fernandez, M.J., Fürst, P., Gundert-Remy, U., Gürtler, R., Husøy, T., Manco, M., Mennes, W., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, I., Wölfle, D., Corsini, E., Cubadda, F., De Groot, D., FitzGerald, R., Gunnare, S., Gutleb, A.C., Mast, J., Mortensen, A., Oomen, A., Piersma, A., Plichta, V., Ulbrich, B., Van Loveren, H., Benford, D., Bignami, M., Bolognesi, C., Crebelli, R., Dusinska, M., Marcon, F., Nielsen, E., Schlatter, J., Vleminckx, C., Barmaz, S., Carfí, M., Civitella, C., Giarola, A., Rincon, A.M., Serafimova, R., Smeraldi, C., Tarazona, J., Tard, A., Wright, M. Safety assessment of titanium dioxide (E171) as a food additive. *EFSA Journal 19*(5): 6585. https://doi.org/10.2903/j.efsa.2021.6585

EU. (2015). Directive (EU) 2015/412 of the European Parliament and of the Council of 11 March 2015 amending Directive 2001/18/EC as regards the possibility for the Member States to restrict or prohibit the cultivation of genetically modified organisms (GMOs) in their territory. http://data.europa.eu/eli/dir/2015/412/oj

EWG. (2022). Environmental Working Group. EWG's Skin Deep. https://www.ewg.org/skindeep/

Gonçalves, S.G.D. (2021). Use of enzymes in cosmetics: proposed enzymatic peel procedure. *CosmEthically Active Journal 1*: 27-33. https://cosmethicallyactive.com/wp-content/uploads/2021/11/Use-of-enzymes-in-cosmetics-proposed-enzymatic-peel-procedure.pdf

Gürkök, S. (2019). Microbial enzymes in detergents: a review. *International Journal of Scientific & Engineering Research 10*(9): 75-81. https://www.ijser.org/researchpaper/Microbial-Enzymes-in-Detergents-A-Review.pdf

Hasan, F., Shah, A.A., Javed, S., Hameed, A. (2010). Enzymes used in detergents: lipases. *African Journal of Biotechnology 9*(31): 4836-4844. https://doi.org/10.5897/AJBx09.026

Health Council of the Netherlands. (2014). Fungal alpha-amylase (derived from the fungus *Aspergillus oryzae*) - Health-based recommended occupational exposure limit. Publication no. 2014/25. https://www.ser.nl/api/Mfiles/DownloadFirstDocument?Id=418e4103-c2f6-49e7-b7ab-8ca94da536ad

Heederik, D.J. (2019). Towards evidence-informed occupational exposure limits for enzymes. *Annals of Work Exposures and Health 63*(4): 371-374. https://doi.org/10.1093/annweh/wxz004

HERA. (2005). Human & Environmental Risk Assessment on ingredients of household cleaning products. α-Amylases, cellulases and lipases. Edition 1.0. November 2005. https://www.heraproject.com/files/38-F-Hera_Bridging_document_28.10.05.pdf

HERA. (2007). Human & Environmental Risk Assessment on ingredients of household cleaning products. Subtilisins (protease). Edition 2.0. February 2007. https://www.heraproject.com/files/22-F-07_PROTEASE_HERA_Final%20Edition%20(unsecured%20-%20PDFA-1b).pdf

Herman, H.A., Good, I., Laufer, A. (1997). Manufacturing and downstream processing of detergent enzymes. In: Enzymes in Detergency (Eds: van Ee ,J.H., Misset, O., Baas E.J.), Marcel Dekker Inc., New York, pp 251–298.

IARC. (2010). IARC (International Agency for Research on Cancer) Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 93. Carbon black, titanium dioxide, and talc. https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono93.pdf

Kelling, C.K., Bartolo, R.G., Ertel, K.D., Smith, L.A., Watson, D.D., Sarlo, K. (1998). Safety assessment of enzyme-containing personal cleansing products: exposure characterization and development of IgE antibody to enzymes after a 6-month use test. *Journal of Allergy and Clinical Immunology 101*(2 Pt 1): 179-187. https://doi.org/10.1016/S0091-6749(98)70382-3

Malgas, S., van Dyk, J.S., Pletschke, B.I. (2015). A review of the enzymatic hydrolysis of mannans and synergistic interactions between β-mannanase, β-mannosidase and α-galactosidase. *World Journal of Microbiology & Biotechnology 31*(8): 1167-1175. https://doi.org/10.1007/s11274-015-1878-2

Maurer, K. H. (2004). Detergent proteases. *Current Opinion in Biotechnology 15*(4): 330-334. https://doi.org/10.1016/j.copbio.2004.06.005

Meesters, J.A.J., Nijkamp, M.M., Schuur, A.G., te Biesebeek, J.D. (2018). Cleaning Products Fact Sheet. Default parameters for estimating consumer exposure. Updated version 2018. RIVM Report 2016-0179. https://www.rivm.nl/bibliotheek/rapporten/2016-0179.pdf

Meesters, J.A.J., te Biesebeek, J.D., ter Burg, W. (2022). Air freshener fact sheet. Default parameters for estimating consumer exposure – version 2021. RIVM Report 2021-0233. https://www.rivm.nl/bibliotheek/rapporten/2021-0233.pdf

Nicholson, P.J. (2022). Enzymes: new uses for old hazards. *Occupational Medicine 72*(6): 351-353. https://doi.org/10.1093/occmed/kqab064

NIH (National Institute of Health). (2022). Enzyme. Retrieved 14 April from: https://www.genome.gov/genetics-glossary/Enzyme

Novozymes. (2020). A beginner's guide to enzymes in detergents. https://biosolutions.novozymes.com/en/dish/insights/article/beginners-guide-enzymes-detergents

Novozymes. (2022). Enzymes. Retrieved 4 April 2022 from: https://www.novozymes.com/en/biology

OECD, 2019. Considerations when assessing children's exposure to chemicals from products. Series on Testing and Assessment No. 310. ENV/JM/MONO(2019)29. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2019)29&docLanguage=en

Olsen, H. S., Falholt, P. (1998). The role of enzymes in modern detergency. *Journal of Surfactants and Detergents 1*(4): 555-567. https://doi.org/10.1007/s11743-998-0058-7

Pepys, J., Longbottom, J.L., Hargreave, F.E., Faux, J. (1969). Allergic reactions of the lungs to enzymes of Bacillus subtilis. *The Lancet 293*(7607): 1181-1184. https://doi.org/10.1016/S0140-6736(69)92166-7

Razenberg, L. B., D., Graven, C., de Jonge, R., Wezenbeek, J. (2020). Microbial cleaning products: an inventory of products, potential risks and applicable regulatory frameworks. RIVM letter report 2020-0160. https://www.rivm.nl/bibliotheek/rapporten/2020-0160.html

Sanchez, S., Demain, A. L. (2011). Enzymes and bioconversions of industrial, pharmaceutical, and biotechnological significance. *Organic Process Research & Development 15*(1): 224-230. https://doi.org/10.1021/op100302x

Sarlo, K., Kirchner, D.B., Troyano, E., Smith, L.A., Carr, G.J., Rodriguez, C. (2010). Assessing the risk of type 1 allergy to enzymes present in laundry and cleaning products: Evidence from the clinical data. *Toxicology 271*: 87-93. https://doi.org/10.1016/j.tox.2010.03.007

SCCS. (2017). Checklists for Applicants submitting dossiers on Cosmetic Ingredients to be evaluated by the SCCS. SCCS/1588/17. https://health.ec.europa.eu/system/files/2021-08/sccs_o_210_0.pdf

SCCS. (2019). Guidance on the safety assessment of nanomaterials in cosmetics. SCCS/1611/19. https://health.ec.europa.eu/system/files/2020-10/sccs_o_233_0.pdf

SCCS. (2021). SCCS Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation - 11th revision. SCCS /1628/21. https://health.ec.europa.eu/publications/sccs-notes-guidance-testing-cosmetic-ingredients-and-their-safety-evaluation-11th-revision_en

SDA. (2005). Soap and Detergent Association (SDA). Risk assessment guidance for enzyme-containing products. https://www.cleaninginstitute.org/sites/default/files/research-pdfs/SDA_Enzyme_Risk_Guidance_October_2005.pdf

SER. (2022a). Sociaal-Economische Raad. Grenswaarde Schimmel alfa-amylase. Retrieved 20 December 2022 from: https://www.ser.nl/nl/thema/arbeidsomstandigheden/Grenswaarden-gevaarlijke-stoffen/Grenswaarden/Schimmel-alfa-amylase

SER. (2022b). Sociaal-Economische Raad. Grenswaarde Proteïnase. Retrieved 20 December 2022 from: https://www.ser.nl/nl/thema/arbeidsomstandigheden/Grenswaarden-gevaarlijke-stoffen/Grenswaarden/Proteinase

Smit, K., Schuur, A.G. (2014). Overview of legislation on sensitisers. With focus on consumer products. RIVM letter report 050013001/2014. https://www.rivm.nl/bibliotheek/rapporten/050013001.pdf

Smithers. (2021). The Future of Sustainable Cleaning Products to 2026. https://www.smithers.com/services/market-reports/materials/future-of-sustainable-cleaning-products-to-2026

van de Plassche, E.J., Bont, P.H.F., Hesse, J.M. (1999). Exploratory report on Fluorescent Whitening Agents (FWAs). RIVM report 601503013.

Van der Putte, I., Qi, S., Affourtit, F., de Wolf, K., Devaere, S., Albrecht, E. (2013). Study on the link between allergic reactions and chemicals in textile products. RPS Report VRM11.8088. https://www.rivm.nl/bibliotheek/rapporten/050013001.pdf

van Rooy, F. G., Houba, R., Palmen, N., Zengeni, M. M., Sander, I., Spithoven, J., Rooyackers, J. M., Heederik, D. J. (2009). A cross-sectional study among detergent workers exposed to liquid detergent enzymes. *Occupational Environmental Medicine 66*(11) : 759-765. https://doi.org/10.1136/oem.2008.045245

Vanhanen, M., Tuomi, T., Tiikkainen, U., Tupasela, O., Voutilainen, R., Nordman, H. (2000). Risk of enzyme allergy in the detergent industry. O*ccupational and Environmental Medicine 57*(2): 121-125. https://doi.org/10.1136/oem.57.2.121

Warenwet. (2022). Warenwet. Retrieved 20 December 2022 from: https://wetten.overheid.nl/BWBR0001969/2021-07-01

Warenwetbesluit Algemene Productveiligheid. (1993). Warenwetbesluit Algemene Productveiligheid. https://wetten.overheid.nl/BWBR0006158/2020-06-09

Weeks, J.A., Harper, R.A., Simon, R.A., Burdick, J.D. (2011). Assessment of sensitization risk of a laundry pre-spotter containing protease. *Cutaneous and Ocular Toxicology 30*(4): 272-279. https://doi.org/10.3109/15569527.2011.565010

Wijnhoven, S., Affourtit, F. (2018). Approaches and tools to better support consumer exposure assessment under REACH. final report of the DG Environment Project "Approaches and tools to better support consumer exposure assessment under REACH" executed by RIVM, RPA and FoBiG. https://ec.europa.eu/environment/chemicals/reach/pdf/Full%20Report%20RIVM%20Cons.pdf

Working Group on Cosmetic Products (2020). Manual of the Working Group on Cosmetic Products (Sub-Group on Borderline Products) on the scope of application of the Cosmetics Regulation (EC) No 1223/2009 (Art. 2(1)(A)) Version 5.2 (September 2020). Available at: https://ec.europa.eu/docsroom/documents/42850

Zheng, L., Xu, Y., Li, Q., Zhu, B. (2021). Pectinolytic lyases: a comprehensive review of sources, category, property, structure, and catalytic mechanism of pectate lyases and pectin lyases*. Bioresources and Bioprocessing 8*: 79. https://doi.org/10.1186/s40643-021-00432-z

Annex 1 Regulatory frameworks

This Annex provides an overview of regulatory frameworks within the European Union and the Netherlands which are relevant for non-food consumer products which can contain enzymes. For each framework a number of aspects will be discussed. These aspects include the aim of the framework, the requirements for access to the market, possible requirements with regard to the safety evaluation and quality of the product or substance (i.e. the enzyme) falling under that regulation. Also possible specific requirements for enzymes or microbes as well as possible labelling requirements of the product under the regulation are described.

A1.1. Chemicals Strategy for Sustainability (CSS)

As part of the EU Green Deal, the European Commission (EC) adopted the Chemicals Strategy for Sustainability (CSS) in October 2020. The CSS is a EU strategy for the future and lays down targets for a non-toxic environment and safe and sustainable by design chemicals.
To be able to meet the CSS targets, rules under existing EU law will change in the coming years. An important instrument for these changes are the revision of the REACH and CLP regulations that are currently at stake. Proposals for changes have been made and currently (September 2022) an impact assessment is performed.

Three potential changes that may be relevant specifically for enzymes, are mentioned below. Formal decision on the actual changes are expected in 2023.

One substance one assessment
Currently, different approaches for hazard and risk assessment exist in different EU legislations. The aim of CSS is to simplify and harmonize this over various regulations. So that an assessment for a substance performed under one legislative framework can be used in other frameworks as well. To realize this, the current idea is to use CLP as backbone for hazard classification. Also, methodologies need to be more coherent/harmonized. Furthermore, it requires transparent assessments and exchange of information/data. The aim is also to move towards assessing and regulating groups of substances with structural and functional similarities instead of single substances.

Extending Generic Risk management Approach (GRA)
The EC has the intention to extend the Generic Risk management Approach (GRA) that is regulated via article 68(2) of REACH. This article regulates that hazard itself is enough to warrant restriction of a chemical or a group of substances. Article 68(2) currently regulates this for CMR 1A/1B substances in consumer products. The proposal is to gradually extend this to other hazard categories:

- in a first step to ED HH/ENV, PBT/vPvB, PMT/vPvM[2] categories;

[2] endocrine disrupters to human health and environment, persistent bioacumulative and toxic and very persistent and very bioacumulative substances, persistent mobile and toxic and very persistent and very mobile substances

- in a second step to immunotoxicity, neurotoxicity, specific target organ toxicity and respiratory sensitisation.

Furthermore, the proposal is to extend the GRA to consumer articles and professional uses of substances and mixtures.

The current Restriction Roadmap – in which (groups of) substances targeted for restriction, e.g. skin sensitisers, are taken up – will still be in place.

SVHC identification triggers higher information requirements
Currently, the available information on use and exposure is often limited. The EC has the intention to increase information requirement on use and exposure to be able to better estimate potential risk of SVHC substances. The idea is that once a substance is included in the Candidate List, this automatically triggers the increased information requirements on use and exposure.

From the above suggested changes for the REACH revision, especially the GRA extension to respiratory sensitisers may have significant consequences for the use of enzymes. How this will actually work out for enzymes, depends on the details of the regulatory changes and whether or not exemptions for essential uses are possible and under what conditions, and on the priority COM gives to certain groups (and uses) of substances for restrictions under art. 68(2).

Substances of very high concern (SVHC) play a key role. Their identification is central in REACH and could be strengthened and the scope and application could be widened to more hazard classes such as endocrine disruptors, persistent and bioaccumulative substances, neurotoxicants, immunotoxicants, substances with effects on specific target organs and on the respiratory system. Identified SVHCs undergo scrutiny for regulatory management measures to address the possible risks of uses. In doing this the Commission intends to extend the generic risk management principle under which (groups of) substances can be restricted in applications such as consumer and professional product formulations through a simplified procedure
As many enzymes have the hazard property of being respiratory sensitisers it will be pivotal to follow up on the policy discussions on the implementation of changes in REACH as regards SVHC identification and the consequential downstream effects.

A1.2. REACH Regulation

Aim of REACH
REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) (EC, 2006) is a regulatory framework for chemicals in the European Union. The aim of REACH is to ensure a high level of protection of human health and the environment, and the free circulation of substances on the internal EU market. REACH lays down provisions on substances and mixtures that apply to the manufacture, placing on the market or use of substances on their own, in mixtures or in articles and to the placing on the market of mixtures.

Enzymes under REACH
Enzymes are considered substances[3] under REACH. This means that all obligations for substances, like registration and information requirements, apply equally to enzymes. Specifically, enzymes are UVCB substances (substances of unknown or variable composition, complex reaction products or of biological materials) under REACH. This does not alter the obligations but may influence how they are fulfilled, e.g. the identification of the substance in the registration dossier. REACH also applies to enzymes created using recombinant DNA techniques.
Many enzymes exhibit irritation and sensitisation properties. Information on the irritation and skin sensitisation properties of substances placed on the market are required from market volumes above 1 tonne/year. As no validated tests are available for respiratory sensitisation, no information requirements are included in REACH Annex VII through X. However, if information on respiratory sensitisation is available (e.g. incidents in the workplace), it must be included in the registration dossier.

Access to market and responsibilities
REACH is based on the principles that it is the responsibility of manufacturers, importers and downstream users to ensure that they manufacture, place on the market or use substances that do not adversely affect human health or the environment. A manufacturer or importer putting a substance (on its own or in a mixture) on the market in quantities of 1 tonne per year or more is required to register the substance and prepare a registration dossier fulfilling the information requirements specified in REACH. The accuracy and correctness of the information contained in the dossier and maintenance of that dossier, remain the responsibility of the registrant.

Information requirements for safety assessment
The information requirements of REACH are tonnage dependent. For substances that are placed on the market in quantities above 1 tonne/year, information is required according to the appropriate Annexes of REACH (VII through X) and applying the considerations on testing and waiving laid down in Annex XI. With increasing market volume, additional hazard information is required. For substances that are registered in quantities of 10 tonnes/year or more, a chemical safety assessment (CSA) is required as part of the registration. The chemical safety assessment starts with a hazard assessment. Only in case the substance is classified according to CLP (Classification, Labeling and Packaging Regulation) or when it is PBT (Persistent, Bioaccumulative and Toxic) or vPvB (very Persistent and very Bioaccumulative), an exposure assessment and risk characterisation needs to be included in the CSA. Where a risk is shown, additional risk reduction measures have to be taken to ensure safe use. If this is not possible, the substance may not be used for that application.

[3] A substance is defined as "*a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition*."

Labelling and communication requirements
Hazard classification of chemicals is a central aspect within the EU chemicals legislation. Classification, labelling and packaging requirements for substances and mixtures are regulated by the CLP regulation which is based on the UN Globally Harmonised System (GHS). The REACH Regulation requires that the relevant (safety) information of substances and mixtures is communicated in the supply chain. For substances or mixtures that obey certain criteria (e.g. those that are classified under the CLP regulation), a safety data sheet (SDS) should be provided with the product to the professional downstream user. The SDS contains information on hazards, composition of mixtures, exposure limits, risk management measures, emergency measures etc. Any downstream user should take this information into account to work safely with the product, and if relevant forward the information to their own clients.

Other relevant aspects
Certain product categories, like food additives, personal care products or medicine, are exempted from specific obligations under REACH (e.g. registration or information requirements). Usually these categories fall under specific product legislation and the obligations are set elsewhere. REACH requirements do not apply to waste because waste is not considered a substance, mixture or article under REACH. Materials recovered from waste in the EU are however in the scope of REACH but registration exemptions apply under certain conditions. In addition, registrants of regular chemicals need to include the waste stage in their registration dossiers and chemical safety assessments proving the registered chemicals is used safely across all life cycle stages.

A1.3. CLP Regulation

Aim of CLP
The CLP (Classification, Labelling and Packaging of substances and mixtures) Regulation (EC, 2008) has two aims:

- to protect human health and the environment by providing criteria for hazard classification of substances and mixtures placed on the EU market, and rules on the labelling and packaging of hazardous substances and mixtures that are on the market, and
- ensure the free movement of substances, mixtures and articles (Article 1).

Enzymes under CLP
Enzymes meet the definition of a substance[4] under the CLP Regulation and are therefore subject to all the requirements of the CLP Regulation, including classification, labelling, packaging and downstream communication. Enzymes may also be present as a mixture[5] or in a product.
For the purpose of this section, it is important to note that the primary hazard concern of enzymes is respiratory sensitisation. Various enzymes

[4] The definition of a substance under CLP equals the REACH definition as mentioned in 3.2.2: "*a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition*."
[5] A mixture is defined as "*a mixture or solution composed of two or more substances*" (Article 2).

are classified as respiratory sensitisers. Further information on the hazard properties of enzymes is discussed in Chapter 5 of the current report.
The generic concentration limit of a component of a mixture that is classified as respiratory sensitiser is ≥0.1% for liquid or solid respiratory sensitisers of category 1A, and ≥1.0 % for liquid or solid respiratory sensitiser of category 1 or category 1B (CLP Annex I, Table 3.4.5).
If the concentration of the enzyme in the mixture (product) is below this concentration, the mixture is considered non-hazardous and does not need to be classified or labelled.[6]

Access to market and responsibilities
The CLP Regulation contains obligations for manufacturers, importers and downstream users to classify substances and mixture based on their hazardous properties before placing them on the market, using the criteria specified in the CLP Regulation. These requirements are independent of market volume. Furthermore, manufacturers, importers and producers of articles must also classify the substances not placed on the market, such as for example intermediates, that are subject to registration or notification under REACH.
The CLP Regulation obliges suppliers to label and package substances and mixtures placed on the market. Furthermore, the manufacturers and importers of hazardous substances must notify the European Chemicals Agency (ECHA) of the classification and labelling elements that apply for their substance (Article 1 and 4). The notifications are collected in the online ECHA C&L inventory database (C&L Inventory - ECHA (europa.eu)).
Certain substances and mixtures in the finished state, intended for the final user, are exempted from the CLP Regulations. Among these are cosmetic products as defined in Regulation (EC) No 1223/2009.

Information requirements for safety assessment
A substance or a mixture fulfilling the criteria (as specified in CLP Parts 2 to 5 of Annex I (Article 3)) relating to physical hazards, health hazards or environmental hazards is considered hazardous and shall be classified in relation to the respective hazard classes. The CLP Regulation contains detailed provision on how to gather and evaluate data that can be used for classification, and how to derive the classification. However, testing is only required for the physical hazards in case insufficient data are available to derive a classification. The CLP also contains provision on the concentration limits of components in mixtures below which a hazardous property no longer needs to be considered. These concentration limits apply when there are no data on the whole mixture except for CMR hazards which are always based on the concentration of the components. Where a substances is included in the list of substances with a harmonised classification (CLP, Annex VI), the classification stated for that substance must be used.
Interestingly, for substances which are (self)classified as respiratory sensitisers, it is noted that these classifications are usually based on self-classification rather than on harmonised classifications (Smit & Schuur, 2014; van der Putte et al., 2013).

[6] Unless there is a specific concentration limit set for a certain enzyme that is lower than the values presented here.

Labelling and communication requirements
Hazardous substances and mixtures must be labelled before they are placed on the market, except if they meet certain exemptions specified in Article 29 of CLP. The CLP hazard label must contain specific elements (see Article 17) including the product identifier, hazard pictogram, signal word and hazard statement, and any precautionary statements and supplementary information where applicable.
The product identifier shall contain the identity of all substances in the mixture that contribute to the classification of the mixture as regards acute toxicity, skin corrosion or serious eye damage, germ cell mutagenicity, carcinogenicity, reproductive toxicity, respiratory or skin sensitisation, specific target organ toxicity (STOT) or aspiration hazard. The CLP specifically states that statements such as 'non-toxic', 'non-harmful', 'non-polluting', 'ecological' or any other statements indicating that the substance or mixture is not hazardous or any other statements that are inconsistent with the classification of that substance or mixture shall not appear on the label or packaging of that substance or mixture (Article 25, paragraph 4).

The classification of a substance under CLP often leads to regulatory obligations for the substance under other legislative frameworks.

A1.4. Biocidal Product Regulation (BPR)

Aim of the BPR
The Biocidal Product Regulation (BPR) (EC, 2012) concerns the placing on the market and use of biocidal products intended to protect humans, animals, materials or articles against harmful organisms by the action of active substances contained in the biocidal product. The purpose of the BPR is to improve the free movement of biocidal products within the EU through harmonisation of the rules, while ensuring a high level of protection of both human and animal health and the environment.

Enzymes under the BPR
Annex V of the BPR describes the biocidal product[7] types that fall within the scope of this regulation. Potentially relevant groups for products containing enzymes are disinfectants (main group 1). Main group 1 is divided into five product types (PTs): disinfectants for human hygiene (PT1), disinfectants and algaecides not intended for direct application to humans or animals (PT2), disinfectants for veterinary hygiene (PT3), disinfectants for the food and feed area (PT4) and disinfectants for drinking water (PT5). For products containing enzymes, PT2 seems to be the most relevant one. Some products might fall within the scope of PT1 (e.g. hand soaps) or PT4 (e.g. kitchen cleaners).

However, the first sentence on main group 1 of Annex V says "These product-types exclude cleaning products that are not intended to have a biocidal effect, including washing liquids, powders and similar products". As stated above, the intention of having a biocidal effect and the

[7] According to the BPR a 'biocidal product' means '*any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action*' (BPR, art. 3, 1a).

presence of active substances[8] determine whether the BPR applies. Enzymes can meet the definition of active substances. Products with enzymes may fall within the scope of the BPR if the enzymes are active against harmful organisms.

Until now we have not heard of the intention of regulators to bring this kind of products containing enzymes under the BPR. Bringing these product under the BPR would set a high threshold, because the BPR makes high demands (high costs, long procedures) on the approval of active substances and the authorisation of biocides. There are no enzymes allowed as active substances yet. Bringing enzymatic cleaners under the BPR is only possible by a coordinated European action. The below mentioned 'Darie-arrest' (Textbox 1 below) and the art.3.3 decision of the European Commission could be used by regulators or enforcers to broaden the scope of the BPR to cleaning products containing enzymes with the intention to prevent the growth of microorganisms or to control the effects of them (for instance unwanted odours). Especially products against unwanted odours seem to be also active against unwanted microorganisms.

Access to market and responsibilities
According to the BPR, biocidal products must be authorised by national Competent Authorities or by the European Chemical Agency (ECHA) before they can be placed on the market. Biocidal products must contain active substances. Active substances must be approved by the European Commission or be included in de review programme for the PT in which they will be used. Active substances (in a biocidal product) are defined as *'a substance or microorganism with an action on or against harmful organisms'* (BPR, art. 3, 1c). At the moment (end of 2022) there are no enzymes approved as active substances under the BPR or included in the review programme. Consequently, currently enzymes are not allowed to be used as active substances in biocidal products. Enzymes could be used in (biocidal) products for other purposes, such as for stain removal.

[8] *An active substance means 'a substance or a micro-organism that has an action on or against harmful organisms' (BPR, art. 3, 1c).*

Textbox 1 'Darie-arrest'.

In 2019, the European Court of Justice ruled that a microbial cleaning product is a biocide ('Darie-arrest': CJEU, 2019), despite the lack of a biocidal claim. The product concerned was a 'probiotic cleaning product' containing Bacillus ferment. The producer claimed that the product was not a biocide, because it *'generates enzymes that assimilate and consume all the organic waste on which micro-organisms feed, so that, on the surfaces treated with that product, no biotope favourable to the development of micro-organisms such as fungi can form*'. This legal judgement shows that products containing enzymes can be assessed to be biocides. The case was returned to the Dutch 'College van Beroep voor het bedrijfsleven' which had to make a final decision. In November 2020 this administrative court decided to take over the ruling of the European Court of Justice, i.e. that this specific product was a biocide. In 2021, the European Commission (EC) is preparing a decision (regarding BPR, art. 3.3) on a cleaning product without a clear biocidal claim, but containing a known biocidal active substance. The Commission proposes to classify the product as a biocidal product under the BPR. In 2022, the EC decided that this cleaning product should be considered a biocidal product, despite the lack of a biocidal claim (EC, 2022b).

Information requirements for safety assessment
For the approval of an active substance, an applicant has to prepare a dossier. This dossier is evaluated by a national Competent Authority. After evaluation, the Biocidal Product Committee of the European Chemicals Agency (ECHA) writes an opinion for approval or non-approval of the active substance and the European Member States make the final decision which is published by the European Commission.
The information requirements for active chemical substances are described in Annex II (under Title 1) of the BPR. Those information requirements are quite specific, but in general terms the following information is required:

- Identity;
- Physical and chemical properties;
- Physical hazards and respective characteristics;
- Methods of detection and identification;
- Effectiveness against target organisms;
- Intended uses and exposure;
- Toxicological profile for human and animal including metabolism;
- Ecotoxicological studies;
- Environmental fate and behaviour;
- Measures necessary to protect humans, animals and the environment; and
- Classification, labelling and packaging.

For the authorisation of a biocidal product, the applicant has to submit a dossier to the national Competent Authority. In the Netherlands this is the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb). Annex III of the BPR describes the information requirements for biocidal products. These information requirements are more or less the same as the requirements for active substances.

Labelling and communication requirements
In the BPR, some requirements on the labelling of biocidal products (BPR, art. 69) are included. It is not allowed to use claims as 'non-toxic', 'harmless', 'natural', 'environmentally friendly', 'animal friendly' or similar indications. Extra labelling requirements for specific substances can be part of the decisions on the approval of active substances published by the European Commission, e.g. information on the risk of skin sensitisation. In addition, the requirements of the CLP Regulation apply to biocidal products.

A1.5. Cosmetic Product Regulation (CPR)

Aim of the CPR
The Cosmetic Product Regulation (EC) 1223/2009 (CPR) establishes rules to be complied with by any cosmetic product made available on the EU market, in order to ensure the functioning of the internal market and a high level of protection of human health (EC, 2009a).
Note that the CPR, just like the BPR, is a product regulation that includes provisions for substances, and not a chemical regulation such as REACH or CLP. The focus of the CPR is on safety of the consumer. Restrictions due to environmental or worker safety concerns may apply to cosmetic products through other regulations, mainly via REACH.

Enzymes under the CPR
Enzymes meet the definition of substances[9] under the CPR and have to comply with all provisions that apply to substances in cosmetic products. For instance, enzymes have to be included in the ingredient list and may not be tested on animals specific for their use in cosmetic products.
As the CPR does not include a specific provision for enzymes, it is the responsibility of the Applicant (responsible person) to ensure that a cosmetic product containing enzymes is safe for the consumer.
However, if there is a specific concern due to the toxicological properties of the enzyme or exposure route of the product in which it is used, the Commission can mandate the SCCS to evaluate the safety of the use of the enzyme in cosmetic products. Depending on the outcome of this evaluation, specific restrictions for the use of the enzyme in cosmetic products may be adopted. Environmental concerns are not regulated under CPR and should be addressed under REACH.

Access to market and responsibilities
Only cosmetic products shall be placed on the market for which a legal or natural person is designated within the European Community as 'responsible person'. This responsible person is the manufacturer, importer, or in some cases the distributor of the product, unless they designate another party as responsible person.
The responsible person shall ensure that:

- the cosmetic product has undergone a safety assessment on the basis of the relevant information and that a cosmetic product safety report is set up in accordance with Annex I of the CPR;

[9] The definition of a substance under CPR equals the REACH definition as mentioned in 3.2.2: "*a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition*."

- there is a product information file readily accessible in electronic or other format to the competent authority of the Member State in which the file is kept;
- the product is notified to the European Commission;
- the product complies with the ingredient restrictions in the Annexes of the CPR;
- nanomaterials in the product are specifically notified to the Commission; and
- the product complies with the labelling requirements in Art. 19 and 20 of the CPR.

Information requirements for safety assessment
The CPR contains specific requirements and restrictions for substances that might pose a risk:

- All substances on Annex II of the CPR are prohibited. This includes pharmacologically active substances, pesticides, CMR substances, catalase, and other substances that cannot be used safely in cosmetic products;
- Substances on Annex III have to comply with specific restrictions. These include amongst others product restrictions, concentration limits, and/or warnings on the label;
- Only colorants, preservatives and UV-filters that are included in respectively Annex IV, V, and VI may be used;
- Substances with a harmonized classification as carcinogenic, mutagenic, or reprotoxic (CMR) are prohibited unless a derogation is granted according to Art. 15; and
- Nanomaterials not included in Annex IV, V, or VI have to be notified and specific information as described in Art. 16 has to be provided to the Commission.

If the Commission has a concern for the use of a substance or if industry requests a derogation for a restricted substance (including colorants, preservatives, UV-filters), the Commission requests the Scientific Committee for Consumer Safety (SCCS) to give its opinion on the safety of the substance for use in the relevant categories of cosmetic products and on the reasonably foreseeable exposure conditions (see Textbox 2 below). Based on the outcome of the SCCS opinion, which is made public, the Commission and Member States decide on the adaptation of the Annex(es) of the CPR.

Textbox 2 Evaluation by the Scientific Committee for Consumer Safety.

The SCCS, consisting of independent experts, gives opinions on health and safety risks of non-food consumer products and services. The SCCS provides risk assessments of cosmetic ingredients for which there is a concern from one or more MS or the Commission. The Applicant of the cosmetic product has to provide a full dossier in which all the required information (on physical chemical characteristics, exposure and hazard of the cosmetic ingredient) is included. This information can be complemented with data from any interested party as response to a "call for data" if launched by the Commission. Stakeholders, including Member States and individuals, can send comments on every preliminary version of the SCCS Opinion in a public consultation prior to publication of the final Opinion. More information on the studies required and the risk assessment performed are provided in the Checklist for Applicants (SCCS/1588/17), the SCCS Notes of Guidance (SCCS /1628/21), and the Guidance on Nanomaterials in Cosmetic Products (SCCS/1611/19), all published on the website of the SCCS (SCCS - Opinions 2016 - 2021 | Public Health (europa.eu)). The information required always minimally includes substance characterization and purity, physico-chemical data, intended use and concentrations, dermal absorption, irritation and sensitisation potential, mutagenicity, and a point of departure (usually from a repeated dose study). The performance of animal testing with cosmetic products or ingredients therein in order to meet the requirements of the CPR is prohibited. Only animal studies performed to meet the requirements of other regulations or performed before 11 March 2013 may be used in the safety evaluation.

Labelling and communication requirements
Cosmetic products are exempted from labelling under CLP. Instead, they have to comply with the labelling requirements in Articles 19 and 20 of the CPR.
These requirements include the provision that all ingredients have to be listed in descending order of weight of the ingredients at the time they are added to the cosmetic product. Perfume and aromatic compositions and their raw materials shall be referred to by the terms 'parfum' or 'aroma'. Allergenic fragrances included in Annex III have to be listed on the label. Claims on the label have to comply with the Working document on claims (EC, 2017).

To know if enzymes are on the ingredients list apart from the word "enzymes", a name finishing by the suffix "ase(s)" can be an indicator of the presence of enzymes.

A1.6. Regulation on detergents

Aim of the Regulation on detergents
The Regulation (EC) 684/2004 on detergents (EC, 2004) applies to cleaning products including detergents and surfactants that are placed on the EU market, and ensures the free movement of detergents and surfactants within the EU a high degree of protection of the environment and human health. The rules are complementary to REACH, CLP and BPR. In 2021 the European Commission launched an inception impact assessment targeted at amending the detergents Regulation as part of

the Chemicals Strategy on Sustainability. The detergents Regulation will be updated with the aim of providing clearer information to consumers by addressing the existing overlaps between the Regulation and other pieces of EU chemicals legislation such as CLP, REACH and BPR.

The detergents Regulation includes among others:

- stipulation of the biodegradability of surfactants in detergents;
- restrictions or bans on surfactants on grounds of biodegradability;
- restrictions or bans on surfactants on grounds of biodegradability;
- the additional labelling of detergents, including fragrance allergens; and
- the information that manufacturers must hold at the disposal of the Member States competent authorities and medical personnel.

According to the definition of the Regulation, products falling under the scope of the Regulation are:

- Detergents: meaning any substance or preparation containing soaps and/or other surfactants intended for washing and cleaning processes. Detergents may be in any form (liquid, powder, paste, bar, cake, moulded piece, shape, etc.) and marketed for or used in household, or for institutional or industrial purposes;
- Other products to be considered as detergents are:
    - Auxiliary washing preparation: intended for soaking (pre-washing), rinsing or bleaching clothes, household linen, etc.;
    - Laundry fabric-softener: intended to modify the feel of fabrics in processes which are to complement the washing of fabrics;
    - Cleaning preparation: intended for domestic all purposes cleaners and/or other cleaning of surfaces (e.g.: materials, products, machinery, mechanical appliances, means of transport and associated equipment, instruments, apparatus, etc.);
    - Other cleaning and washing preparations: intended for any other washing and cleaning processes.

Enzymes under the Regulation on Detergents
In this Regulation, detergents are defined as 'any substance or mixture containing soaps and/or other surfactants intended for washing and cleaning processes'. The definition does not explicitly include nor exclude products containing enzymes (added enzymes or *in situ* produced enzymes) for cleaning purposes. Enzymes fall under the scope of the "substance" definition which is the same as under REACH. This means that cleaning products containing enzymes (such as laundry detergents) fall within the scope of the Regulation on detergents.

Access to market and responsibilities
When products are placed on the market, manufacturers have the responsibility to declare detergents and surfactants for detergents referred to in Article 1. Furthermore, manufacturers are responsible for the conformity of detergents and/or of surfactants for detergents with the provisions of this Regulation and its Annexes. In addition, manufacturers shall be established within the Community.

Manufacturers shall conform with the conditions, characteristics and limits laid down in this Regulation and its Annexes and, where relevant, with Directive 98/8/EC (EC, 1998) and with any other relevant Community legislations. Surfactants that are also active substances within the meaning of Directive 98/8/EC and that are used as disinfectants are exempt from the provisions of Annexes II, III, IV and VIII of this Regulation provided: they are listed in Annex I or IA of Directive 98/8/EC, or they are constituents of biocidal products authorised under Article 15(1) or 15(2) of Directive 98/8/EC, or they are constituents of biocidal products allowed under the transitional measures or subject to the 10 year work programme provided for in Article 16 of Directive 98/8/EC. Instead, such surfactants are deemed to be disinfectants and the detergents of which they are ingredients are subject to labelling provisions for disinfectants of Annex VII A.

Information requirements for safety assessment
Article 10 states that: "Member States' competent authorities may apply, as appropriate, all necessary control measures to detergents placed on the market which ensure the compliance of the product with the provisions of this Regulation. The reference method shall be the test and analytical methods referred to in Annex VIII. These control measures shall not oblige manufacturers to repeat tests made by laboratories fulfilling the conditions indicated in Article 8(2), or to pay for any repeat or additional test, provided the initial test has shown compliance of detergents, or surfactants used as ingredients in detergents, with this Regulation.
In cases of concern that a test carried out in accordance with the methods listed in Annex II, III, IV or VIII has produced false positive results, the Member States' competent authorities shall notify the Commission and the Commission shall, in accordance with the procedure laid down in Article 12(2), verify those results and take the necessary measures."

Labelling and communication requirements
The Regulation on detergents also sets requirements on the information present on the label of the detergent. This should include:

- the name of the product;
- contact details of the party placing the product on the market;
- information on the content and the ingredients;
- instructions for use and special precautions, if required; and
- in case of laundry detergents and consumer automatic dishwasher detergents: dosing instructions.

For the list of ingredients, some ingredients may be given in weight classes (less than 5%; 5 – 15%, 15 - 30%; 30% or more). This holds for soap, surfactants, phosphates, phosphonates and bleaching agents. Some other ingredients – including perfumes, enzymes, optical brighteners and preservatives – should be mentioned on the label regardless of the amount present in the product. Allergenic fragrances should be mentioned as individual substances if added in concentrations exceeding 0.01%. Preservatives should be mentioned irrespective of the concentration in the product.
Besides this more general information on the composition of the product, the label should also mention a website where the complete

chemical composition of the detergent is available. The Regulation on detergents does not provide specific labelling requirements on enzymes present in the detergents, except that enzymes, no matter their concentrations, should be mentioned in the complete ingredient list.

A1.7. Genetically modified organisms (GMO) legislation

Aim of the GMO legislation
EU legislation on GMOs has two main aims:

- to protect human and animal health and the environment in accordance with the precautionary principle; and
- to ensure the effective functioning of the internal market.

Accordingly, it establishes harmonised and centralised procedures requiring an authorisation for placing a GMO on the market or for its deliberate release into the environment.

The EU's GMO authorisation system is based on an assessment of the risks to human and animal health and the environment, and includes requirements for post-authorisation monitoring, labelling and traceability. The legislation also has an important international dimension, embodied in the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, to which EU law on GMOs is enshrined in six main pieces of legislation (food and feed legislations are not relevant for this report, as they fall outside the scope of non-food consumer products):

- Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (EC, 2001a);
- Directive 2009/41/EC on the contained use of genetically modified micro-organisms (EC, 2009b);
- Directive (EU) 2015/412 amending Directive 2001/18/EC as regards the possibility for the Member States to restrict or prohibit the cultivation of GMOs in their territory (EU, 2015);
- Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003a);
- Regulation (EC) No 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC (EC, 2003b); and
- Regulation (EC) No 1946/2003 on transboundary movements of genetically modified organisms. (EC, 2003c).

Overall, the legislation covers:

- GMOs intended for deliberate release into the environment for purposes other than being placed on the market;
- GMOs to be placed on the market;
- GM food or feed; and
- genetically modified micro-organisms (GMMs) to be used under containment conditions.

In the Netherlands, the legislation for contained use is not limited to micro-organisms but covers all genetically modified organisms. In general enzyme production by genetically modified (micro)organisms is conducted under the framework for contained use.

Enzymes under the GMO legislation
Directive 2009/41/EC and Directive 2001/18/EC apply to living genetically modified organisms[10][11]. Enzymes are no organisms and are therefore not regulated by the GMO regulations. When enzymes are produced by GMOs, the GMOs are subject to the requirements of Directive 2009/41/EC and/or 2001/18/EC.

Access to market and responsibilities
When enzymes are produced by GMOs under contained use conditions in conformity with the Directive 2009/41/EC, the final product, being the enzyme preparation, must be free of any residual living GMOs. As a result the final product no longer consists of or contain GMOs and thus the GMO regulations are no longer applicable.
In case the enzymes are produced under the framework of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms prior consent for the deliberate release of GMOs issued by the relevant authorities is required. In order to obtain consent, an application ('notification') has to be submitted to a national competent authority, accompanied inter alia by an environmental risk assessment. The risk assessment must comply with the general principles and the methodology set out in the Directive and draw conclusions for each relevant area of risk.
The authorisation procedure for the placing on the market of GMOs (as such or in products) is conducted at EU level. The national competent authority to which the notification has been submitted must deliver an assessment report. In cases where the Commission or another Member State have expressed objections to the assessment report and no agreement has been reached, the Commission adopts a decision after obtaining the scientific opinion of EFSA. The national competent authority that prepared the report then gives written consent for the placing on the market of the GMO as such or in a product. It must set out the conditions for the placing on the market, and labelling and monitoring requirements. It can be valid for a renewable period of up to 10 years.

Information requirements for safety assessment
If the production with the GMO takes place under containment conditions, Directive 2009/41/EC applies. A notification to the national competent authorities is required and in some cases their prior consent for the use of GMMs. To that end, the user has to carry out an assessment of the contained uses as regards risks to human health and the environment.
If the production with the GMO is considered as deliberate release in accordance with Directive 2001/18/EC, an application ('notification') has to be submitted accompanied by an environmental risk assessment in order to obtain consent for placing the GMO on the market. The risk assessment must comply with the general principles and the methodology set out in the Directive and must draw conclusions for each relevant area of risk. Annex II of Directive 2001/18/EC describes the

[10] In the EU directives 2001/18/EC and 2009/41/EC a GMO is defined as 'an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'.
[11] The directives define an organism as '*organism means any biological entity capable of replication or of transferring genetic material*'.

objective to be achieved, the elements to be considered and the general principles and methodology to be followed to perform the environmental risk assessment.
When the product is produced by a GMO but the product itself doesn't contain or consists of GMOs, the product falls outside the scope of Directives 2001/18/EC and 2009/41/EC and thus the GMO regulations are no longer applicable.

Labelling and communication requirements
If a product containing GMOs is authorized for commercial release under Directive 2001/18/EC, the consent sets out the conditions for the placing on the market, and labelling and monitoring requirements.
The words "This product contains genetically modified organisms" shall appear either on a label or in an accompanying document.
For GM plants a public EC register records the location of GMOs grown under part C, so that the possible effects of such GMOs on the environment may be monitored.

A1.8. General Product Safety Directive (GPSD) and Dutch Commodities Act (Warenwet)

Aim of the GPSD and Dutch Commodities Act
If none of the above described regulatory frameworks is applicable to a consumer product, the product falls within the scope of the General Product Safety Directive 2001/95/EC (EC, 2001b), which is implemented in the (broader) Dutch Commodities Act (in Dutch "Warenwetbesluit algemene productveiligheid) (Warenwet, 2022). The GPSD defines general EU rules on product safety. It does not consider pharmaceuticals, medical devices and food.
The Dutch Commodities Act applies to consumer products (food and non-food) for which no specific European Regulation applies (e.g. the Cosmetic Products Regulation or Biocidal Product Regulation). It is a framework act, providing general rules on public health, product safety, fair trading and adequate information.

Responsibilities and requirements
General product safety is one of the main elements of both the GPSD and the Commodities Act. The producer is responsible for the safety of the product and information about the product, for instance on potential risks. According to the Commodities Act, if the producer is located outside of the European Union (EU), the responsibility falls to the importer and distributor. They must ensure the product is safe and the product information is correct.

Labelling requirements
The GPSD does not state specific requirements on labelling, other than the fact that labelling has to be taken into account. Under the Commodities Act, requirements for general product safety are stated in Article 2 of the Commodities Act decree on general product safety (Warenwetbesluit Algemene Productveiligheid, 1993).

Annex 2 Search strategy

For the market evaluation, an online search was performed for the cleaning products containing enzymes sold on websites that deliver in the Netherlands. Considering the fact that there is a vast and changing amount of such products sold on the internet, the goal of this search was not to identify all products. Instead, the goal was to obtain an explorative, non-exhaustive overview of the types of products that are sold on Dutch web shops at the time of the search (Q3 of 2021).

Terms used to search for products include "enzymen" (enzymes), "detergent" (detergents), "allesreiniger" (all-purpose cleaner), "sanitair" (sanitary), "tuin" (garden), "keuken" (kitchen), "cosmetica" (cosmetics), "scrub" (scrub). Different combinations of these terms were used and websites advertising enzyme cleaning products often referred to other websites on which enzymes cleaning products were sold. On websites of producers and/or distributors, a search of "enzymen" (enzymes) was also performed.
A criterion for the selection of the search results was that only products claimed to contain enzymes were included.

A search in retail stores was also performed. The search focused on cleaning and cosmetic products present in the store. Labels were searched for mentioning 'enzymes' or specific names of enzymes. When enzymes were mentioned on the labels, the product was added to the overview.

Annex 3 Survey

Q001 - Intro: **Text**

With this questionnaire, the Dutch National Institute for Public Health and the Environment (RIVM) would like to gain insight into the composition and production of enzyme-containing consumer products. Enzyme-containing consumer products cover products that contain single enzymes or enzyme preparations, or products in which enzymes are produced in situ by added micro-organisms.

Insight into the type and concentration of enzymes in consumer products can be used as input for evaluation of potential hazards, consumer exposure and possible risks involved in the use of these products. This research is commissioned by the Office for Risk Assessment and Research of the Netherlands Food and Consumer Product Safety Authority (NVWA-BuRO).

Q002 - Toestemming: **Single coded**

The answers you give in this survey will be summarized in outline by Kantar Public and RIVM. No data that can be traced back to your person or company/institution will be reported to other parties besides the people involved at Kantar Public and RIVM. The publications about this do not contain any personally identifiable results or data. We only process personal data that is necessary to contact you. Only RIVM employees involved in this survey have access to this data. No data that can be traced back to your person, company or institution will be reported to our commissioning party. RIVM does not sell your data to third parties.

RIVM and Kantar Public respect you privacy. Kantar's privacy policy can be found at: https://www.kantar.com/nl/privacy. The RIVM privacy statement can be found at: https://www.rivm.nl/documents/privacy statement-rivm.

RIVM stores your personal data for a period up to and including the processing of your answers to this survey. After processing your answers, we will keep your personal data for a maximum of 5 years in order to be able to reach you in the future for any new developments related to your answers.

Participation in this study is voluntary and you can withdraw from the study and withdraw your consent at any time without giving a reason. The research data (without contact details) will be kept at the RIVM for at least 10 years. This is based on the statutory retention periods applicable to RIVM.

The questionnaire will take about 10 minutes of your time. If you have any questions regarding this study, you can send an e-mail to Marsha.Hilhorst@kantarpublic.com or Jolinde.Kettelarij@rivm.nl

Do you agree to participate in this study and consent to the processing of my data as mentioned?

1	Yes, I will take part
2	No, I will not take part

Q003 - Product_categories: **Multi coded**

In which category does the production of your company fall?

Multiple answers possible

1	Production of products with enzymes
2	Production of pure enzymes, enzyme mixtures or micro-organisms that produce enzymes
996	Other (please specify) **Open *Fixed*

Scripter notes: If ONLY answer 2 is chosen GOTO Q009

Q004 - Enzym_produce: **Single coded**

Does your company produce consumer products that contain enzymes?

1	Yes, we produce consumer products	
2	Yes, we produce consumer products and products for the professional market	
3	No, we only produce products for the professional market	➔ GO TO **Q024 - Future_use1**
4	No, we do not produce products that contain enzymes	➔ GO TO **Q026 - Comments**

Ask only if **Q004 - Enzym_produce**,1,2

Q005 - Number_products: **Single coded**

The next questions will only be about consumer products.

How many consumer products with enzymes does your company produce (approximately)?

1	1-10
2	11-100
3	101-500
4	More than 500
999	I don't know **Fixed *Exclusive*

Q006 - Source: **Multi coded**

What is the source of the enzymes that you use?

Multiple answers possible

1	My company produces the enzymes
2	My company purchases them from **Open*
996	Other (please specify) **Open *Fixed*

Ask only if **Q004 - Enzym_produce**,1,2

Q007 - Products: **Matrix**

Does your company produce the following products with enzymes?

If you cannot find the exact category, please choose the product group that fits best.

	Yes	No
Hair care	❍	❍
Bathing, showering	❍	❍
Skin care	❍	❍
Make-up and nail care	❍	❍
Sun care	❍	❍
Oral hygiene	❍	❍
Laundry products	❍	❍
Dishwashing products	❍	❍
All-purpose cleaners	❍	❍
Sanitary products	❍	❍
Floor and furniture cleaning products	❍	❍
Miscellaneous cleaning products	❍	❍
Veterinary hygiene purposes	❍	❍
Disinfectants and general biocidal products	❍	❍

Ask only if **Q004 - Enzym_produce**,1,2

Q008 - Most_product: **Single coded**

We would like to ask you some more questions about the consumer product that contains the highest concentration of enzymes.
What type is the consumer product you produce that contains the highest amount of enzymes?

If your company produces multiple consumer products that contain the same amount of enzymes, please choose the product that is sold most.

1	Hair care product
2	Bathing, showering product
3	Skin care product
4	Make-up and nail care product
5	Sun care product
6	Oral hygiene product
7	Laundry product
8	Dishwashing product
9	All-purpose cleaner
10	Sanitary product
11	Floor and furniture cleaning product
12	Miscellaneous cleaning product
13	Veterinary hygiene purposes product
14	Disinfectants and general biocidal product

Scripter notes: Only show the answers named in Q007
If every answer in Q011=no, then goto Q024

Ask only if **Q004 - Enzym_produce**,1,2

Q009 - Form: **Single coded**

What form does that <answer Q008> with enzymes have?

1	Spray
2	Gel
3	Liquid
4	Solid
996	Other, please specify **Open *Fixed*

Ask only if **Q004 - Enzym_produce**,1,2

Q010 - Mix_single: **Single coded**

Enzymes are often produced or purchased as enzyme mixtures. The mixture includes preservatives and other substances or impurities, besides the active enzymes. The actual enzyme concentration in that mixture might therefore be lower than 100%. This information can be found on the safety data sheet (SDS) provided with the enzyme mixture.

Do you use an enzyme mixture in your <answer Q008>, or do you use a single enzyme in that consumer product?

If you produce (many) different consumer products per product type, please provide the product with highest concentration enzyme used.

1	Enzyme mixture
2	Single enzyme

Ask only if **Q012 - Mix_single**,1

Q011 - Name: **Open**

What is the name of the enzyme mixture used in your <answer q008>?
This information can be found on the documentation that is provided with the enzyme mixture (e.g. certificate of analysis, SDS).

999 Don't know **Fixed *Exclusive*

Ask only if **Q012 - Mix_single**,1

Q012 - Concentration_product: **Numeric**

What is the concentration of enzyme mixture in your <answer q008>?
We mean the concentration in the final product.
This information can be found on the documentation that is provided with the enzyme mixture (e.g. certificate of analysis, SDS).

999 Don't know **Fixed *Exclusive*

Ask only if **Q012 - Mix_single**,1

Q013 - Which_enzymes: **Multi coded**

Which enzymes does the mixture in your <answer Q008> contain?
This information can be found on the documentation that is provided with the enzyme mixture (e.g. certificate of analysis, SDS).

1 (Alpha-) Amylase
2 Cellulase
3 Glucose oxidase
4 Lipase
5 Mannanase
6 Pectinase
7 Protease (including subtillisin)
8 Xanthan lyase
9 Enzymes from natural sources (papain, bromelain, superoxide dismutase, lactoperoxidase, lysozyme, etc.)
996 Other (please specify) **Open *Fixed*

Ask only if **Q012 - Mix_single**,2

Q014 - Enzyme_single: **Multi coded**

What are the names of the single enzymes used in your <answer Q008>?

1	(Alpha-) Amylase
2	Cellulase
3	Glucose oxidase
4	Lipase
5	Mannanase
6	Pectinase
7	Protease (including subtillisin)
8	Xanthan lyase
9	Enzymes from natural sources (papain, bromelain, superoxide dismutase, lactoperoxidase, lysozyme, etc.)
996	Other (please specify) **Open *Fixed*

Ask only if **Q012 - Mix_single**,2

Q015 - Enzyme_concentration: **Numeric**

In what concentration (what percentage) is the single enzyme <answer q016> used in the <answer q008>?

999	Don't know **Fixed *Exclusive*

Ask only if **Q012 - Mix_single**,1

Q016 - Concentration_mix: **Numeric**

Not back | Min = 0 | Max = 100

What is the concentration of <answer Q015> in the mixture that you use for the <answer q008>? Before it is added to the final product. *Please provide a percentage with a maximum of two digits after the decimal point.*
This information can be found on the documentation that is provided with the enzyme mixture (e.g. certificate of analysis, SDS).

999	Don't know **Fixed *Exclusive*

Ask only if **Q012 - Mix_single**,1,2

Q017 - Encapsulated: **Single coded**

Is the enzyme <Q015/Q016> that you use for the <answer Q008> encapsulated? *This information can be found on the documentation that is provided with the enzyme mixture (e.g. certificate of analysis, SDS).*

1	Yes, with **Open*
2	Yes, but I don't know with what/am not allowed to tell with what
3	No
999	Don't know **Fixed *Exclusive*

Ask only if **Q012 - Mix_single**,1

Q018 - Impurities: **Single coded**

Do you have information on possible impurities (e.g. contaminants, preservatives, additives) in the enzyme mixture that you use for <answer q008>?

1 Yes, there are impurities
2 There are no impurities
3 I don't have any information on that

Ask only if **Q020 - Impurities**,1

Q019 - Type_impurities: **Open**

What impurities does the enzyme mixture you use for <Q008> contain?

This information can be found on the documentation that is provided with the enzyme mixture (e.g. certificate of analysis, SDS).

Ask only if **Q004 - Enzym_produce**,2

Q020 - Compare: **Single coded**

Compared to the consumer products you produce, what type of enzymes do your products for the professional market contain?

1 The same types of enzymes
2 Different types of enzymes

Ask only if **Q004 - Enzym_produce**,2

Q021 - Compare_2: **Single coded**

Compared to the consumer products, the enzymes that you use in professional products contain:

1 A lower concentration of enzymes
2 A similar concentration of enzymes
3 A higher concentration of enzymes

Ask only if **Q003 - Product_categories**,2

Q022 - Enzyme_sold: **Open**

What is the name of the most sold enzyme, or most sold enzyme mixture that you produce?

Ask only if **Q003 - Product_categories**,2

Q023 - Enzyme_use: **Open**

For what type of products is that enzyme (mixture) most commonly used?

Q024 - Future_use1: **Single coded**

Please give your opinion on the following statement.
In the following years, I expect the number of products with enzymes will...

1 increase
2 stay the same
3 decrease

Q025 - Future_use2: **Single coded**

In the following years, I expect enzymes to be used...

1 in a wider range of products
2 in the same range of products
3 in a narrower range of products

Q026 - Comments: **Open**

Do you have any comments, suggestions or additional information regarding this questionnaire?

999 No **Fixed *Exclusive*

Q027 - Contact_details: **Open**

We would like to be able to contact you to clarify your answers, if needed. No data that can be traced back to your person or institution will be reported to parties besides the people involved at RIVM and Kantar. Could we get your contact details?

State name, company, job title, phone number, email adress below.

999 No, I don't want provide my contact details **Fixed *Exclusive*

Annex 4 ConsExpo calculations

1. Trigger spray stain remover (subtilisin)

Input parameters

ConsExpo modelling Trigger spray stain remover exposure

Substance	subtilisin
Product	Weight fraction: 0.014%
Population	Adult, 68.8 kg
Scenario	Application – spot treatment (non-volatile substances)
Frequency	128 per year
Inhalation	
Exposure model	Exposure to spray – Spraying
Spray duration	0.05 minute
Exposure duration	10 minute
Weight fraction substance	0.014%
Room volume	10 m^3
Room height	2.5 m
Ventilation rate	2 per hour
Inhalation rate	25 l/min
Spraying towards person	No
Mass generation rate	1.6 g/s
Airborne fraction	0.2
Density non volatile	1.8 g/cm^3
Inhalation cut off diameter	15 µm
Aerosol diameter distribution	LogNormal
Median diameter	100 µm
Arithmetic coefficient of variation	0.6
Maximum diameter	50 µm
Include oral non-respirable material exposure	yes
Absorption model	n.a.
Dermal	
Exposure model	Direct contact – Instant application
Exposed area	450 cm^2
Weight fraction substance	0.014%
Product amount	1 g
Retention factor	1
Absorption model	n.a.
Oral	
Exposure model	Non-respirable spray model
No parameters exposure route	Parameters are set in Inhalation
Absorption model	n.a.

Results

Inhalation	
Mean event concentration	1.6×10^{-6} mg/m^3
Peak concentration (TWA 15 min)	1.6×10^{-6} mg/m^3
Mean concentration on day of exposure	1.1×10^{-8} mg/m^3
Year average concentration	3.9×10^{-9} mg/m^3
External event dose	5.9×10^{-9} mg/kg bw
External dose on day of exposure	5.9×10^{-9} mg/kg bw
Dermal	
Dermal load	3.1×10^{-4} mg/cm^2
External event dose	2.0×10^{-3} mg/kg bw
External dose on day of exposure	2.0×10^{-3} mg/kg bw
Oral	
External event dose	3.2×10^{-7} mg/kg bw
External dose on day of exposure	3.2×10^{-7} mg/kg bw

2. Trigger spray odour control (Subtilisin)

Input parameters

ConsExpo modelling Trigger spray odour control (Subtilisin)	
Substance	subtilisin
Product	Weight fraction: 0.02%
Population	Adult, 68.8 kg
Scenario	Spraying furniture with non-volatile substances – adult users
Frequency	52 per year
Inhalation	
Exposure model	Exposure to spray – Spraying
Spray duration	0.167 minute
Exposure duration	240 minute
Weight fraction substance	0.02%
Room volume	58 m^3
Room height	2.5 m
Ventilation rate	0.5 per hour
Inhalation rate	9.17 l/min
Spraying towards person	No
Mass generation rate	1.7 g/s
Airborne fraction	0.018
Density non volatile	1.13 g/cm^3
Inhalation cut off diameter	15 µm
Aerosol diameter distribution	LogNormal
Median diameter	2 µm
Arithmetic coefficient of variation	0.39
Maximum diameter	50 µm
Include oral non-respirable material exposure	yes

Absorption model	n.a.
Dermal	
Exposure model	Direct contact – Constant rate
Exposed area	2200 cm^2
Weight fraction substance	0.02%
Contact rate	46 g/min
Release duration	0.333 minute
Absorption model	n.a.
Oral	
Exposure model	Non-respirable spray model
No parameters	Parameters are set in Inhalation exposure route
Absorption model	n.a.

Results

Inhalation	
Mean event concentration	$3.5x10^{-4}$ mg/m^3
Peak concentration (TWA 15 min)	$9.7x10^{-4}$ mg/m^3
Mean concentration on day of exposure	$5.9x10^{-5}$ mg/m^3
Year average concentration	$8.4x10^{-6}$ mg/m^3
External event dose	$1.1x10^{-5}$ mg/kg bw
External dose on day of exposure	$1.1x10^{-5}$ mg/kg bw
Dermal	
Dermal load	$1.4x10^{-6}$ mg/cm^2
External event dose	$4.5x10^{-5}$ mg/kg bw
External dose on day of exposure	$4.5x10^{-5}$ mg/kg bw
Oral	
External event dose	$2.6x10^{-14}$ mg/kg bw
External dose on day of exposure	$2.6x10^{-14}$ mg/kg bw

3. Trigger spray odour control (Cellulase)

Input parameters

ConsExpo modelling Trigger spray odour control (Cellulase)	
Substance	Cellulase
Product	Weight fraction: 0.05%
Population	Adult, 68.8 kg
Scenario	Spraying furniture with non-volatile substances – adult users
Frequency	52 per year
Inhalation	
Exposure model	Exposure to spray – Spraying
Spray duration	0.167 minute
Exposure duration	240 minute
Weight fraction substance	0.05%
Room volume	58 m^3
Room height	2.5 m

Ventilation rate	0.5 per hour
Inhalation rate	9.17 l/min
Spraying towards person	No
Mass generation rate	1.7 g/s
Airborne fraction	0.018
Density non volatile	1.13 g/cm^3
Inhalation cut off diameter	15 µm
Aerosol diameter distribution	LogNormal
Median diameter	2 µm
Arithmetic coefficient of variation	0.39
Maximum diameter	50 µm
Include oral non-respirable material exposure	yes
Absorption model	n.a.
Dermal	
Exposure model	Direct contact – Constant rate
Exposed area	2200 cm^2
Weight fraction substance	0.05%
Contact rate	46 g/min
Release duration	0.333 minute
Absorption model	n.a.
Oral	
Exposure model	Non-respirable spray model
No parameters exposure route	Parameters are set in Inhalation
Absorption model	n.a.

Results

Inhalation	
Mean event concentration	8.9x10^{-4} mg/m^3
Peak concentration (TWA 15 min)	2.4x10^{-3} mg/m^3
Mean concentration on day of exposure	1.5x10^{-4} mg/m^3
Year average concentration	2.1x10^{-5} mg/m^3
External event dose	2.8x10^{-5} mg/kg bw
External dose on day of exposure	2.8x10^{-5} mg/kg bw
Dermal	
Dermal load	3.5x10^{-6} mg/cm^2
External event dose	1.1x10^{-4} mg/kg bw
External dose on day of exposure	1.1x10^{-4} mg/kg bw
Oral	
External event dose	6.6x10^{-14} mg/kg bw
External dose on day of exposure	6.6x10^{-14} mg/kg bw

4. Trigger spray odour control (Alpha-amylase)

Input parameters

ConsExpo modelling Trigger spray odour control (Amylase)	
Substance	Amylase
Product	Weight fraction: 0.03%
Population	Adult, 68.8 kg
Scenario	Spraying furniture with non-volatile substances – adult users
Frequency	52 per year
Inhalation	
Exposure model	Exposure to spray – Spraying
Spray duration	0.167 minute
Exposure duration	240 minute
Weight fraction substance	0.03%
Room volume	58 m^3
Room height	2.5 m
Ventilation rate	0.5 per hour
Inhalation rate	9.17 l/min
Spraying towards person	No
Mass generation rate	1.7 g/s
Airborne fraction	0.018
Density non volatile	1.13 g/cm^3
Inhalation cut off diameter	15 µm
Aerosol diameter distribution	LogNormal
Median diameter	2 µm
Arithmetic coefficient of variation	0.39
Maximum diameter	50 µm
Include oral non-respirable material exposure	yes
Absorption model	n.a.
Dermal	
Exposure model	Direct contact – Constant rate
Exposed area	2200 cm^2
Weight fraction substance	0.05%
Contact rate	46 g/min
Release duration	0.333 minute
Absorption model	n.a.
Oral	
Exposure model	Non-respirable spray model
No parameters	Parameters are set in Inhalation exposure route
Absorption model	n.a.

Results

Inhalation	
Mean event concentration	5.3×10^{-4} mg/m^3
Peak concentration (TWA 15 min)	1.5×10^{-3} mg/m^3
Mean concentration on day of exposure	8.9×10^{-5} mg/m^3
Year average concentration	1.3×10^{-5} mg/m^3
External event dose	1.7×10^{-5} mg/kg bw
External dose on day of exposure	1.7×10^{-5} mg/kg bw
Dermal	
Dermal load	2.1×10^{-6} mg/cm^2
External event dose	6.7×10^{-5} mg/kg bw
External dose on day of exposure	6.7×10^{-5} mg/kg bw
Oral	
External event dose	3.9×10^{-14} mg/kg bw
External dose on day of exposure	3.9×10^{-14} mg/kg bw

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