Health Council of the Netherlands

Fungal alpha-amylase (derived from the fungus *Aspergillus oryzae*)

Health-based recommended occupational exposure limit

2014/25

GSW/1696
416-05

Health Council of the Netherlands

Fungal alpha-amylase (derived from the fungus *Aspergillus oryzae*)

Health-based recommended occupational exposure limit

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *schimmel alfa-amylase*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-8232/JR/cn/459-S70
Bijlagen : 1
Datum : 6 november 2014

Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan schimmel alfa-amylase.

In het advies *Preventie van werkgerelateerde luchtwegallergieën* (rapportnr. 2008/03) heeft de Gezondheidsraad een werkwijze voorgesteld voor het afleiden van gezondheidskundige advieswaarden of voor het vaststellen van op risico gebaseerde referentiewaarden voor allergene stoffen. Het voorliggende advies over schimmel alfa-amylase maakt gebruik van deze werkwijze. De commissie heeft de concentratie schimmel alfa-amylase in de lucht berekend waarbij een werknemer een extra kans van één procent gedurende zijn arbeidzame leven heeft om door beroepsmatige blootstelling gesensibiliseerd te raken ten opzichte van de kans hierop in de niet beroepsmatige blootgestelde algemene bevolking.

De conclusie van het advies is opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad. De commissie heeft daarbij gebruik gemaakt van commentaren die zijn ontvangen op een openbaar concept van dit advies en van de oordelen die intern zijn ingewonnen bij de Beraadsgroep Gezondheid en omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. J.L. Severens,
vicevoorzitter

Bezoekadres
Rijnstraat 50
2515 XP Den Haag
E-mail: jm.rijnkels@gr.nl
Telefoon (070) 340 66 31

Postadres
Postbus 16052
2500 BB Den Haag
www.gr.nl

Fungal alpha-amylase (derived from the fungus *Aspergillus oryzae*)

Health-based recommended occupational exposure limit

Dutch Expert Committee on Occupational Safety,
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2014/25, The Hague, November 6, 2014

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

This report can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. Fungal alpha-amylase (derived from the fungus *Aspergillus oryzae*) - Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, 2014; publication no. 2014/25.

ISBN: 978-94-6281-017-4

Contents

Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen, een commissie van de Gezondheidsraad, gezondheidskundige advieswaarden af voor stoffen in de lucht waaraan mensen blootgesteld kunnen worden tijdens hun beroepsuitoefening. Deze advieswaarden vormen vervolgens de basis voor grenswaarden waarmee de gezondheid van werknemers beschermd kan worden. Dit advies gaat over de gevolgen van blootstelling aan schimmel alfa-amylase, een enzym dat afkomstig is van de schimmel *Aspergillus oryzae*. Vooral mensen die in bakkerijen of meelfabrieken werken kunnen hiermee te maken hebben. De conclusie van de commissie is gebaseerd op wetenschappelijke publicaties die vóór augustus 2014 zijn verschenen.

Fysische en chemische eigenschappen

Het enzym alfa-amylase van de schimmel *Aspergillus oryzae*, is een glycoproteene met 478 aminozuren. In zuivere vorm is het een witgeel hygroscopisch poeder. Het enzym zet langketenige koolhydraten om tot maltose en glucose. Extracten van de schimmel worden vooral toegepast als deegverbeteraar bij de bereiding van bakkerijproducten.

Blootstelling

Blootstelling aan schimmel alfa-amylase in de lucht kan worden gemeten als de hoeveelheid allergeen in inhaleerbaar stof en is voornamelijk gemeten in bakkerijen. Gemiddelde concentraties in de inademingslucht over een volledige werkdag, lopen uiteen van de detectielimiet tot enkele honderden ng/m^3, met maxima tot 30 µg/m^3. De hoogste blootstellingen zijn gemeten tijdens het wegen en mengen van meel en bij werkzaamheden met deeg. Omdat deegverbeteraars in verschillende hoeveelheden worden gebruikt voor brood en gebak, is de blootstelling ook afhankelijk van de soort bakkerij. Gedurende een interventieprogramma tussen 2000 en 2007 is de blootstelling aan alfa-amylase in Nederlandse industriële bakkerijen en meelfabrieken afgenomen, maar toegenomen bij producenten van bakingrediënten.

Kinetiek

Blootstelling aan schimmel alfa-amylase vindt plaats via stofdeeltjes of aerosolen. Er zijn geen specifieke gegevens over de kinetiek voor enzymen, maar men verwacht dat deze enzymen zich net zo gedragen als andere deeltjes. De plaats van depositie in het luchtwegsysteem is afhankelijk van de deeltjesgrootte. In bakkerijen is alfa-amylase voornamelijk aangetoond in stofdeeltjes met een aerodynamische diameter groter dan 5 µm. De aerodynamische diameter van meer dan de helft van de stofdeeltjes was zelfs groter dan 9 µm. De meeste van deze stofdeeltjes zullen waarschijnlijk in de neus, mond en bovenste luchtwegen terecht komen.

Effecten

Inademing van schimmel alfa-amylase veroorzaakt zowel immunologische als niet-immunologische reacties. Immunologische reacties leiden tot sensibilisatie die bij voortdurende blootstelling allergische luchtwegreacties kan veroorzaken. Mensen krijgen dan bijvoorbeeld astma of ontstekingen in neus, luchtwegen en ogen. Deze symptomen kunnen echter ook worden veroorzaakt door irritatie, een niet-immunologisch mechanisme. De enige manier om deze twee reacties te onderscheiden is de mensen te testen op sensibilisatie voor schimmel alfa-amylase. Uit de beschikbare onderzoeken kan verder worden opgemaakt dat specifieke sensibilisatie binnen enkele maanden na het begin van de blootstelling kan

optreden, maar dat het ook jaren kan duren voordat allergische symptomen zich ontwikkelen.

De meeste gegevens over effecten van beroepsmatige blootstelling aan schimmel alfa-amylase zijn afkomstig van onderzoek onder medewerkers van bakkerijen en meelfabrieken. Het aantal gevallen van sensibilisatie onder deze medewerkers loopt uiteen van 1 tot 30 procent, afhankelijk van het arbeidsverleden, de werkomstandigheden en persoonlijke kenmerken. Ter vergelijking: het aantal gevallen van sensibilisatie door blootstelling aan schimmel alfa-amylase in de algemene, niet-blootgestelde bevolking ligt tussen de 1 en 2 procent.

Er zijn geen onderzoeken uitgevoerd naar mogelijke andere schadelijke gezondheidseffecten onder werknemers. Ook zijn er nauwelijks diergegevens uit proefdieronderzoek gepubliceerd.

Evaluatie en advies

Voor het afleiden van een advieswaarde hecht de commissie de meeste waarde aan gegevens over sensibilisatie. Iemand die gesensibiliseerd raakt, loopt bij voortdurende blootstelling een grote kans om allergisch klachten te krijgen. Aangezien sensibilisatie niet omkeerbaar is zal deze persoon voor de rest van zijn of haar leven gesensibiliseerd zijn en bij blootstelling allergische klachten kunnen krijgen. In bakkerijen worden behalve alfa-amylase ook tarwemeel en andere additieven gebruikt. Ook deze stoffen kunnen luchtwegklachten veroorzaken die niet zijn te onderscheiden van de klachten die schimmel alfa-amylase veroorzaakt. Met speciale immunologische tests kan dat onderscheid bij sensibilisatie wel gemaakt worden. De commissie heeft geen bewijs gevonden dat luchtwegklachten bij een lagere blootstelling optreden dan sensibilisatie. Dit betekent dat een advieswaarde die gebaseerd is op gegevens van sensibilisatie tevens luchtwegklachten helpt te voorkomen.

Om een gezondheidskundige advieswaarde te kunnen afleiden, zijn gegevens nodig over de relatie tussen blootstelling en het optreden van sensibilisatie. In veel onderzoeken is zo'n relatie niet goed onderzocht vanwege een gebrek aan blootstellingsgegevens. In twee afzonderlijk van elkaar uitgevoerde onderzoeken zijn echter wel zulke relaties bestudeerd. In beide gevallen gaat het om medewerkers in bakkerijen, is de blootstelling uitgedrukt in allergeengehalte in de stof in de lucht (bepaald met dezelfde techniek) en zijn gegevens over het optreden van sensibilisatie gerapporteerd. De uitkomsten zijn iets verschillend, maar de commissie ziet dit als normale statistische variatie bij een verschillende samenstelling van deelnemers.

De commissie is van mening dat voor het effect ‘sensibilisatie’ geen drempelwaarde is aan te wijzen. In de algemene (niet beroepsmatig blootgesteld) bevolking komen al gevallen van sensibilisatie voor schimmel alfa-amylase voor; er is dus geen nulsituatie. Dit laatste impliceert dat blootstelling, ongeacht de hoogte, een risico geeft op het ontwikkelen van sensibilisatie en dat daarom het beste een referentiewaarde kan worden afgeleid. Een referentiewaarde is een concentratie van alfa-amylase in de lucht waarbij beroepsmatige blootstelling leidt tot een vooraf bepaalde extra kans op het optreden van luchtwegsensibilisatie ten opzichte van het aantal gevallen in de (niet blootgestelde) algemene bevolking.

Vanwege de grote overeenkomsten tussen de twee onderzoeken heeft de commissie besloten de gegevens van beide samen te voegen en daaruit een referentiewaarde te berekenen met behulp van een lineair regressiemodel. De referentiewaarde is gebaseerd op 1 procent extra kans (additioneel risico van 1 procent). Voor schimmel alfa-amylase heeft de commissie een waarde afgeleid van 0,9 ng/m^3.

Gezondheidskundige referentiewaarden voor sensibilisatie

De commissie beveelt een referentiewaarde van 0,9 ng enzym/m^3 aan voor beroepsmatige blootstelling aan schimmel alfa-amylase, gemiddeld over een achturige werkdag. Bij deze concentratie hebben werkers ten opzichte van de algemene bevolking een extra kans van 1% op sensibilisatie voor schimmel alfa-amylase allergenen.

Executive summary

Scope

At request of the Minister of Social Affairs and Employment, the Dutch expert Committee on Occupational Exposure Safety (DECOS), one of the permanent committees of experts of the Health Council, proposes health-based recommended occupational exposure limits for chemical substances in the air in the workplace. These recommendations serve as basis in setting legally binding occupational exposure limits by the minister.

In this advisory report, the committee evaluates the consequences of exposure to fungal alpha-amylase, an enzyme from the fungus *Aspergillus oryzae*. Workers in bakeries and flour mills may be exposed to this enzyme. The committee's conclusions are based on scientific papers published before August 2014.

Physical and chemical properties

The enzyme alpha-amylase from the fungus *Aspergillus oryzae*, is a 478 amino acid glycoprotein. In pure form it is whitish-yellow and a hygroscopic powder. The enzyme catalyses the hydrolysis of long-chain carbohydrates to maltose and glucose. Fungal alpha-amylase extracts are primarily used as a dough improver in the preparation of bakery products.

Exposure

Exposure to airborne fungal alpha-amylase should be monitored as the content of the allergen in inhalable dust, and has mainly been determined in bakeries. Average concentrations in full-shift inhalable air ranged between the detection limit and a few hundred ng/m^3, with maxima up to 30 µg/m^3. The highest exposures were observed for workers involved in weighing and mixing, and dough handling activities. Because different quantities of dough improver are used for bread and pastry, exposure is also dependent on the type of bakery. During an intervention programme between 2000 and 2007 in the Netherlands, exposure to alpha-amylase decreased in industrial bakeries and flour mills, but increased at ingredient producers.

Kinetics

Exposure to alpha-amylase occurs from dust particles or liquid aerosols. There are no kinetics data specifically relating to enzymes, but they are considered to behave as other particles. The place of deposition in the airway system depends on the size of the particle. In bakeries, fungal alpha-amylase was predominantly present in dust particles with an aerodynamic diameter larger than 5 µm, with over fifty percent associated with particles larger than 9 µm. The majority of these particles are therefore likely to be deposited in the nose, mouth and upper airways.

Effects

Inhalation of fungal alpha-amylase elicits immunological as well as non-immunological responses. Immunological responses lead to sensitisation, which may induce – at continuing exposure – allergic respiratory symptoms, such as asthma, rhinitis, and rhinoconjunctivitis. The respiratory symptoms can also be caused by irritation, a non-immunological response. The only way to distinguish between the two types of responses is to test people on being sensitised to fungal alpha-amylase. The available studies suggest that sensitisation may occur within months after starting of exposure, but it can take several years to develop symptoms.

Most data on the effects of occupational exposure to fungal alpha-amylase are retrieved from studies on bakery workers and flour millers. The number of cases of specific sensitisation among these workers varies between 1 to 30

percent. For comparison, the number of cases in the general, non-exposed population varies between 1 and 2 percent.

No studies have been performed on other possible adverse health effects in humans, nor were there relevant animal studies reported.

Evaluation and recommendation

In deriving a health-based OEL, the committee considered data on sensitisation as the most relevant. Somebody who is sensitised has a high risk in developing allergic reactions at continuing exposure. Because sensitisation is an irreversible effect, the person in question will be sensitised for the rest of his or her life, and at exposure, may show allergic symptoms. In bakeries not only fungal alpha-amylase is used, but also wheat flour and other additives. Also these substances can induce respiratory symptoms, which are not distinguishable from the symptoms described for alpha-amylase exposure. Such a distinguish can be made for sensitisation by using special immunological tests. Further, the committee did not find evidence that respiratory symptoms occur at lower exposure levels than sensitisation. This means that an occupational exposure limit based on data on sensitisation also prevents the development of respiratory symptoms.

In deriving a health-based occupational exposure limit, data are needed on exposure-response relationships. However, in most studies such a relationship was not investigated due to lack of exposure data. In two independent studies an exposure-response relationship was assessed. Both studies were carried out among bakery workers, used airborne enzyme levels as exposure parameter (measured by the same technique), and tested on sensitisation to fungal alpha-amylase. The outcomes differ somewhat, but according to the committee this is explained by normal statistical variation due to differences in the composition of the population under study.

The Committee is of the opinion that for the effect ‘sensitisation’ no threshold level can be assessed. The reason being that in the general (not occupationally exposed) population already cases of sensitisation to fungal alpha-amylase have been reported. That implies that exposure, irrespective the level, gives a risk in developing sensitisation, and that the setting of reference values is warranted. A reference value is a concentration of alpha-amylase in the air, at which occupational exposure leads to a predefined accepted level of risk of allergic airway sensitisation, compared to the background risk in the general, non-exposed population.

Since the two studies show large similarities, the committee decided to combine the data and to use a linear regression model to derive a reference value.

The reference value is based on an additional sensitisation risk of 1 percent. For fungal alpha-amylase, the committee derived a value of 0.9 ng/m^3.

Health-based recommended reference values for sensitisation

The committee recommends a reference value of 0.9 ng enzyme/m^3 for occupational exposure to fungal alpha-amylase, as an eight-hour time-weighted average concentration. At this concentration workers have an additional sensitisation risk for fungal alpha-amylase of 1 percent compared to the background risk in the general population.

Chapter 1

Scope

1.1 Background

At request of the minister of Social Affairs and Employment, the Dutch expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations on the toxicity of substances that are used in the workplace (Annex A). The purpose of these evaluations is to recommend health-based occupational exposure limits, which specify levels of exposure to airborne substances, at or below which it may be reasonably expected that there is no risk of adverse health effects.

In this advisory report, such an evaluation and recommendation is made for alpha-amylase from the fungus *Aspergillus oryzae*.

1.2 Committee and procedure

The present document contains the assessment of DECOS, hereafter called the committee. The members of the Committee are listed in Annex B. The submission letter to the Minister can be found in Annex C.

In 2014, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in

deciding on the final version of the report. The received comments, and the replies by the Committee, can be found on the website of the Health Council.

1.3 Data

The Committee's recommendations on the health-based occupational exposure limit or reference values of alpha-amylase from *A. oryzae* are based on scientific data, which are publicly available. Published literature was retrieved from the on-line databases Medline and Toxline, supplemented with subject searches in journals and internet sources. The final search was carried out in August 2014.

Chapter 2

Identity, properties and monitoring

2.1 Identification

2.1.1 Fungal alpha-amylase

Alpha-amylase is a naturally occurring enzyme that catalyses the hydrolysis of starch into sugars. It is present in the saliva of humans and some other mammals, in seeds containing starch as a food reserve (e.g. wheat), and is secreted by bacteria and many fungi. It is also produced industrially, both from bacteria (e.g. *Bacillus spp*. for use in the detergent industry) and fungi (e.g. *Aspergillus spp.* for use in the food industry).[1]

Regarding occupational exposure, the most used alpha-amylase is the enzyme produced by the fungus *Aspergillus oryzae*. Since techniques are available to monitor specifically the alpha-amylase from this fungus, and data do not indicate allergenic cross-reactions among the different types of alpha-amylases, the committee restricted this advisory report to the fungal alpha-amylase from *Aspergillus oryzae*.

2.1.2 Combined exposure

Fungal alpha-amylase is mainly used in the bakery industry. The dust in the bakery industry may contain several other ingredients used in the bakery process, such as flour dust from wheat and rye, other enzymes (malt enzymes), and

ingredients (e.g. baker's yeast, egg powder, sugar). The committee is aware that these ingredients might contribute to the biological effects of fungal alpha-amylase. For instance, flour dusts from wheat and rye are known to have sensitizing properties.[2,3] However, the present risk evaluation is restricted to fungal alpha-amylase.

2.2 Identity

Until the 1960's, the food industry mainly employed acid hydrolysis for processing starch. Since then, this traditional method was replaced by enzymes, mainly fungal alpha-amylase from *Aspergillus oryzae*. Alpha-amylases (EC 3.2.1.1) catalyze the hydrolysis of internal α-1,4 glycosidic bonds in long-chain carbohydrates yielding maltose and glucose. Commercial flour additives contain up to 0.3% w/w alpha-amylase, usually from *Aspergillus oryzae*.[4] In bread production, amylases are added to baking flour at 0.001 to 0.02% w/w, to compensate for the low natural amylase content.[5-7] The liberation of yeast-fermentable carbohydrates (dextrins and maltose) by alpha-amylase stimulates the growth of yeast, improving the rising of the dough and the quality of the bread. Alpha-amylase is also used to delay the progressive hardening and drying in time, thereby prolonging the shelf life of bread.[8]

Name	:	alpha-amylase from *Aspergillus oryzae*
EC number	:	232-565-6
CAS number	:	9000-90-2; 9001-19-8
Enzyme Commission no.	:	3.2.1.1
Synonyms	:	1,4-alpha-D-glucan glucanohydrolase; Asp o 2; Fungamyl; Taka-amylase
Appearance	:	whitish-yellow, very hygroscopic powder
Molecular weight	:	53 kD
pH-optimum	:	7.0

2.3 Physical and biochemical properties

Alpha-amylase from *A. oryzae* is a 478 amino acid glycoprotein.[9] The molecule consists of three structural domains; the structure is stabilized by four disulfide bonds. Binding to calcium is necessary for enzyme activity; two calcium binding sites are located near the enzymatically active site cleft of the enzyme.[10] By purifying the commercial alpha-amylase extract from *Aspergillus oryzae*, Baur et al. (1994) demonstrated that the enzyme is the main allergenic component of the commercial baking enzyme products.[5] The enzyme activity rapidly declines

upon heating, but the antigenic properties are more resilient. Heating the enzyme for 20 minutes at 99.8°C (the interior temperature of dough and bread during baking) caused a partial decrease of the IgE-binding capacity.[11]

2.4 EU classification and labelling

In the European Union, fungal alpha-amylase is classified as a category 1 respiratory sensitiser and labelled with H334 (May cause allergy or asthma symptoms or breathing difficulties if inhaled).[12]

2.5 Validated analytical methods

2.5.1 Environmental monitoring

Several methods are available for determining airborne fungal alpha-amylase, namely as non-specific, inhalable dust which is sampled using gravimetric techniques (calculated using the mass of the sampled particles and the air volume monitored), and by measuring the amount of alpha-amylase in inhalable dust.

Inhalable dust sampling

In The Netherlands, inhalable dust is usually sampled with the Dutch 'PAS-6' sampling head, but other methods are also available. For determining the aerodynamic particle size of dust, personal air samplers can be used. The Institute of Occupational Medicine (IOM) in Edinburgh, Scotland, developed the IOM inhalable dust sampling head and cassette to meet the sampling criteria for inhalable particulate mass.[2] Within Europe, size fractions for measurement of airborne particles in workplace atmospheres have been standardized since 1993 (European Standard EN 481:1993). In this standard three size fractions have been defined (inhalable, thoracic and respirable).[13]

Alpha-amylase content in inhalable dust

Basically, there are two ways to monitor the presence of alpha-amylase in inhalable dust. One is measuring its allergen content by using an immunoassay, the other is measuring the enzyme activity.

In the case of alpha-amylase, the most relevant adverse health effect is specific sensitisation, an immunological reaction which may lead to allergic symptoms (see Chapter 7 and 9). Such an immunologic reaction starts with the

recognition of epitopes by the immune system. Epitopes are components (antigenic determinants) which are present in the protein of the enzyme. By means of immunoassays using antibodies, low levels of epitopes can be recognized. In addition, these antibodies are unique in that they only recognize certain epitopes in the fungal alpha-amylase, and not the epitopes in alpha-amylase from other sources. Thus by using such an immunoassay, the concentration of fungal alpha-amylase can be measured, even in the presence of alpha-amylase from other sources (e.g. the alpha amylase as natural content of wheat flour), and therefore the technique may be considered both sensitive and specific.

The other technique, measuring enzyme activity, is not preferred, because inactive or denaturated enzyme molecules may still be allergenic and therefore have adverse health properties, whereas these are not measured enzymatically. Furthermore, by measuring enzyme activity no distinction can be made between the different sources of alpha-amylase.[14]

Overall the committee concludes that it is best to use an immunoassay in determining exposure levels of airborne fungal alpha-amylase. Details on the methods available are summarized below.

Immunoassays. Houba et al. (1997) developed a method using an enzyme immunoassay with affinity-purified polyclonal rabbit IgG antibodies to fungal alpha-amylase (sandwich-immunoassay).[15] In addition, Sander et al. (1997) and Elms et al. (2001) developed an enzyme (inhibition) immunoassay using monoclonal antibodies to fungal alpha-amylase (ELISA-immunoassay).[16,17] Both types of immunoassays are specific for fungal alpha-amylase and do not detect cereal alpha-amylases occurring naturally in flour.

In an interlaboratory comparison study, Lillienberg et al. (2000) compared the outcomes of both types of immunoassays using 80 representative whole-shift inhalable dust samples (PAS-6 sampler), taken in four bakeries in Sweden, Germany and The Netherlands.[18] Extracts of the samples were distributed to laboratories in the three countries. Two sandwich-immunoassays were used, using polyclonal antibodies from two different origins. Also two other ELISA-immunoassays were used, one based on a polyclonal antibody, the other on a monoclonal antibody. The geometric means for samples analysed by the sandwich-immunoassays varied about a factor 2 among laboratories; the geometric means of alpha-amylase determined by the other ELISA-immunoassay was 3-6 times higher. The detection limit was 65-150 pg/mL for the sandwich-immunoassay, and 600 pg/mL for the other ELISA-immunoassay.

With the aim to develop an international standard for measuring exposure to fungal alpha-amylase, Sander et al. (2007) conducted a study to compare the sandwich- and the ELISA-immunoassays, and to establish optimal elution and assay conditions.[19] Inhalable dust was collected in bakeries and mills in Germany, England, The Netherlands and Spain. The assays for measuring fungal alpha-amylase in dust extracts correlated well for log-transformed values of positive samples (Pearson's correlation coefficient ≥ 0.88), but the absolute concentrations differed up to 5.8-fold. Of the extraction methods employed, phosphate-buffered saline with 0.05% Tween-20 proved to be the best. Storage of dust/filter extracts at -20°C resulted in an approximate loss of 40% in alpha-amylase antigenic activity after 3-4 months.

In epidemiological studies, usually those in which the sandwich-immunoassay was used, airborne dust was sampled on Teflon (PTFE) filters.[14,15,20-22] In other studies, glass fibre filters were used.[16,23-25] The effect of filter type on the yield of alpha-amylase has not yet been examined.

More recently, a semiquantitative lateral flow immunoassay was developed for fungal alpha-amylase, allowing rapid on-site detection of the allergen in airborne dust. The detection limit for this method is 320 pg/mL.[26,27]

Correlation between inhalable dust concentration and fungal alpha-amylase content

The correlation between inhalable flour dust and fungal alpha-amylase is highly variable. A significant correlation was found in some studies (Page et al. 2010[28], Bulat et al. 2004[20]), whereas the correlation in other studies was poor (Houba et al. 1997[15], Elms et al. 2003[29], Baatjies et al. 2010[30]). A possible explanation for the variability between inhalable dust and alpha-amylase concentration are the variable enzyme contents of the premix products used in the bakeries.[30] For example, cake and pastry baking differ from bread baking in that flour improvers containing alpha-amylase do not play a vital part in the process.[31] For these reasons, the committee is of the opinion that dust exposure measurements cannot be used as a surrogate for exposure to fungal alpha-amylase. Cereal flour allergen concentrations in airborne dust are also an inadequate surrogate for exposure to fungal alpha-amylase, because there is hardly any cross-reactivity between cereal flour allergens and fungal alpha-amylase.(Houba et al. 1996, Sandiford et al. 1994, Nieuwenhuijsen et al. 1999).[32-34]

2.5.2 *Biological effect monitoring*

Tests are available to screen for sensitised persons against specific allergens. A useful clinical method for determining a rough approximation of the person's sensitivity to an allergen is the skin prick test. In this test, allergens are introduced into the skin, after which the extent of local inflammation (wheal and flare diameter (mm)) is measured, as a result of the pharmacological effects of mediators, such as histamine, on the blood vessels in the skin. Skin prick tests resulting in a wheal diameter of at least 3 mm larger than the negative diluent (saline) control after fifteen minutes are usually considered positive for sensitisation.

Alternatively, analysis of the presence of relevant specific IgE-antibodies, for instance in blood and nasal secretions, may be carried out. Serum concentrations of IgE antibodies to fungal alpha-amylase can be determined by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). In both assays, fungal alpha-amylase is conjugated to commercially available cyanogen bromide activated cellulose or paper discs. In the Radio-Allergo-Sorbent-Test (RAST) developed by Brisman and Belin (1991), an ^{125}I-labelled anti-human IgE preparation is used as a tracer.[35] Baur et al. (1994) used an enzyme-allergo-sorbent-test (EAST) for determining alpha-amylase specific IgE in sera.[5] In this assay, an anti-IgE-beta-galactosidase conjugate is used for detection. The EAST and RAST techniques were found to correlate to a high degree. The standard cut-off level for considering a test positive is $\geq$ 0.35 kU/L. More recently a commercial test has become available for quantifying specific IgE antibodies to fungal alpha-amylase in blood, employing a highly sensitive enzyme-enhanced chemiluminescent enzyme immunoassay (CAP-FEIA, Page et al. 2010, Baatjies et al. 2009).[28,36] In contrast to the standard cut-off level generally used of $\geq$ 0.35 kU/L, in this commercial the test cut-off level is 0.10 kU/L.

Specific bronchial challenge testing is employed for the assessment of bronchial hyperresponsiveness, and if occupational asthma is suspected. The test provoces a physical response (rhinitis, asthma), and involves inhalation of a low dose of an allergen. Since there is serious risk of the patient suffering a serious asthmatic attack, it is important to perform the test in a good clinical setting.

2.6 Summary

In the present report alpha-amylase from the fungus *Aspergillus oryzea* is evaluated. It is an enzyme that catalyses long-chain carbohydrates yielding maltose and glucose. Alpha-amylase is a glycoprotein that is composed of antigenic determinants, so-called epitopes, which can be recognized by the immune system. In this way specific sensitisation followed by allergic reactions can be provoked. Fungal alpha-amylase content in inhalable dust can be monitored by using an immunoassay. Monitoring inhalable dust as parameter for alpha-amylase exposure is not preferred, because of the poor correlation between inhalable dust and alpha-amylase content. Persons can be tested on sensitising responses by a skin or blood test.

Chapter 3

Sources

3.1 Natural sources

Alpha-amylase form *Aspergillus oryzea* is a naturally occurring enzyme that catalyses the hydrolysis of starch into sugars. It is present in the saliva of humans and some other mammals, in seeds containing starch as a food reserve (e.g. wheat), and is secreted by bacteria and many fungi.

3.2 Man-made sources

3.2.1 Production

Fungal alpha-amylase is also produced industrially, both from bacteria (e.g. *Bacillus spp*. for use in the detergent industry) and fungi (e.g. *Aspergillus spp.* for use in the food industry).[1] The enzyme is manufactured for the food and feed industry.[37,38] Production volumes are not available.

3.2.2 Use

Bakers use fungal alpha-amylase as a supplement to cereal flour to improve carbohydrate fermentation by yeasts. This supplement can be added to the cereal flour by the bakers themselves, or is added already during flour milling to the cereal flour, which then is sold to the bakeries as a ready-for-use flour product.

Inhalable dust in the atmosphere of the enzyme producing industry, bakeries, flour mills and manufacturers of dough improvers is the main sources of occupational exposure to the enzyme. Because it is added to flour from finely milled or otherwise processed cereal grains, fungal alpha-amylase is usually associated with flour dust in bakeries and related facilities. The most common tasks associated with alpha-amylase exposure involve dust-generating activities such as dispensing, sieving, weighing and mixing.[39,40]

Fungal contamination of cereals as a possible source of alpha-amylase was proposed by Moneo et al. (1994).[41] In a study of 259 millers in Spain, skin tests and specific serum IgE to fungal alpha-amylase were obtained in 12 of the 73 workers. Since the workers did not use fungal alpha-amylase, the authors suggest that the enzyme is secreted by moulds growing on wheat or wheat flour. However, Houba et al. (1996) reported having detected only low concentrations of airborne fungi in (Dutch) bakeries, of which only a small fraction appeared to originate from *A. oryzae*.[14]

Chapter 4

Exposure

4.1 General population

Almost no studies have been published concerning exposure levels to fungal alpha-amylase in non-occupational settings. Vissers et al. (2001) measured wheat and fungal alpha-amylase allergens in floor dust samples, collected in the private homes of 34 bakers in The Netherlands.[42] The concentration of fungal alpha-amylase allergens (geometric mean) ranged from 10.5 ng eq/m^2 (living room) to 76.7 ng eq/m^2 (baker's bedroom). The highest levels were found in houses that could be reached directly through the bakery, in houses with textile floor covers, and if bakers brought their work clothes into the house.

Fungal alpha-amylase was shown to retain to some extent its antibody binding capacity and allergenicity after baking. In comparison with the content of fungal alpha-amylase in dough, between 0.1 and 20% of the antibody-binding capacity remained in bread and rolls.[43] Fungal alpha-amylase may be added to certain washing powders, but exposure from dust from these agents in households is probably negligible.[44]

4.2 Working population

Characteristic exposure data for fungal alpha-amylase are available for bakeries, flour mills and bakery ingredient producers in the Netherlands.[14,15,18,45] The results of full-shift personal measurements of fungal alpha-amylase in inhalable

dust show exposures similar to those found in studies from other countries (Table 1).

Table 1 Full-shift personal exposure to airborne inhalable dust-associated fungal alpha-amylase in various industries.

Industry and country	AM (ng/m^3)	GM (ng/m^3)	GSD (ng/m^3)	Range (ng/m^3)	Reference
Sandwich-immunoassay (polyclonal antibodies)					
14 Bakeries, The Netherlands; 454 personal samples	1.9 - 39.4[a]	0.7 - 18.1[a]	-[a]	ND - 221.8	Houba et al. 1996[14]
21 Bakeries, The Netherlands;					Houba et al. 1997[15]
• large bakery: 406 personal samples		ND - 18.1[a]	-[a]		
• small bakery: 101 personal samples		ND - 0.3[a]	-[a]		
7 Bakeries (5 small, 2 large), Canada; 96 personal samples	22.0	2.8	10.4	ND - 307.1	Burstyn et al. 1998[21]
Flour mills, packing stations and large bakeries (n=7), the UK; 256 personal samples	0.7 - 46.7[a]				Nieuwenhuijsen et al. 1999[22]
4 Bakeries, UK; 26 personal samples				ND - 29.8	Elms et al. 2001[16]
Bakeries, mills and flour improver producer/packer, UK;					Elms et al. 2003[29]
• 8 small; 27 personal samples		1.7[e]		<0.4 - 173	
• 5 medium; 28 personal samples		1.4[e]		<0.4 - 12.1	
• 6 large; 41 personal samples		4.2[e]		<0.4 - 1,370	
• 2 mills; 17 personal samples		1.5[e]		<0.4 - 11.1	
• 1 improver producer/packer; 4 personal samples		455.9[e]		71.2 - 1,178	
Bakeries, Belgium;					Bulat et al. 2004[20]
• 4 industrial; 54 personal samples[b]		0.2 - 0.5	-[a]	0.1 - 136.2[a]	
• 66 traditional; 218 personal samples[b]		0.5 - 0.6	-[a]	0.1 - 51.1[a]	
Bakeries, The Netherlands;					Meijster et al. 2007[45]
• 65 traditional; 169 personal samples		0.8	6.5	0.1 - 115	
• 20 industrial; 344 personal samples		0.4	6.0	0.1 - 910	
• 7 flour mills; 142 personal samples		8.4	9.7	0.2 - 30,009	
• 7 ingredient producers; 113 personal samples		33.5	22.4	0.2 - 889,054	
Bakery, USA; 83 personal samples	0.1 - 2.1[f]			0.02 - 11,000	Page et al. 2009[46], 2010[28]
17 Small bakeries, Scotland; 114 personal samples[c]	-[c]	-[c]	-[c]	3 - 710	HSE 1999[31]

ELISA-immunoassay (monoclonal antibodies)

1 Conventional bakery, Germany; 17 personal samples	335	13	-[c]	ND - 4,836	Sander et al. 1998[25]
1 "Vollwertbäckerei", Germany; 14 personal samples	-	-		ND	Sander et al. 1998[25]
Bakeries in:					Lillienberg et al. 2000[18]
• Sweden; 29 personal samples	7.2 - 22.0[d]	0.8 - 4.7[d]	5.0 - 9.0[d]	0.1 - 237[d]	
• Germany; 29 personal samples	9.5 - 30.1[d]	0.1 - 614.6[d]	0.6 - 3.8[d]	5.0 - 9.0[d]	
• The Netherlands; 22 personal samples	7.9 - 25.6[d]	0.1 - 165.5[d]	1.3 - 6.2[d]	5.0 - 10.0[d]	
20 Supermarket bakeries, UK; 89 personal samples	-	-	-	ND - 12.4	Brant et al. 2005[23]
Bakeries (n=55), UK; 195 personal samples		5.9	-[c]	<0.78 - 33.7	Elms et al. 2006[47]
18 Supermarket bakeries, South-Africa; 211 personal samples	0.29	0.13	2.08	0.1 - 19.6	Baatjies et al. 2009[36], 2010[30]

AM: arithmetic mean; GM: geometric mean; GSD: geometric standard deviation; ND: below the detection limit.
[a] No overall AM and/or GM was presented in the original publication, but only for each occupational title; the means presented are the lowest and highest AM and GM for the occupational titles;
[b] Calculated from the reported fraction of alpha-amylase in inhalable dust;
[c] This information could not be obtained from the original publication;
[d] Interlaboratory comparison study. The range of values determined by the 4 participating laboratories is shown;
[e] Only the median was reported;
[f] GM for entire population not reported; separate values for current low exposure (range 0.019 - 1.2 ng/m^3) and for current and past higher exposure (range 0.095 - 11,000 ng/m^3).

In bakeries, the highest exposures to fungal alpha-amylase were observed for workers involved in weighing, mixing, pouring and dough handling activities, as well as during the production of croissants, puff-pastry and bread-bun.[4,21,29,48]

In a study in supermarket bakeries in the UK, fungal alpha-amylase was detected in whole-shift personal respirable dust samples of only three of nine sensitised individuals, showing the difficulty of capturing relevant sensitising exposures even in whole-shift, personal measurements.[23] Elms et al. (2001) analysed four consecutive 15-minute inhalable dust samples from eight bakery workers, and found a large inter-sample variation of the alpha-amylase concentration, revealing alternating periods of high and low exposure.[16] Short-term peak exposures should therefore be taken into consideration for sensitised individuals who have low exposure levels if measured over the full shift period. Short-term exposure measurements during performance of dust-generating tasks were reported by Brisman and Belin (1991).[35] Using an enzymatic method for analysing fungal alpha-amylase in inhalable airborne dust in a Swedish factory producing bread mixes and dough improvers, they determined a concentration of 30 µg/m^3 in a 30-60 min personal sample for a worker at a packing station.

In The Netherlands, an intervention programme was enrolled as part of a covenant in the flour processing industry (industrial bakeries, flour mills and

ingredient producers). Worker's health was monitored through the instalment of a sector-wide health surveillance system and all employers and employees were informed about the risks of occupational exposure and were provided information on good work practices and control measures. The campaign included site visits by a trained consultant and distribution of a specially developed dust control manual. The effect of the programme on exposure to airborne inhalable fungal alpha-amylase was investigated in a large study using exposure data from studies conducted in bakeries, flour mills and ingredient producing companies between 1993 and 2007 (see Table 2).[14,15,24,32,45]

Despite several changes in work practices (e.g., the increased use of liquid instead of powdery fungal alpha-amylase containing bread improver), only limited effect on exposure levels was observed from the sector-wide intervention programme. The overall trends in exposure, not corrected for co-variables, indicated a slight downward trend for fungal alpha-amylase in bakeries. Per sector, a significant yearly downward trend was observed of 8% for fungal alpha-amylase among the population of bakery workers. However, trends varied substantially between job categories: a downward trend was observed for pastry bakers and cleaners, a strong yearly increasing exposure trend was observed (>20%) for general bakers, whereas no trend was observed for bread bakers, dough makers, maintenance workers and low-exposed jobs. In flour mills, no trend was observed in fungal alpha-amylase exposure over time. On job level, a significant decreasing trend was shown only for maintenance workers. For workers in the ingredients production industry, there was a strong increase in occupational exposure to fungal alpha-amylase for all job titles. Overall, a non-significant increasing trend of almost 30% annually was observed over the covenant period for this sector (2001-2007). There was no apparent explanation for the exposure increase to fungal alpha-amylase, as information from the sector did not indicate an increased use or changed work practices. The authors stated that the results should be interpreted with caution because data per job were limited for this industry.[24]

Table 2 Average exposure level to fungal alpha-amylase (ng/m^3) per sector for the period 2000-2001 and 2007.[49]

Sector	2000 - 2001			2007		
	N	GM	GSD	N	GM	GSD
Industrial bakeries	175	1.0	3.3	131	0.4	7.1
Flour mills	143	8.0	9.8	88	6.3	15
Ingredient producers	114	32	22	89	177	16

N, number of companies; GM, geometric mean; GSD, geometric standard deviation.

Chapter 5

Kinetics

Occupational exposure to fungal alpha-amylase occurs from inhalation of dust or liquid aerosols. There are no data on absorption, distribution, metabolism, and excretion specifically relating to enzymes, but they are considered to behave as other particulate matter. Therefore, below the kinetics of particles (with no or very low toxic potential) is summarized.

Deposition

Upon inhalation, particles are deposited on the mucous membranes of the airways. The place of deposition in the airways is dependent on the size of the particle.[38] Based on the aerodynamic diameter, particles are divided in inhalable, thoracic and respiratory fractions (see European Standard EN 481:1993). Inhalable particle (or dust) fractions are defined as fractions in which 50% of the particles have an aerodynamic diameter of 100 µm. These particles are mainly deposited in the nose and nasopharyngeal region of the respiratory tract. In thoracic fractions about 50% of the particles have an aerodynamic diameter of 10 µm, and these can be found the trachea and bronchial region of the respiratory tract. Finally, particles in the respiratory fraction are the smallest, and may reach the lungs (particles with an aerodynamic diameter of 3.5-4 µm or smaller). In crispbake and small bakeries in The Netherlands, fungal alpha-amylase was predominantly present in dust particles with an aerodynamic diameter larger than

5 µm, with over 50% associated with particles larger than 9 µm, and less than 10% associated with particles with an aerodynamic diameter lower than 5 µm.[15]

Dust particles are cleared from the lungs by macrophages and the mucociliary system. However, heavy exposure may lower the ability of macrophages to eliminate particles, which may result in penetration of dust particles into the interstitium. The individual (anatomic) characteristics of an exposed person are also of importance in the development of disease.[2,3]

Chapter 6

Mechanism of action

Respiratory exposure to fungal alpha-amylase elicits immunological (allergic) and non-immunological (irritation) responses, such as rhinitis, rhinoconjunctivitis, asthma. The symptoms of irritation, are similar to allergic symptoms. For interpreting the symptoms and its consequences on health, it is important to make a distinction between the non-immunological and immunological responses. In practice, symptoms are ascribed to irritation if immunological responses are ruled out

6.1 Immunological responses

Sensitisation is an immunological mechanism (type I hypersensitivity reaction), which may occur at a first exposure, and is characterised by little or no response against the sensitising agent, in this case alpha-amylase. However, when a person is sensitised, subsequent exposure may cause intense responses, such as asthma, rhinitis and conjunctivitis. This may occur at low exposure concentrations. The responses may be life threatening and may have an immediate or delayed onset. The key mechanism of sensitisation is the formation of specific IgE-antibodies against alpha-amylase. These IgE-antibodies are incorporated at the surface of mast cells. Following a second encounter with the same allergens, mast cells may overreact when these allergens bind to the antibodies presented at the surface of the mast cells. Mast cells form the starting point of a cascade of chemical reactions resulting in clinical symptoms. Specific IgE-antibodies against alpha-

amylase have been demonstrated in workers who were sensitised after inhalation of dust, by in vivo and in vitro tests. Workers who have become sensitised to a particular agent may also exhibit cross-reactivity to other agents with similar chemical structures. However, cross-reactivity was not found between fungal alpha-amylase and pancreas alpha-amylase from pigs (Losada et al. 1992)[50] or cereal alpha-amylase (Nieuwenhuijsen et al. 1999; Sandiford et al. 1994).[22,34,51]

A special group among the population are the atopics, that are persons with a family history of similar clinical symptoms or persons who are allergic to common allergens. In general, atopic individuals may be slightly more prone to sensitisation to alpha-amylase than non-atopic individuals. In the human population it is estimated that 5.1 to 20.8% are atopic. Most investigators found that atopic workers were more vulnerable to develop work-related respiratory symptoms than non-atopic workers.

In addition to being atopic, other factors may increase the risk to become sensitised to work-related allergens, such as the level of airborne allergens. In none of the available studies involving fungal alpha-amylase, a positive association was found between sensitisation to the enzyme and smoking habits (Brisman and Belin 1991; Cullinan et al. 1994, 2001; Houba 1996; Vanhanen et al. 1997),[35,52-55] except in atopic workers (Droste et al. 2003; Harris-Roberts et al. 2009; Page et al. 2010),[28,40,56] although others did not always confirm this.

Age has not been reported to be a determinant of sensitisation to fungal alpha-amylase. Gender was a potential confounder in one study but the authors did not stratify their results (Brisman et al. 2004).[57]

6.2 Non-immunological responses

A strong association between exposure to fungal alpha-amylase and respiratory symptoms has been shown in a number of studies (see Chapter 7), of which up to 70% of work-related asthmatic symptoms in bakery employees has been attributed to non-specific irritation to dust.[7] This led Smith et al. (1999) to suggest that sensitisation to alpha-amylase is not a relevant endpoint for the prevention of bakery-related respiratory disease.[58]

As is written in Section 5, fungal alpha-amylase is considered to behave as dust particles. In general, exposure to large dust particles, irrespective of its chemical activity properties, may lead to local irritation to the eyes, nose and ears. In addition, inhalable dust particles may lead to irritation and inflammation of the bronchioles, alveolar ducts and alveoli. When dust particles are deposited in the respiratory system, the body tries to clear the material, in which the mucociliary defence system, and/or inflammatory cells, such as macrophages,

are involved. Macrophages produce inflammatory mediators, which induce inflammatory responses with symptoms of irritation.

6.3 Conclusion

Respiratory exposure to fungal alpha-amylase elicits immunological and non-immunological responses. Immunological responses, primarily IgE-mediated, lead to sensitisation and allergic respiratory symptoms, such as rhinitis, rhinoconjunctivitis, asthma, or in some cases to contact dermatitis (on dermal exposure). The non-immunological responses are most likely due to irritation of the upper and lower airways. Irritation may result in similar symptoms as described for allergic responses.

Chapter 7

Effects

7.1 Observations in humans

Observations in humans are limited to effects in the skin, eyes, airways and lungs. No data are available on effects on other organ systems, nor on carcinogenic effects and reproduction toxicity.

7.1.1 Irritation

In a prospective cohort study, involving 300 UK bakery and flour mill workers not previously exposed to flour, only 15% of the workers with respiratory symptoms or eye, nose or skin symptoms, were sensitised to wheat flour or alpha-amylase (as revealed by positive skin prick tests), suggesting that a high proportion of the work-related symptoms did not have an immunologic basis (Cullinan 1994, 2001).[52,53]

Houba et al. (1996) performed a survey among 393 Dutch bakery workers.[54] Twenty-three percent of these workers reported work-related rhinitis and/or chest tightness, but only 30% (7% of all bakery workers) showed an immunological response to wheat flour or fungal alpha-amylase. Although the authors noted that the sensitivity of the IgE test was not perfect, and that there are also other potential bakery allergens that have not been tested, they considered a non-specific reaction to the dusty environment in the bakeries as one of the likely explanations for this finding. Most workers with work-related symptoms, but

without an immunologic response (sensitisation) to wheat flour or fungal alpha-amylase, had IgE antibodies to non-occupational allergens (58%), had a history of allergic symptoms to common allergens (27%), or reported chronic respiratory symptoms outside the job environment (55%).

Smith and Lumley (1996) screened 3,450 workers in a large food company in the UK using a questionnaire.[7] The overall prevalence of work-related asthmatic symptoms was 4.4%. After an examination by a physician, only 9% of this group had occupational asthma. The majority of the complaints was thought to have arisen from irritation or aggravation of pre-existing asthma.

In a group of workers (n=679) from 18 different UK flour mills, Smith et al. (2000) examined the prevalence of respiratory symptoms and their relationship to sensitisation to wheat flour allergens and fungal amylases.[59] Of the examined workers, 20.5% described upper respiratory tract symptoms of an occasional nature, which the investigators related to short-term exposures to high levels of dust. Of the workers, 0.9% was sensitised to fungal alpha-amylase (positive skin prick test); three individuals had symptoms due to wheat flour allergy. Total inhalable dust exposure was measured for personnel exposed to flour dust at 10 different sites (116 samples). Median levels of 6.2 mg/m^3 were measured (range 1-10 mg/m^3; TWA 8 h) for production personnel, and a median of 18.7 mg/m^3 for hygiene operatives. The authors concluded that the principal causation of symptoms experienced by workers were non-specific irritant effects related to short-term exposures to high levels of total inhalable dust.

The committee noted that the investigators of the studies above did not describe (extensively) relationships between exposure levels and occurrence of symptoms.

7.1.2 Sensitisation

Prevalence

General population

In two studies among apprentice bakers in Canada and Poland, the prevalence early in the training was 0% (0/230, Gautrin et al. 1997[60]; 0/287, Walusiak et al. 2004[61]). In a study among trainee bakers in Italy (n=144, 81 attending the first year and 63 attending the second year), the prevalence for sensitisation to fungal alpha-amylase was 0.7% (1 case, also sensitised to wheat and with a personal history of allergy). In the same study, no persons sensitised to fungal alpha-

amylase were found in the reference group (81 graphic artist students; De Zotti et al. 1995).[62]

The prevalence of fungal alpha-amylase specific IgE antibodies in US blood donors was 1.0% (5/529).[63] The prevalence of sensitisation to fungal alpha-amylase among Dutch laboratory animal workers (positive skin prick test), reported by Houba et al. (1996), was 1.7% (7/416).[14] The prevalence of sensitisation among workers in a Belgian petrochemical plant was 0.8% (2/251).[56]

These studies suggest that there is at least some background level of sensitisation to fungal alpha-amylase (0 - 1.7%) in the general population. That in the general population people may be sensitised to fungal alpha-amylase could be explained by consumption of alpha-amylase containing food products, and the use of ready for use flour products (containing fungal alpha-amylase as supplement) for preparing bread and other bakery products themselves. Other reasons may be: cross-reactivity with as yet unknown other allergens; atopy; a lack of standardised methods for testing specific sensitisation resulting in false-positive outcomes; and, the use of anonymous blood donors, which may have been employed in companies using fungal alpha-amylase.

Working population

In Table 3 data from varies studies (mainly cross-sectional studies) are shown on the percentages of workers who are sensitised to fungal alpha-amylase, irrespective if they showed also symptoms of respiratory allergy or not. Detailed descriptions on study design, and results are given in Annex E. The most common tests used to determine the presence of specific sensitisation to fungal alpha-amylase were the skin prick test, and the radioallergosorbent test (RAST) to measure serum specific IgE levels.

Overall, prevalence rates vary between 1 and 19% among workers in bakeries and flour mills. Higher prevalence rates (up to 31%) have been reported for workers in enzyme producing and handling companies. The variability in prevalence rates could be explained by differences in exposure levels, duration and frequency of exposure, and the use of different protocols in testing sensitisation (e.g., variations in origin of fungal alpha-amylase, test concentrations, and cut-off points). In spite of these differences, the available studies clearly show that immunological sensitisation to fungal alpha-amylase is common.

Table 3 Sensitisation rates in workers exposed to fungal alpha-amylase. See Annex E for study details.

Reference	Type of industry	N	Skin prick test (% positive)	Specific serum IgE (% positive)
Bakeries				
Houba et al 1996[14]	bakery	169	9	8
Smith et al 1997[44]	bakery	385	16	
Baur et al 1998[64]	bakery	88/89	19	19
Smith and Wastell Smith 1998[65]	bread bakery	392	16	
Smith and Wastell Smith 1998[65]	cake bakery	77	1	
Jeffrey et al 1999[66]	bakery	205		15
Smith et al 2000[59]	flour mill	678	1	
Droste et al 2003[56]	bakery	222	8	
Walusiak et al 2004[61]	baker's training	287		11 (third year)
Storaas et al 2005[67]	bakery	183	7	2
Brant et al 2005[23]	supermarket bakery	233/210		4
Page et al 2009[46]	bakery	96		4 (7 at low threshold)
Harris-Roberts et al 2009[40]	bakery	160		6
Baatjies et al 2009[36]	supermarket bakery	507/513	4	4
Flour milling				
Cullinan et al 1994[52]	bakery + flour mill	256	5	
Enzyme producing industry and other sources				
Brisman and Belin 1991[35]	dough improver	20	30	5
Johnsen et al 1997[68]	enzyme producer	140	4	
Vanhanen et al 1997[55]	enzyme producer	173	3	3
Biagini et al 1996[69]	biotechnology plant	36	6	
Losada et al 1992[50]	pharmaceutical	83	31	

Dose-response relationships

A few studies have been performed in which exposure-response relationships to sensitisation were investigated. These include one longitudinal cohort study among bakery and flour mill workers in the United Kingdom, and three cross-sectional studies performed in bakeries in the Netherlands, Belgium, and the USA. Details on study design and results are given in Annex F. All used the

sandwich-immunoassay to determine airborne exposure levels of fungal alpha-amylase. Furthermore, the majority of the studies used a skin prick test to assess specific sensitisation to the enzyme; only two studies reported (also) on serum specific IgE levels. Since studies were performed in bakeries, co-exposure to other potential allergens was inevitable.

Two studies showed a statistically significant increase in number of sensitised workers with increasing exposure: Nieuwenhuijsen et al. (1999), and Houba et al. (1996).[22,70] Data from these studies are shown in Table 4.

Table 4 Summary of dose-response relationships between fungal alpha-amylase exposure and specific sensitisation with statistical significance.

Study characteristics	Exposure levels (fungal alpha-amylase, ng/m^3)	Sensitisation	Reference
Initial cross-sectional phase of cohort; 264 new workers Cohort, 3 bakeries, 1 flour packing factory, and 3 mills, UK	Method: Sandwich-immunoassay. Exposure at time of study (AM±SD): • low: 0.7±0.8 ng/m^3 • medium: 10.7±2.2 ng/m^3 • high: 46.7±16.6 ng/m^3 Highest exposure ever worked in: • low: 0.8±0.8 ng/m^3 • medium: 10.5±2.3 ng/m^3 • high: 48.0±16.6 ng/m^3	Method: Skin prick test. Prevalence, exposure at time of study: • low: 3.1% (7/225) • medium: 16.7% (3/18) • high: 15.4% (2/13) Prevalence, highest exposure ever: • low: 2.5% (5/203) • medium: 9.5% (2/21) • high: 29.4% (5/17) Prevalence ratio, highest exposure ever: • medium: 3.9 (95% CI, 0.8-20.2) • high: 9.9 (95% CI, 2.8-34.6)	Nieuwenhuijsen et al. 1999[22], 1994[71] Cohort details: Cullinan et al. 1994[52]
Cross-sectional, 14 bakeries in the Netherlands; 178 workers	Method: Sandwich-immunoassay Highest exposure ever worked in (GM±GSD): • low: 0.7±4.0 ng/m^3 • medium: 1.3±3.8 ng/m^3 • high: 18.1±4.6 ng/m^3 • indistinct/variable: 6.1±8.2 ng/m^3	Prevalence, skin prick test: • low: 1.4% (1/71) • medium:12.8% (5/39) • high: 30.4% (7/23) • indistinct: 8.3% (3/36) • reference population: 1.7% (7/416) Prevalence ratio, skin prick test: • medium:8.6 (95% CI, 1.01-74) • high: 15.9 (95% CI, 1.95-129) • indistinct: 4.6 (95% CI, 0.48-45) Prevalence, specific IgE: • low: 2.5% (2/71) • medium: 13% (5/39) • high: 15% (4/23) Prevalence ratio, specific IgE: • medium: 4.6 (95% CI, 0.85-22) • high: 3.9 (95% CI, 0.65-24)	Houba et al. 1996[70]; Doekes et al. 1998[6]

AM, average mean; 95% CI, 95% confidence interval; GM, geometric mean; SD, standard deviation.

The study by Nieuwenhuijsen et al. (1999) was a nested case-control study within a longitudinal cohort study among bakeries, flour mills and a flour packaging factory in the United Kingdom.[22] Workers (n=264) were screened for the presence of sensitisation to wheat flour allergens, and fungal alpha-amylase. The investigators divided the workers in three exposure groups (AM±SD, values represent highest exposure ever worked in): 0.7±0.8 ng/m^3 (low), 10.5±2.3 ng/m^3 (medium), and 48.0±16.6 ng/m^3 (high). The prevalence to sensitisation to fungal alpha-amylase increased by increasing exposure from 2.5% (low), 9.5% (medium) to 29.4% (high), resulting in prevalence ratios of: 3.9 (95% CI, 0.8-20.2; medium versus low exposure), and 9.9 (95% CI, 2.8-34.6; high versus low exposure). Analyses showed a significant exposure-response relationship to fungal alpha-amylase. Atopics showed a statistically non-significant increased risk of sensitisation compared to non-atopics. The authors noted that the number of workers in the medium- and high exposure group was small, the reason being that most workers were exposed to low concentrations of fungal alpha-amylase.

Houba et al. (1996) carried out a cross-sectional study in bakeries in the Netherlands. Workers (n=178) were screened for the presence of sensitisation to common allergens, cereal flour allergens, bakers' yeast, storage mites and fungal alpha-amylase.[70] In this study the workers were divided into three exposure groups (GM±SD, values represent highest exposure ever worked in): 0.7±4.0 ng/m^3 (low), 1.3±3.8 ng/m^3 (medium), and 18.1±4.6 ng/m^3 (high). Increased percentages of sensitised workers (to fungal alpha-amylase) were found with increasing exposure: 1.4% (low), 12.8% (medium) to 30.4% (high). The corresponding prevalence ratios were: 8.6 (95% CI, 1.01-74, medium versus low), and 15.9 (95% CI, 1.95-129, high versus low). Sensitisation to fungal alpha-amylase was more common in atopic workers, which was shown by a strong exposure-response relationship, whereas in the group of non-atopic workers no such strong relationship was observed. For IgE sensitisation, the authors found a positive trend for alpha-amylase exposure, but it did not reach statistical significance. In the other studies no clear statistically significant positive trends were found, although the number of sensitised workers tended to increase with increasing exposure.

From the same cohort-study as Nieuwenhuijsen et al., Brisman et al. (2004) reported on a nested case-control study using more recent data and making a distinction between different exposure groups.[57] However, the committee noted the poor reporting, in that: it is not clear whether the persons in the groups are the same persons as in the Nieuwenhuijsen-study; no data are given on the number of persons in the groups; and, it is unclear whether data include new cases only

or all (new and old) cases. For this reason the committee considers this study not useful for quantitative risk assessment.

The committee noted that exposure categories based on 'the highest exposure a worker ever worked in' are preferred over exposure categories based on current exposure, because it is plausible that a worker may become sensitised when working at a high exposure level and develops respiratory symptoms later, even if relocated to a lower exposure area.

7.1.3 *Allergic respiratory symptoms*

The committee is aware that respiratory symptoms may be induced by other factors than exposure to fungal alpha-amylase (see Section 7.1.1). Since in the majority of the studies co-exposure with other potential allergens, for instance in wheat and rye flour, was inevitable, these may have also induced respiratory symptoms. For this reason, associations between exposure to fungal alpha-amylase and respiratory symptoms should be interpreted with caution.

Studies have been published on exposure to fungal alpha-amylase, on which respiratory symptoms (cough, sneezing, watery eyes, runny nose, rhinitis, shortness of breath, asthma) could not be attributed to other causes than exposure to the enzyme. For instance, a few cases of bakers' asthma have been described in bakers who were sensitised to fungal alpha-amylase, and not to other potential allergens in bakeries (see Annex F and G). Also some studies described hyperresponsiveness after sensitised subjects underwent a specific bronchial provocation test (see Annex F).

7.1.4 *Allergic contact dermatitis*

Allergic contact dermatitis in bakery workers, sensitised to fungal alpha-amylase, has been reported in a number of studies. For instance, Schirmer et al. (1987) reported a case of a German baker, sensitised to alpha-amylase in a dough additive.[72] Specific IgE, IgG and IgM antibodies were detected in his serum. In a retrospective observational study, Hernández-Bel et al. (2011) confirmed sensitisation to fungal alpha-amylase by skin prick test in two Spanish bakers among 27 cases of protein contact dermatitis on hands and forearms.[73] Morren et al. (1993) examined 32 Belgian bakers with hand dermatitis, seven of whom had an immediate wheal-and-flare reaction to fungal alpha-amylase upon scratch chamber testing, and two also had delayed contact eczema.[33] All were found positive to skin prick testing. In a cross-sectional study, Brisman and Belin

(1991) found one person with contact dermatitis among six Swedish bakers, sensitised to alpha-amylase (skin prick test positive).[35]

7.1.5 *Food allergy*

Heating of fungal alpha-amylase, simulating bread baking, only partially removes the allergenic properties of the enzyme.[11] Allergenicity from enzyme residues in bread can therefore not be excluded. A case of a Spanish farmer with sneezing, cough and oral angioedema after eating bread was reported.[74] Skin prick testing revealed sensitisation to olive tree pollen and to fungal alpha-amylase. Since the farmer had never worked in pharmaceutical industry or in a bakery-related job, the authors concluded that the only source of sensitisation to alpha-amylase was bread. No other studies were found on fungal alpha-amylase induced food allergies in regular bread consumers.

7.2 Animal studies

No animal data on long-term exposure were available. Also no data were presented on carcinogenic and reproduction toxic properties.

7.2.1 *Single exposure*

In Japanese studies, acute oral LD_{50}'s in rat and mouse between 7.5 and 15 g/kg body weight, respectively, have been reported.[75] The WHO/FAO working group reviewed several toxicity studies, and reported on acute oral LD_{50} in mouse of less than 20 g/kg bodyweight (data obtained from industry).[76] In a Russian journal, the lowest concentration published for inhalation toxicity in mammals (species unspecified) was 15 mg/m^3.[75]

7.2.2 *Repeated exposure*

The WHO/FAO working group also reported on two dietary animal studies with repeated exposure to fungal alpha-amylase (data obtained from industry).[76] The first is a 3-week toxicity study with groups of 5 male and 5 female Wistar rats, which received 0, 0.5, or 5% fungal alpha-amylase in the diet. Only minor differences were observed in body weight change and food intake. At study termination, no compound-related haematological effects, changes in organ weights or gross pathology were noted. The second is a 3-month study in Sprague-Dawley rats (10-20 male and female animals/group), which received diets containing 0%,

5% or 10% of the enzyme (equivalent to 3.5 or 7 g enzyme/kg bw/day). No signs of toxicity were observed and no effect on body weight gain or food consumption. Differential blood counts were normal after 4 and 8 weeks. Furthermore, at study termination, haematology, organ weights, gross examination and histopathology revealed no compound-related effects.

7.2.3 *Specific sensitisation to fungal alpha-amylase*

Robinson et al. (1998) studied the sensitisation potencies of enzymes, including commercial fungal alpha-amylase (Fungamyl), using the Guinea-pig intratracheal test (GPIT) and the Mouse intranasal test (MINT) models.[77] In each dosing group, 10 female Hartley guinea-pigs, and five female BDF1 mice were used.

Regarding the GPIT, alpha-amylase was administered intratracheally, once a week for 10 weeks. Sensitisation was determined by observing the animals for signs of respiratory distress. Intratracheal instillation of 0.3, 1 or 3 µg (per animal) of the enzyme caused sensitisation in guinea-pigs at the highest dose only.

In the MINT, the enzyme was administered intranasally in a saline-detergent matrix on days 1, 3 and 10. Sensitisation was determined by measuring serum specific IgG1 on day 15. Intranasal administration of the enzyme resulted in an ED_{50} (the dose resulting in half of the maximum serum IgG titre) of 2.35 µg (per animal per installation). The lowest dose resulting in an increase of enzyme-specific serum IgG1 was 0.2 µg (per animal).

Experiments in animals confirm the findings in humans that exposure to fungal alpha-amylase may lead to specific sensitisation and allergy. For instance, allergenicity of enzymes was studied by Sarlo et al. (1997) in an animal model, the Guinea-pig intratracheal test.[78] The animals received alpha-amylase by intratracheal instillation, once per week for ten weeks. Passive cutaneous anaphylaxis antibody (IgG1) titres against the enzyme in sera of the treated animals were measured by intradermally injecting the sera in shaven naïve Guinea-pigs. The findings were compared with alcalase, an enzyme for which much information is available. The test was shown to have predictive value for IgE-mediated responses to inhalation of alpha-amylase by humans. Sarlo et al. compared antibody titres to alpha-amylase from *Bacillus licheniformis* with those from alcalase treatment, and found that the alfa-amylase showed a 10-fold higher allergenicity then alcalase.

7.3 Summary

Most data on the effects of occupational exposure to fungal alpha-amylase are retrieved from human studies on bakery workers and flour millers. Effects are mainly described on the upper airways and lungs, such as asthma (baker's asthma), rhinitis, and rhinoconjunctivitis. Part of the symptoms are explained by irritation responses, whereas another part of the symptoms by (specific IgE mediated) immune responses (sensitisation, allergy). Sensitisation to fungal alpha-amylase may occur soon after start of exposure (months), but it can take several years to develop symptoms.

The percentage of workers who show irritation to the respiratory tract due to exposure to fungal alpha-amylase, is difficult to estimate. The reason for this is that allergic symptoms are comparable with the symptoms of irritation. Another factor is that it is inevitable that workers in bakeries and flour mills are exposed to other compounds as well, such as wheat and rye flour dust, and other additives, which are known to cause respiratory irritation (and sensitisation) as well. Based on a few studies in which also investigations were performed on specific sensitisation, it is estimated that a considerable proportion of the workers with respiratory symptoms are due to irritation only.

Regarding sensitisation to fungal alpha-amylase, prevalence values of up to 30% have been reported among workers in bakeries and flour mills. Also in the enzyme producing industry, workers were found to be sensitised to fungal alpha-amylase (3-31%). In the general population, prevalence values up to 1.7% were found.

In a few epidemiological studies, investigators put effort in assessing exposure-response relationships between occupational exposure to fungal alpha-amylase and specific sensitisation and respiratory symptoms. The lowest exposure levels reported on were less than 1 ng alpha-amylase/m^3. In two studies (Nieuwenhuijsen et al. 1999, and Houba et al. 1996) statistically significant positive trends were observed with increased exposure. None of the two studies showed an exposure level below which no signs of sensitisation or respiratory symptoms were observed.

The number of animal studies on the toxicity of fungal alpha-amylase is very limited. In one study using guinea pigs and mouse, the allergenic potential of fungal alpha-amylase was confirmed.

Chapter 8

Existing guidelines, standards and evaluation

8.1 General population

Not available.

8.2 Occupational population

No occupational exposure limits have been established for fungal alpha-amylase in The Netherlands or in other countries.

Chapter 9

Hazard assessment

The committee limited this evaluation to alpha-amylase derived from *Aspergillus oryzae*.

9.1 Hazard identification

Available epidemiological studies have shown that the main health effects of exposure to airborne fungal alpha-amylase are symptoms observed in the respiratory tract and the eyes, such as rhinitis, asthma (baker's asthma), and conjunctivitis. Occasionally, upon skin contact, also dermatitis is recorded. Part of the symptoms have been shown to be of allergic origin, mediated by immunoglobulin E (IgE) antibodies to fungal alpha-amylase. However, the respiratory symptoms may also be explained by non-allergic irritation responses, as is shown in some studies among bakery workers and apprentices. The allergic potency of fungal alpha-amylase is confirmed in animals.

No relevant human and animal data were available on other possible adverse health effects, nor were there data presented on the carcinogenic potential and reproduction toxicity.

In bakeries and flour mills it is inevitable that workers are simultaneously exposed to dust of other compounds, such as cereal flour dust, and other additives. In particular cereal flour dust may induce respiratory symptoms which are indistinguishable from the symptoms caused by alpha-amylase exposure.[2]

Also cereal flour dust contains allergens that may induce IgE mediated immune responses and thus sensitisation. However, tests are available (skin prick tests and IgE-based immunoassays) that can distinguish between sensitisation to alpha-amylase and other allergens. Furthermore, data do not indicate that persons who are sensitised to cereal flour allergens will also be sensitised to alpha-amylase without previous exposure to the enzyme (cross-reactivity). Overall, in assessing a health-based occupational exposure limit for fungal alpha-amylase, the committee prefers to use the data on sensitisation to the enzyme as critical endpoint, instead of allergic symptoms. In addition, another reason in using data on sensitisation rather than data on allergic symptoms is that sensitisation often precedes the onset of allergic symptoms, although sensitisation per se is not an illness.[3] Since most persons who are sensitised will develop allergic symptoms at continuing exposure, the committee considers sensitisation a crucial step in developing an allergy: by preventing sensitisation also allergic symptoms will be prevented, whereas the other way round might not be the case.

Overall, the prevalence of sensitisation to fungal alpha-amylase among workers reached up to 19% in bakeries and flour mills, and up to 31% in the enzyme producing factories. The variations within an occupational group is explained by differences in duration, frequency, the level of exposure, and the use of different protocols in testing sensitisation (variations in origin of alpha-amylase, concentrations, and cut-off points). Despite these variations, the studies show a clear association between exposure to fungal alpha-amylase and induction of sensitisation. In the background population, which is not occupationally exposed to the enzyme, the highest prevalence of sensitisation to alpha-amylase was found to be 1.7%.

A few investigators assessed relationships between exposure levels and sensitisation to fungal alpha-amylase. Of most interest are the studies in which exposure was determined by measuring airborne fungal alpha-amylase using an immunoassay, instead of airborne dust levels. The reason being that the latter exposure parameter is less reliable, because measurements have shown a high variability of alpha-amylase content in dust.

Focussing on airborne alpha-amylase levels and specific sensitisation, in two studies a statistically significant increase in number of sensitised workers was observed with increasing exposure (Nieuwenhuijsen et al. 1999; Houba et al. 1996; see Table 4 in Chapter 7).[14,22] Also other studies showed positive trends, but the relationships were weak and did not reach statistical significance (Brisman et al. 2004; Cullinan et al. 2001; Droste et al. 2003; Page et al.

2010).[28,53,56,57] The committee noted that in the studies by Brisman et al. (2004) and Cullinan et al. (2001) the same group of workers participated as in Nieuwenhuijsen et al. (1999) (data from a same cohort).

The committee is aware that the results could have been influenced by bias and confounding which may have resulted in an underestimation or overestimation of the findings. For instance, in some studies potential sources of bias could not be ruled out, such as the healthy worker effect (the working population appears be more healthy, because workers who are ill have left the workplace and are not taken into account). This may explain why in higher exposure groups respiratory symptoms levelled off.[14,22,44] Other bias are the presence of atopic workers (considered more vulnerable in developing a specific allergy), and missing data of an individual person on exposure in the past. Furthermore, exposure-response relationships are expressed as mean exposure levels during a full work-shift (8 hour time weighed average concentrations). However, it is reasonable to believe that peak exposure (high exposures in a short time (minutes)) may have occurred.[16,23,35] Peak exposure is strongly related to short work activities, and plays a role in inducing sensitisation and work-related symptoms. As such it may have influenced the exposure-response relationships. On the other hand, no data are available to the committee on the influence of the height and frequency of these peak exposures on the exposure-response relationships. Consequently, the committee is not able to take this confounding quantitatively into account in assessing a health-based occupational exposure limit.

Overall, despite the presence of bias, confounding, variations in study design, exposure monitoring and sensitisation tests, the outcomes of the studies support the suggestion that with increasing exposure the number of sensitised persons increases as well.

9.2 Selection of the study suitable for risk estimation

Of interest in selecting a suitable study are the two cross-sectional studies, one performed in the United Kingdom and the other in the Netherlands, which showed statistically significant increases in prevalence to sensitisation with increasing exposure to fungal alpha-amylase: Nieuwenhuijsen et al. (1999), and Houba et al. (1996); see Table 4 and Annex G for study details).[14,22]

There are many similarities between the studies with respect to: design (cross-sectional); exposure monitoring (sandwich-immunoassay; performed in the same laboratory); sensitisation testing (skin prick test for fungal alpha-amylase); division into groups (three exposure groups based on highest exposure

ever worked in); and occupational group (bakery workers). Difference include: design (the Nieuwenhuijsen-study was part of a cohort study); the number of participating workers (264 and 178 workers in the Nieuwenhuijsen-study and Houba-study, respectively); occupational groups (the Nieuwenhuijsen-study also included workers from flour mills and a flour packing factory); mean duration of employment (Nieuwenhuijsen-study, 28 months (range: 1 month to maximal 4 years); Houba-study, 10.2 ± 8.7 years (range 0.2 - 43 years)); and sensitisation testing (Houba et al. also used a serum specific IgE immune-assay). The committee noted the disproportional group sizes in the Nieuwenhuijsen-study, in that the majority of the workers were assigned in the low-exposure group. This phenomenon was however also noted in the Houba-study, although to a lesser extent. Overall, the committee concludes that there are many similarities between the two studies and that any difference in outcome are most likely explained by statistical variation due to differences in random test effects.

Concerning the Nieuwenhuijsen-study, as is mentioned earlier, data are obtained from a cohort study on which also other investigators reported on exposure-response relationships (Brisman et al. 2004; Cullinan et al. 2001).[53,57] Using data from the same participants as Nieuwenhuijsen et al. (1999), Brisman et al. (2004) found trends of increasing sensitisation to fungal alpha-amylase with increasing exposure, but this did not reach statistical significance. However, the prevalence of chest symptoms statistically significantly increased with increasing exposure. Also Cullinan et al. (2001) observed positive trends for sensitisation and chest symptoms (using total dust inhalable dust as exposure measure). Overall, the findings by Brisman et al. (2004) and Cullinan et al. (2001) are in line with those by Nieuwenhuijsen et al. (1999).

Overall, the committee has no preference for the Houba- or the Nieuwenhuijsen-study, and thus decided to use them both as point of departure in deriving a value.

9.3 Quantitative hazard assessment

In none of the two studies a clear threshold level was observed, below which no cases of sensitisation to fungal alpha-amylase were found. In addition, in the general (not occupationally exposed) population already cases of sensitisation to fungal alpha-amylase have been reported. This means that an exposure level, at which sensitisation to airborne fungal alpha-amylase will not occur, cannot be identified; thus no threshold-based occupational exposure level can be attained. Earlier, the Health Council reported on this issue.[3] The council concluded that in theory a threshold level exists for allergic sensitisation by inhaled allergens. This

implies that a health-based recommended occupational exposure limit can be calculated, using the same procedures and methods as those for other non-carcinogenic substances. However, the council emphasized that for most allergens, in practice it will not be possible to calculate a reliable health-based recommended occupational exposure limit. The reason being that the threshold level will be too low to discern using the techniques presently available. For those allergens, the Health Council proposed an alternative approach, involving determination of reference values, i.e. concentration levels that correspond to predefined accepted levels of extra risk of allergic sensitisation. In addition, the Ministry of Social Affairs and Employment has requested the council to base a reference value on an additional sensitisation risk to fungal alpha-amylase of 1 percent due to occupational exposure, compared to the background risk in the general population (zero exposure). In the case of fungal alpha-amylase such a reference value is derived.

Since the Houba and Nieuwenhuijsen-studies have many similarities, the committee decided to combine their data and to perform a meta-regression analysis. By doing so, the power of the statistical analyses increases, improving the precision of the estimated effect. Details on the meta-regression analysis are shown in Annex G. Using a linear regression model, the committee derived an exposure level of fungal alpha-amylase of 0.9 ng enzyme/m^3, which corresponds to an additional sensitisation risk of 1% compared to the background sensitisation rate.

The committee discussed whether the reference value should be adjusted for inter-individual differences in vulnerability among humans. In case of developing allergies, a group of vulnerable people are the atopics. Since atopics were included in the study populations, no adjustments are needed.

The available literature does not suggest that non-allergic symptoms occur at lower exposure levels than allergic symptoms. Therefore, the committee is of the opinion that a risk assessment based on sensitisation not only protects against allergic symptoms, but most likely against non-allergic symptoms as well.

9.4 Recommendation for a health-based reference value

The committee recommends a reference value for occupational exposure to fungal alpha-amylase of 0.9 ng enzyme/m^3, as an eight-hour time-weighted average concentration. At this concentration workers have an additional

sensitisation risk for fungal alpha-amylase of 1 percent compared to the background risk in the general (not exposed) population.

9.5 Groups at extra risk

Three groups of workers have or may have an increased risk when exposed to fungal alpha-amylase. First, workers sensitised to fungal alpha-amylase will likely develop symptoms after repeated exposure to low levels of the enzyme. Second, workers with an atopic status or an allergic constitution may have an increased risk to develop work-related allergic sensitisation and subsequently respiratory symptoms and lung function changes. Third, workers with pre-existing asthma or those with more general respiratory symptoms may also have an increased risk to develop symptoms most likely because of non-specific irritation.

References

1 Souza PM de, de Oliveira MP. Application of microbial alpha-amylase in industry - A review. Braz J Microbiol 2010; 41(4): 850-861.

2 Health Council. Wheat and other cereal flour dusts. An approach for evaluating health effects from occupational exposure. Health Council of The Netherlands; 2004: publication no. 2004/02OSH.

3 Health Council. Prevention of work-related respiratory allergies. Recommended occupational exposure limits and periodic screening. Health Council of The Netherlands; 2008: publication n.o 2008/03E.

4 Jauhiainen A, Louhelainen K, Linnainmaa M. Exposure to dust and alpha-amylase in bakeries. Applied Occupational and Environmental Hygiene 1993; 8(8): 721-725.

5 Baur X, Chen Z, Sander I. Isolation and denomination of an important allergen in baking additives: alpha-amylase from Aspergillus oryzae (Asp o II). Clinical and Experimental Allergy 1994; 24(5): 465-470.

6 Doekes G, Houba R, Heederik D. Enzyme allergy: Standards for airborne exposure in the workplace? Zentralblatt für Arbeitsmedizin, Arbeitsschutz und Ergonomie 1998; 48(11): 480-484.

7 Smith TA, Lumley KPS. Work-related asthma in a population exposed to grain, flour and other ingredient dusts. Occupational Medicine 1996; 46(1): 37-40.

8 Schäfer T, Borchert TW, Nielsen V, Skagerlind P, Gibson K, Wenger K et al. Industrial Enzymes. In: Ulber R, Sell D, editors. Advances in Biochemical Engineering/Biotechnology. Springer Berlin / Heidelberg; 2007: 59-131. Internet: http://dx.doi.org/10.1007/10_2006_039 consulted: 14-12-2014.

9 Toda H, Kondo K, Narita K. The Complete Amino Acid Sequence of Taka-amylase A. Proceedings of the Japan Academy, Series B 1982; 58(7): 208-212.

10 Swift HJ, Brady L, Derewenda ZS, Dodson EJ, Dodson GG, Turkenburg JP et al. Structure and molecular model refinement of Aspergillus oryzae (TAKA) alpha-amylase: an application of the simulated-annealing method. Acta Crystallogr B 1991; 47(Pt 4): 535-544.

11 Baur X, Czuppon AB, Sander I. Heating inactivates the enzymatic activity and partially inactivates the allergenic activity of Asp o 2. Clin Exp Allergy 1996; 26(2): 232-234.

12 European Union. 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. Official Journal of the European Union 2008; L 353: 1-1355.

13 CEN. EN 481:1993, Workplace atmospheres - Size fraction definitions for measurement of airborne particles. Brussels: Comité Européen de Normalisation; 1993.

14 Houba R, Heederik DJ, Doekes G, Van Run PE. Exposure-sensitization relationship for alpha-amylase allergens in the baking industry. American Journal of Respiratory and Critical Care Medicine 1996; 154(1): 130-136.

15 Houba R, van RP, Doekes G, Heederik D, Spithoven J. Airborne levels of alpha-amylase allergens in bakeries. Journal of Allergy and Clinical Immunology 1997; 99(3): 286-292.

16 Elms J, Denniss S, Smith M, Evans PG, Wiley K, Griffin P et al. Development and validation of a monoclonal based immunoassay for the measurement of fungal alpha-amylase: focus on peak exposures. Ann Occup Hyg 2001; 45(2): 89-95.

17 Sander I, Neuhaus-Schroeder C, Borowitzki G, Baur X, Raulf-Heimsoth M. Development of a two-site enzyme-linked immunosorbent assay for alpha-amylase from Aspergillus oryzae based on monoclonal antibodies. Journal of Immunological Methods 1997; 210(1): 93-101.

18 Lillienberg L, Baur X, Doekes G, Belin L, Raulf-Heimsoth M, Sander I et al. Comparison of four methods to assess fungal alpha-amylase in flour dust. Ann Occup Hyg 2000; 44(6): 427-433.

19 Sander I, Zahradnik E, Bogdanovic J, Raulf-Heimsoth M, Wouters IM, Renström A et al. Optimized methods for fungal alpha-amylase airborne exposure assessment in bakeries and mills. Clin Exp Allergy 2007; 37(8): 1229-1238.

20 Bulat P, Myny K, Braeckman L, Van Sprundel M, Kusters E, Doekes G et al. Exposure to inhalable dust, wheat flour and alpha-amylase allergens in industrial and traditional bakeries. Annals of Occupational Hygiene 2004; 48(1): 57-63.

21 Burstyn I, Teschke K, Bartlett K, Kennedy SM. Determinants of wheat antigen and fungal alpha-amylase exposure in bakeries. Am Ind Hyg Assoc J 1998; 59(5): 313-320.

22 Nieuwenhuijsen MJ, Heederik D, Doekes G, Venables KM, Newman Taylor AJ. Exposure-response relations of alpha-amylase sensitisation in British bakeries and flour mills. Occup Environ Med 1999; 56(3): 197-201.

23 Brant A, Berriman J, Sharp C, Welch J, Zekveld C, Nieuwenhuijsen M et al. The changing distribution of occupational asthma: a survey of supermarket bakery workers. Eur Respir J 2005; 25(2): 303-308.

24 Meijster T, Tielemans E, Heederik D. Effect of an intervention aimed at reducing the risk of allergic respiratory disease in bakers: change in flour dust and fungal alpha-amylase levels. Occup Environ Med 2009; 66(8): 543-549.

25 Sander I, Neuhaus-Schroder C, Raulf-Heimsoth M, Doekes G, Heederik D, Baur X. Quantifizierung der inhalativen Belastung mit alpha-amylase in zwei Bäckereien. Pneumologie 1998; 52(8): 440-443.

26 Bogdanovic J, Koets M, Sander I, Wouters I, Meijster T, Heederik D et al. Rapid detection of fungal alpha-amylase in the work environment with a lateral flow immunoassay. J Allergy Clin Immunol 2006; 118(5): 1157-1163.

27 Koets M, Sander I, Bogdanovic J, Doekes G, van AA. A rapid lateral flow immunoassay for the detection of fungal alpha-amylase at the workplace. J Environ Monit 2006; 8(9): 942-946.

28 Page EH, Dowell CH, Mueller CA, Biagini RE, Heederik D. Exposure to flour dust and sensitization among bakery employees. Am J Ind Med 2010; 53(12): 1225-1232.

29 Elms J, Beckett P, Griffin P, Evans P, Sams C, Roff M et al. Job categories and their effect on exposure to fungal alpha-amylase and inhalable dust in the U.K. baking industry. AIHA Journal 2003; 64(4): 467-471.

30 Baatjies R, Meijster T, Lopata A, Sander I, Raulf-Heimsoth M, Heederik D et al. Exposure to flour dust in South African supermarket bakeries: modeling of baseline measurements of an intervention study. Ann Occup Hyg 2010; 54(3): 309-318.

31 Health and Safety Executive. Flour dust: Risk assessment document. HSE Books, Sudbury, Suffolk, United Kingdom., 76p. [EH71/11]. 1999.

32 Houba R, van RP, Heederik D, Doekes G. Wheat antigen exposure assessment for epidemiological studies in bakeries using personal dust sampling and inhibition ELISA. Clin Exp Allergy 1996; 26(2): 154-163.

33 Morren MA, Janssens V, Dooms-Gossens A, Van HE, Cornelis A, De Wolf-Peeters C et al. alpha-Amylase, a flour additive: an important cause of protein contact dermatitis in bakers. J Am Acad Dermatol 1993; 29(5 Pt 1): 723-728.

34 Sandiford CP, Tee RD, Taylor AJ. The role of cereal and fungal amylases in cereal flour hypersensitivity. Clin Exp Allergy 1994; 24(6): 549-557.

35 Brisman J, Belin L. Clinical and immunological responses to occupational exposure to alpha-amylase in the baking industry. Br J Ind Med 1991; 48(9): 604-608.

36 Baatjies R, Lopata AL, Sander I, Raulf-Heimsoth M, Bateman ED, Meijster T et al. Determinants of asthma phenotypes in supermarket bakery workers. Eur Respir J 2009; 34(4): 825-833 consulted: 14-12-2014.

37 Association of Manufacturers and Formulators of Enzyme Products (AMFEP). Amfep/09/68. List of commercial enzymes. http://www.amfep.org. 2009. Internet: http://www.amfep.org consulted: 14-12-2014..

38 Brisman J. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 111. Industrial Enzymes. Arbete och Hälsa 1994; 28: 1-25.

39 Green BJ, Beezhold DH. Industrial fungal enzymes: an occupational allergen perspective. J Allergy 2011; 2011: 1-11.

40 Harris-Roberts J, Robinson E, Waterhouse JC, Billings CG, Proctor AR, Stocks-Greaves M et al. Sensitization to wheat flour and enzymes and associated respiratory symptoms in British bakers. Am J Ind Med 2009; 52(2): 133-140.

41 Moneo I, Alday E, Gonzalez-Muñoz M, Maqueda J, Curiel G, Lucena R. Alpha-Amylase hypersensitivity in non-exposed millers. Occupational Medicine 1994; 44(2): 91-94.

42 Vissers M, Doekes G, Heederik D. Exposure to wheat allergen and fungal alpha-amylase in the homes of bakers. Clin Exp Allergy 2001; 31(10): 1577-1582.

43 Sander I, Raulf-Heimsoth M, Van K, V, Baur X. Is fungal alpha-amylase in bread an allergen? Clin Exp Allergy 2000; 30(4): 560-565.

44 Smith TA, Lumley KPS, Hui EHK. Allergy to flour and fungal amylase in bakery workers. Occupational Medicine (London) 1997; 47(1): 21-24.

45 Meijster T, Tielemans E, de PN, Heederik D. Modelling exposure in flour processing sectors in the Netherlands: a baseline measurement in the context of an intervention program. Ann Occup Hyg 2007; 51(3): 293-304.

46 Page EH, Dowell CH, Mueller CA, Biagini RE. Exposure to flour dust and sensitization among bakery employees. Washington, DC 200910: National Inst. for Occupational Safety and Health; 2009: Health Hazard Evaluation Report: HETA-2005-0248-3077, Sara Lee Bakery, Sacramento, California.

47 Elms J, Robinson E, Mason H, Iqbal S, Garrod A, Evans GS. Enzyme exposure in the British baking industry. Ann Occup Hyg 2006; 50(4): 379-384.

48 Vanhanen M, Tuomi T, Hokkanen H, Tupasela O, Tuomainen A, Holmberg PC et al. Enzyme exposure and enzyme sensitisation in the baking industry. Occupational and Environmental Medicine 1996; 53(10): 670-676.

49 Heederik D, Meijster T, Spaan S, Schinkel J, Tielemans E, van Niftrik M. Eindmeting Arboconvenant grondstofallergie. Resultaten van de herhalingsmetingen piekblootstelling, stof- en allergeenbelasting. Utrecht, Zeist: IRAS, TNO; 2007.

50 Losada E, Hinojosa M, Quirce S, S nchez-Cano M, Moneo I. Occupational asthma caused by alpha-amylase inhalation: clinical and immunologic findings and bronchial response patterns. J Allergy Clin Immunol 1992; 89(1): 118-125.

51 Sandiford CP, Nieuwenhuijsen MJ, Tee RD, Taylor AJ. Measurement of airborne proteins involved in Bakers' asthma. Clin Exp Allergy 1994; 24(5): 450-456.

52 Cullinan P, Lowson D, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM et al. Work Related Symptoms, Sensitisation, and Estimated Exposure in Workers not Previously Exposed to Flour. Occupational and Environmental Medicine 1994; 51(9): 579-583.

53 Cullinan P, Cook A, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM et al. Allergen and dust exposure as determinants of work-related symptoms and sensitization in a cohort of flour-exposed workers; a case-control analysis. Ann Occup Hyg 2001; 45(2): 97-103.

54 Houba R, Doekes G, Heederik D. Occupational respiratory allergy in bakery workers: a review of the literature. Am J Ind Med 1998; 34(6): 529-546.

55 Vanhanen M, Tuomi T, Nordman H, Tupasela O, Holmberg PC, Miettinen M et al. Sensitization to industrial enzymes in enzyme research and production. Scand J Work Environ Health 1997; 23(5): 385-391.

56 Droste J, Myny K, Van SM, Kusters E, Bulat P, Braeckman L et al. Allergic sensitization, symptoms and lung function among bakery workers as compared with a nonexposed work population. Journal of Occupational and Environmental Medicine 2003; 45(6): 648-655.

57 Brisman J, Nieuwenhuijsen MJ, Venables KM, Putcha V, Gordon S, Taylor AJ. Exposure-response relations for work related respiratory symptoms and sensitisation in a cohort exposed to alpha-amylase. Occup Environ Med 2004; 61(6): 551-553.

58 Smith TA, Bennett N, Bithell MT. Exposure-response relations of alpha-amylase sensitization in British bakeries and flour mills. Occup Environ Med 1999; 56(11): 788-792.

59 Smith TA, Parker G, Hussain T. Respiratory symptoms and wheat flour exposure: a study of flour millers. Occupational Medicine 2000; 50(1): 25-29.

60 Gautrin D, Infante-Rivard C, Dao T-V, Magnan-Larose M, Desjardins D, Malo J-L. Specific IgE-dependent sensitization, atopy, and bronchial hyperresponsiveness in apprentices starting exposure to protein-derived agents. American Journal of Respiratory and Critical Care Medicine 1997; 155(6): 1841-1847.

61 Walusiak J, Hanke W, Gorski P, Palczynski C. Respiratory allergy in apprentice bakers: do occupational allergies follow the allergic march? Allergy 2004; 59(4): 442-450.

62 De Zotti R., Molinari S, Larese F, Bovenzi M. Pre-employment screening among trainee bakers. Occup Environ Med 1995; 52(4): 279-283.

63 Biagini RE, Mackenzie BA, Sammons DL, Smith JP, Striley CAF, Robertson SK et al. Evaluation of the prevalence of antiwheat-, anti-flour dust, and anti-alpha-amylase specific IgE antibodies in US blood donors. Annals of allergy, asthma & immunology 2004; 92(6): 649-653.

64 Baur X, Degens PO, Sander I. Baker's asthma: still among the most frequent occupational respiratory disorders. J Allergy Clin Immunol 1998; 102(6 Pt 1): 984-997.

65 Smith TA, Wastell Smith P. Respiratory symptoms and sensitization in bread and cake bakers. Occupational Medicine 1998; 48(5): 321-328.

66 Jeffrey P, Griffin P, Gibson M, Curran AD. Small bakeries--a cross-sectional study of respiratory symptoms, sensitization and dust exposure. Occup Med (Lond) 1999; 49(4): 237-241.

67 Storaas T, Steinsvag SK, Florvaag E, Irgens A, Aasen TB. Occupational rhinitis: diagnostic criteria, relation to lower airway symptoms and IgE sensitization in bakery workers. Acta Otolaryngol 2005; 125(11): 1211-1217.

68 Johnsen CR, Sorensen TB, Ingemann Larsen A, Bertelsen Secher A, Andreasen E, Kofoed GS et al. Allergy risk in an enzyme producing plant: a retrospective follow up study. Occupational and Environmental Medicine 1997; 54(9): 671-675.

69 Biagini RE, Driscoll RJ, Bernstein DI, Wilcox TG, Henningsen GM, Mackenzie BA et al. Hypersensitivity reactions and specific antibodies in workers exposed to industrial enzymes at a biotechnology plant. Journal of Applied Toxicology 1996; 16(2): 139-145.

70 Houba R. Occupational respiratory allergy in bakery workers - Relationships with wheat and fungal alpha-amylase aeroallergen exposure. [Proefschrift]. Thesis, Landbouwuniversiteit Wageningen; 1996.

71 Nieuwenhuijsen MJ, Sandiford CP, Lowson D, Tee RD, Venables KM, McDonald JC et al. Dust and flour aeroallergen exposure in flour mills and bakeries. Occup Environ Med 1994; 51(9): 584-588.

72 Schirmer RH, Kalveram KJ, Kalveram CM, Siebert J, Kunze J. Chronic lichenoid dermatitis in sensitization to alpha-amylase in a baker. Z Hautkr 1987; 62(10): 792-797.

73 Hernández-Bel P, de la Cuadra J, García R, Alegre V. Protein contact dermatitis: review of 27 cases. Actas Dermosifiliogr 2011; 102(5): 336-343.

74 Moreno-Ancillo A, Domínguez-Noche C, Gil-Adrados AC, Cosmes PM. Bread eating induced oral angioedema due to alpha-amylase allergy. J Investig Allergol Clin Immunol 2004; 14(4): 346-347.

75 RTECS (The Registry of Toxic Effects of Chemical Substances). BU7432500 alpha-Amylase. 2009.

76 FAO and WHO working groups. Enzymes derived from Aspergillus oryzae (alfa-Amylase, Proteases). WHO Food Additives Series 1987; 22(629): 3-9.

77 Robinson MK, Horn PA, Kawabata TT, Babcock LS, Fletcher ER, Sarlo K. Use of the mouse intranasal test (MINT) to determine the allergenic potency of detergent enzymes: comparison to the guinea pig intratracheal (GPIT) test. Toxicol Sci 1998; 43(1): 39-46.

78 Sarlo K, Fletcher ER, Gaines WG, Ritz HL. Respiratory allergenicity of detergent enzymes in the Guinea Pig Intratracheal Test: Association with sensitization of occupationally exposed individuals. Fundamental and Applied Toxicology 1997; 39(1)

79 Gautrin D, Ghezzo H, Infante-Rivard C, Malo J-L. Incidence and determinants of IgE-mediated sensitization in apprentices. American Journal of Respiratory and Critical Care Medicine 2000; 162(4): 1222-1228.

80 Gautrin D, Ghezzo H, Infante-Rivard C, Malo JL. Incidence and host determinants of work-related rhinoconjunctivitis in apprentice pastry-makers. Allergy 2002; 57(10): 913-918.

81 Zotti R de, Bovenzi M. Prospective study of work related respiratory symptoms in trainee bakers. Occup Environ Med 2000; 57(1): 58-61.

82 Storaas T, Irgens A, Florvaag E, Steinsvag SK, Ardal L, Van Do T et al. Bronchial responsiveness in bakery workers: relation to airway symptoms, IgE sensitization, nasal indices of inflammation, flour dust exposure and smoking. Clin Physiol Funct Imaging 2007; 27(5): 327-334.

83 Storaas T, Ardal L, Van Do T, Florvaag E, Steinsvag SK, Irgens A et al. Nasal indices of eosinophilic and exudative inflammation in bakery-workers. Clin Physiol Funct Imaging 2007; 27(1): 23-29.

84 Baur X, Fruhmann G, Haug B, Rasche B, Reiher W, Weiss W. Role of Aspergillus amylase in baker's asthma. The Lancet 1986; 327(8471): 43.

85 Flindt MLH. Allergy to alpha-amylase and papain. Lancet 1979; 314(8131): 1407-1408.

86 Quirce S, Cuevas M, Díez-Gómez M, Fernández-Rivas M, Hinojosa M, González et al. Respiratory allergy to Aspergillus-derived enzymes in bakers' asthma. J Allergy Clin Immunol 1992; 90(6): 970-978.

87 Blanco Carmona JG, Juste PS, Garces SM. Occupational asthma in bakeries caused by sensitivity to alpha-amylase. Allergy 1991; 46(4): 274-276.

88 Birnbaum J, Latil F, Vervloet D, Senft M, Charpin J. Rôle de l'alpha-amylase dans l'asthme du boulanger. Rev Mal Respir 1988; 5(5): 519-521.

89 Houba R, Heederik D, Kromhout H. Grouping strategies for exposure to inhalable dust, wheat allergens and alpha-amylase allergens in bakeries. Annals of Occupational Hygiene 1997; 41(3): 287-296.

90 Droste J, Vermeire P, Van SM, Bulat P, Braeckman L, Myny K et al. Occupational exposure among bakery workers: impact on the occurrence of work-related symptoms as compared with allergic characteristics. J Occup Environ Med 2005; 47(5): 458-465.

A	Request for advice
B	The Committee
C	The submission letter (in English)
D	Comments on the public review draft
E	Prevalence of sensitisation to alpha-amylase and of respiratory symptoms
F	Exposure-response relationships
G	Meta-regression analysis

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- R.A. Woutersen, chairman
  Toxicologic Pathologist, TNO Innovation for Life, Zeist; and professor of translational toxicology, Wageningen University and Research Centre, Wageningen
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- D.J.J. Heederik
  Professor in Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
- H. van Loveren
  Professor in Immunotoxicology, Maastricht University, Maastricht; and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  Occupational Physician; Netherlands Centre for Occupational Diseases, University of Amsterdam, Amsterdam
- A.H. Piersma
  Professor in Reproductive Toxicology, Utrecht University, Utrecht; and National Institute for Public Health and the Environment, Bilthoven

- H.P.J. te Riele
  Professor in Molecular Biology, VU University Amsterdam; and the Netherlands Cancer Institute, Amsterdam
- I.M.C.M. Rietjens
  Professor in Toxicology, Wageningen University and Research Centre, Wageningen
- F.G.M. Russel
  Professor in Molecular Pharmacology and Toxicology, Radboud University, Nijmegen Medical Centre, Nijmegen
- G.M.H. Swaen
  Eepidemiologist, Maastricht University, Maastricht
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- P.B. Wulp
  Occupational Physician, Labour Inspectorate, Groningen
- J.J.A.M. Hendrix, *advisor*
  Social and Economic Council, The Hague
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter (in English)

Subject : Submission of the advisory report *Fungal alpha-amylase*
Your Reference: DGV/MBO/U-932342
Our reference : U-8232/JR/cn/459-S70
Enclosed : 1
Date : November 6, 2014

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to fungal alpha amylase.

In the advisory report *Prevention of work-related airway allergie* (report No. 2008/03), the Health Council proposed a method to derive health-based occupational exposure limits, or on risk-based reference values for allergenic substances. The present advisory report on fungal alpha-amylase makes use of this method. The Health Council has calculated the concentration of fungal alpha-amylase in the air, at which occupational exposure leads to an additional sensitisation risk of 1%, compared to the background risk in the non-exposed, general population.

The conclusions in the advisory report were drawn by the Health Council's Dutch Expert Committee on Occupational Safety (DECOS). The subcommittee has taken comments into account from a public review, and included the opinions by the Health Council's Standing Committee on Health and the Environment.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,

(signed)
Prof. J.L. Severens,
Vice-President

Annex **D**

Comments on the public review draft

A draft of the present report was released in 2014 for public review. The following persons and organisations have commented on the draft review:

- W. Eduard, National Institute of Occupational Health, Norway
- J. Lentz and A. Nayak, National Institute for Occupational Safety and Health, the USA.

Annex **E**

Prevalence of sensitisation to alpha-amylase and of respiratory symptoms

Prospective cohort studies

Study design and population information	Exposure information	Health information	Results	Reference
287 apprentice bakers, examined in the 1st, 2nd and 3rd year of their training, Poland.	No exposure data presented.	Medical examination and physician-administered questionnaire on history of allergic diseases, atopy, smoking, exposure to pet allergens, and housing conditions. The incidence of work-related respiratory symptoms was calculated separately for each follow-up year and cumulative; skin prick tests to common and cereal allergens. In skin prick test positive students and in 20 random subjects in the 1st and 2nd year, and all students in 3rd year also a skin prick test was preformed with IgE antibodies to fungal alpha-amylase (cut-off point, ≥0.35 kU/L). Nasal provocation tests to	Specific sensitisation to fungal alpha-amylase • First year: all negative. • Third year: 31/287 persons (10.8%). In the third year 51/287 cases showed sensitisation to occupational allergens. Of these positive cases: • for 16 cases the presence of specific IgE was the only marker of occupational sensitisation; • in 27 cases, development of occupational hypersensitivity was preceded by skin reactivity to common allergen at an earlier stage. Background prevalence of sensitisation (new bakery apprentices): 0% (0/287).	Walusiak et al. 2004[61]

		cereal flours. Pulmonary function tests examination of persons reporting chest symptoms.		
230 trainee pastry bakers; Canada; follow-up 8 and 16 months after start of training.	No exposure data presented.	Skin prick test to fungal alpha-amylase; questionnaire on allergy history, lung function tests. Study included base-line examination.	No positive cases found at the beginning of the study, and after 8 (n = 189), and 16 months (n = 138) of training. The low incidence is explained by the finding that trainees rarely handled enzymatic additives and flour containing these additives. The prevalence of atopy (skin prick test to 11 common inhalants) was 54.5%. During the study, 8/186 subjects were positive for flour-deriveD allergen sensitisation. Background prevalence of sensitisation (new bakery apprentices): 0% (0/230).	Gautrin et al. 1997[60], 2000[79], 2002[80]
125 trainee bakers; Italy; follow-up was at 6, 18 and 30 months after start of training.	No exposure data presented.	Skin prick tests to fungal alpha-amylase and to wheat flour allergens. Study included base-line examination.	After 30 months: • 3/125 positive for fungal alpha-amylase; • 10/125 positive for wheat flour allergens. All students sensitised for alpha-amylase were also sensitised for wheat flour allergens. • 10/125 showed work-related respiratory symptoms.	De Zotti and Bovenzi, 2000[81]

Cross-sectional studies

Study design and population information	Exposure information	Health information	Results	Reference
Exposure levels expressed as ng fungal alpha-amylase/m^3				
517 employees of 31 supermarket bakeries in South-Africa.	Full-shift personal airborne dust was sampled in 18 bakeries on 2 days (n=211). Analysis for total mass and flour dust fungal alpha-amylase by ELISA-immunoassay.	Self-administered questionnaire (n=517) on respiratory symptoms, employment history and job title, degrees of exposure to flour dust, baking activities at home and smoking habits.	Overall prevalence of sensitisation to fungal alpha-amylase. *Specific IgE* • all workers: 4% (21/513) • atopic: 7% (15/213) • nonatopic: 2% (6/294)	Baatjies et al 2009[36] Exposure measurements: Baatjies et al. 2010[30]

Inhalable dust levels (GM±SD): • bread baker (n=112): 1.33±2.25 mg/m^3; • confectioner (n=38): 0.65±2.08 mg/m^3; • supervisor (n=13): 0.56±2.05 mg/m^3; • manager (n=13): 0.51±2.34 mg/m^3; • counterhand (serving customers, n=35): 0.28±1.89 mg/m^3. Exposure to fungal alpha-amylase (GM±SD) (range): • bread baker: 0.15±2.32 ng/m^3 (0.083-19.62); • confectioner: 0.12±2.14 ng/m^3 (0.083-6.54); • supervisor: 0.10±1.17 ng/m^3 (0.083-1.17); • manager: 0.12±1.58 ng/m^3 (0.083-0.51); • counterhand (serving customers): 0.11±1.40 ng/m^3 (0.083-0.64). Notes: (1) There was no association between job-title and exposure to fungal alpha-amylase (GM) in this study, but weighing tasks (p=0.057) and use of premix products (p=0.010) were associated with increased exposure to the enzyme. (2) There was a poor correlation between alpha-amylase concentration and inhalable dust (Pearson r=0.33, p<0.001). (3) Alpha-amylase concentration was below detection limit (0.083 ng/m^3) in 81% of the inhalable dust samples, which may be explained by the low contents of this enzyme in premix products: 0.75-100 ng/mg.	Skin prick tests (n=507) to common and work-related allergens, including fungal alpha-amylase. Fungal alpha-amylase specific serum IgE was measured by fluorescence enzyme immunoassay (n=513). A result >0.35 kU/L was considered positive. Pulmonary function testing (spirometry and methacholine challenge, n=517).	Difference between atopic and nonatopic were not significant. *Skin prick test* • all workers: 3% (17/513) • atopic: 6% (13/213) • non-atopic: 1% (4/294) Atopy prevalence among all workers was 42%. The authors did not present job-title (or exposure) specific sensitisation prevalence values. Of all workers, 22% showed bronchial hyperresponsiveness. A relationship between sensitisation to fungal alpha-amylase and lung function was not examined.

225 bakers from 22 bakeries, the UK: • 40 general bakers • 31 mixers/sievers/ weighers • 7 cleaner jobs • 47 other jobs.	Exposure (8-hour TWA) to fungal alpha-amylase was determined for several job categories. Personal breathing zone inhalable dust samples from 208 workers in 55 bakeries were analysed for fungal-amylase (ELISA immunoassay). Alpha-amylase was however not detectable in a large proportion of the samples. Therefore, below, the number enzyme-containing samples per total number of samples are listed: • total (11/171): 5.9 ng/m^3; <0.78-33.7 (GM and range) • general bakers (5/90), 1.7 ng/m^3, <0.78-24.5 (GM, range); • mixers/weighers/sievers (4/44), <0.78-13.2 ng/m^3 (range only); • cleaners (1/6), <0.78-22.0 ng/m^3 (range only); • others (1/31), <0.78-33.7 ng/m^3 (range only).	Interviewer-led questionnaire on work-related respiratory symptoms, demographic details, work history and smoking habits; lung function assessment (n=225). Specific serum IgE (RAST assay) to wheat flour, enzymes including a mixture of fungal and bacterial alpha-amylase, and common allergens (n=160). Workers were categorized in 4 groups: • not sensitised; • sensitised to wheat flour only; • sensitised to enzymes only; • sensitised to wheat flour and any enzyme. The authors did not separately examine fungal alpha-amylase.	Prevalence of sensitisation to fungal or bacterial alpha-amylase (IgE-positive): 5.6% (9/160). Prevalence to any enzyme (including enzymes from *Aspergillus niger*, xylanase, and hemicellulase): 7.5% (12/160). Note: the authors did not report the prevalence of sensitisation to fungal alpha-amylase in each of the job categories. Abnormal lung function was more frequent among workers sensitised to enzymes but not to wheat allergen (odds ratios adjusted for job-duration, age, atopy and smoking): • abnormal FVC: OR 22.73 (95% CI 1.91-270.94); • abnormal FEV_1: OR 18.92 (95% CI 1.60-223.18). Atopy was the most important risk factor for sensitisation to workplace allergens (OR 18.4, 5.3-64.3). Correction for atopy was not feasible. Among atopic workers smoking was a strong predictor of sensitisation to wheat or enzymes, corrected for duration of employment and current exposure category (OR 4.7, 1.1-20.8).	Harris-Roberts et al. 2009[40] Exposure assessment: Elms et al. 2006[47]
239 employees of 20 supermarket bakeries, UK	Full-shift personal inhalable dust concentrations measured (IOM sampler; n=89). Alpha-amylase content in dust samples was analysed by an ELISA immunoassay. Only three	Self-administered questionnaire on job-classification, job history and respiratory symptoms and current smoking status (n=239). Skin prick test to common allergens (n=233) and RAST to fungal alpha-amylase specific serum IgE	Prevalence of sensitisation to fungal alpha-amylase (specific serum IgE): • bakers: 8% (5/66) • managers: 11% (3/28) • confectioners: 0% (0/45) • assistants: 1% (1/71) • total: 4% (9/210)	Brant et al. 2005[23]

	samples: • one baker (3.3 ng/m^3) • one manager (12.4 ng/m^3) • one bakery assistant (3.9 ng/m^3). All reported they sometimes performed baking tasks.	(n=210). Atopy was evenly spread across the working groups.	The relationship between alpha-amylase sensitisation and job title was not statistically significant (p=0.19). Prevalence of atopy was 41%, comparable with Baatjies et al 2009.[2]	
20 workers in a factory producing bread mixes and dough improvers, Sweden	Workers were exposed to fungal alpha-amylase during mixing and packing (n=16) or in the laboratory (n=4). Nine persons working in another part of the factory served as referent group. Total dust sampling (1 personal, 1 stationary sampler, during daily task duration of 30-60 min). Enzymatically determined fungal alpha-amylase content at packing station: 30 µg/m^3 (= peak exposure).	Interview by allergologist, and spirometry before and after inhalation of salbutamol/ lactose; skin prick tests to common allergens, and to work-related allergens including fungal alpha-amylase; RAST assay for specific IgE antibodies to fungal alpha-amylase. Workers with positive skin prick test underwent nasal challenge tests with the fungal alpha amylase.	Prevalence of sensitisation to fungal alpha-amylase (skin prick test - IgE - IgG, respectively): • all workers: 6/20 - 1/20 - 3/20; • with symptoms (rhinitis, dermatitis): 4/11 - 0/11 - 0/11; • without symptoms: 2/9 - 1/9 - 3/9; • referents (no symptoms): 1/9 - 1/9 - 0/9. Symptoms reported in prick test positive workers consisted of rhinitis (n=3) and dermatitis (n=1). Four employees of the same factory had previously been diagnosed for asthma and/or rhinitis. All were positive to fungal alpha-amylase in the skin prick test.	Brisman and Belin 1991[35]
Exposure levels expressed as mg inhalable dust/m^3				
197 employees of 6 bakeries, Norway.	Breathing zone personal dust samplers (n=58). Four exposure groups: • <1.0 mg/m^3 (packers, oven workers,a administration); • 1.0-1.9 mg/m^3 (mainly confectionary workers, bread formers); • 2.0-3.9 mg/m^3 (mainly dough makers); • >3.9 mg/m^3 (mainly dough makers).	Interview focusing on occupational rhinitis (n=181) and self-administered questionnaire on work tasks, family history, occupational symptoms, smoking habits and prevalence of allergy and atopic dermatitis/ eczema (n=180). Allergy tests for occupational and common allergens (skin prick, total and specific serum IgE, and histamine release; n=183).	No data were presented on the different exposure groups. Overall prevalence of sensitisation to fungal alpha-amylase: • skin prick test: 7% (13/183) • specific serum IgE: 2% (4/183) Prevalence of sensitisation to storage mites was 20% (37/183). Occupational rhinitis, IgE	Storaas et al. 2005[67], 2007[82], 2007[83]

		Spirometry, bronchial provocation test with metacholine, nasal challenge and lavage. Categorisation of workers in job titles: dough makers, bread formers, oven staff, packers, confectionary workers, administration and cleaning workers.	and non-IgE mediated, preceded lower airway symptoms, and was associated with asthma symptoms. Storage mite sensitisation was related to occupational rhinitis and exposure. Bronchial hyperresponsiveness (BHR), determined by metacholine challenge, was associated with smoking and work-related asthma. BHR, corrected for baseline lung function, was not associated with occupational IgE sensitisation (defined as positive to wheat, alpha-amylase, oats, barley, rye, soybean, storage mites, mold or cockroach). The authors concluded that IgE sensitisation is not the main causative factor for airway hyperresponsiveness and occupational rhinitis in bakery workers. BHR was not associated with current flour dust exposure level, with number of working hours in a bakery, or with a history of dough-making.	
679 employees of 18 flour mills, regularly exposed to flour dust (workers involved in milling, production or packing activities), the UK	Full-shift personal total inhalable dust measurements between 1990 and 1998 (n=116) Exposure to inhalable dust was 8.1 mg/m^3 (0.5-217; median, 8-hour TWA). Exposure by job category (GM and range): • production (n=78): 6.2 mg/m^3 (0.5-54.7); • hygiene (n=38): 18.7 mg/m^3 (1.1-217).	Screening by occupational physician, using structured interview on type, time of onset and duration of work-related respiratory symptoms (n=679). Skin prick testing to common allergens, to wheat, soya and rice flour, and to fungal alpha-amylase (n=678). Mean duration of employment was 12.5 years (2 months - 47 years)	No data were presented on the separate job categories. Overall prevalence of sensitisation to fungal alpha-amylase (skin prick test positive) was 0.9% (6/678). Symptoms in the positive group included allergic rhinitis (n=1), non-specific irritation (n=2), and asymptomatic (n=3). Workers sensitised to wheat flour and alpha-amylase were different. Overall prevalence of atopy	Smith et al. 2000[59]

			was 37% (248/678). Work-related respiratory symptoms were reported by 147/679 workers (22%), mostly occasional and transient, which were classified as non-specific irritation. Allergic respiratory symptoms were reported by 8/679 workers (1%, 4 rhinitis and 4 asthma). Fungal alpha-amylase was thought to be responsible for one of the 4 cases of asthma, because this worker only had symptoms after handling bread improver.	
392 employees from 19 bread bakeries and 77 workers from 3 cake bakeries, UK. Of the 3 large bakeries, one did not use alpha-amylase and had not done so for at least 5 years prior to the study.	Personal sampling of respirable dust (workers involved in sieving, weighing and/or mixing/ dough-making) at various times between 1990 and 1996. The 1990-1996 dust exposure measurements were collated (no local exhaust ventilation, 8-hour TWA, GM±SD and range): *Bread bakeries*: - sieving (n=35): 11.4±73.1 mg/m^3 (0.9-349.5); • weighing (n=26): 8.2±146.7 mg/m^3 (1.0-770); • doughmaking (n=80): 3.3±19.5 mg/m^3 (0.1-142.2). *Cake bakeries*: • sieving (n=12): 35.7±26 mg/m^3 (15.9-90); • weighing (n=8): 19.2±20.7 mg/m^3 (7.4-68.5); • mixing (n=24): 3.8±4.2 mg/m^3 (0.5-16.3).	Structured interview (three occupational physicians with prior agreed criteria for diagnosis), and skin prick tests to common allergens and work-related allergens (wheat, soya and rice flour, and fungal alpha-amylase). Workers were allocated to four categories: • occupational asthma; • occupational rhinitis; • respiratory irritation; • asymptomatic.	No data were presented of the separate exposure groups. Overall prevalence of sensitisation to fungal alpha-amylase: • Bread-bakeries: 16% (63/392); • Cake-bakeries:1% (1/ 77; in the bakery not using fungal alpha-amylase, employee previously worked 1 year in a bread bakery). Overall prevalence of work-related respiratory symptoms: • Bread-bakeries: 20.4% (80/392, occupational asthma, occupational rhinitis or respiratory irritation); • Cake-bakeries:10.4% (8/77, only respiratory irritation). This study shows that dust exposure in bakeries poorly correlates with alpha-amylase allergenicity. The authors hypothesize that	Smith and Wastell Smith 1998[65]

			the low prevalence of alpha-amylase sensitised workers in the cake bakeries is caused by the lower concentration of alpha-amylase in flour, as compared to bread improvers handled in bread bakeries.	
Job- or task-based exposure categories				
224 workers in 18 small bakeries (<50 employees), Scotland.	Job categories: A - workers handling flour directly (dough making, cleaning, weighing, moulding, mixing); B - workers exposed from general contamination of spaces. Full-shift personal sampling of total inhalable and respirable dust (n=144, 8-hour TWA in mg/m^3): • A: 6.7±5.3 (AM±SD) • B: 1.5±1.5 (AM±SD) • A: 4.9±2.3 (GM±SD) • B: 1.0±2.7 (GM±SD)	Physician-administered questionnaire on work-related symptoms, past medical history, smoking status and occupational history (n=224). Serum IgE, specific to common and bakery allergens, including fungal alpha-amylase, was measured using RAST (threshold for positive sera was defined as mean plus 2.5 standard deviations of the background level, established in workers in an electronic factory) (n=205).	Overal prevalence of sensitisation: • fungal alpha-amylase: 15% (31/205) • wheat flour: 24% (49/205) • rye flour: 16% (33/205) • common environmental allergens: 34% (71/205) No separate data presented for categories A and B regarding sensitisation to alpha-amylase. Of the sensitised individuals, 18 reported work-related respiratory symptoms, whereas 43.7% of all the workers reported work-related symptoms. There was a statistically significant association between reported work-related symptoms and IgE to alpha-amylase (p<0.001), to wheat flour (p<0.001), and to common allergens (p=0.03).	Jeffrey et al. 1999[66]
94 workers in an enzyme research laboratory, and 79 workers in an enzyme producing plant, Finland. Enzymes produced: cellulase, xylanase, bacterial alpha-amylase, and fungal alpha-amylase.	Categories: • not/rarely (working in laboratories and office staff, n=59); • occasionally (handling mainly liquid enzymes, n=51); • often (handling mainly dry enzymes, n=63).	Self-administered questionnaire on work history, history of atopy, smoking and work-related symptoms; skin prick tests to common allergens, and to a series of enzymes, among which fungal alpha-amylase; RAST with specific IgE antibodies against enzymes (used if person was skin prick positive to an enzyme). IgG antibodies to fungal alpha-	Prevalence of sensitisation to fungal alpha-amylase: • not/rarely exposed: 1/59 • occasionally exposed: 1/51 • often exposed: 4/63 Most employees sensitised to fungal alpha-amylase (5/6) were also atopic. Only one had symptoms during work. *Note 1*: The study was performed 1.5 yr after the	Vanhanen et al. 1997[55]

		amylase by ELISA using conjugated rabbit antihuman IgG.	appearance of respiratory symptoms, after which hygienic measures were taken. *Note 2*: Workers were exposed to a mixture of enzymes, including bacterial and fungal alpha-amylase.	
3,450 workers in a large food company, UK.	Categories: • flour milling (n=528); • bread baking (n=1,756); • cake and pastry baking (n=209); • other activities (n=957).	Questionnaire and, in selected workers, skin prick tests to workplace allergens (wheat bran and flour, fungal alpha-amylase, and hen egg), and to common allergens. Differentiation between respiratory sensitisation, and non-specific irritation based on pattern of symptoms in relation to exposure.	Prevalence of occupational asthma: • flour milling: 0.2% (1/528) • bread baking: 0.6% (10/1756) • cake baking: 0% (0/209) • other activities: 0.1% (1/957) Fungal alpha-amylase was responsible for 10 cases of sensitisation, all associated with bread baking. Also, all these cases were diagnosed as having occupational asthma (based on work history, and strongly positive prick test).	Smith and Lumley 1996[7]
83 workers in pharmaceutical industry, exposed to fungal alpha-amylase powder, Spain.	Categories (based on questionnaire): • low (intermittent and occasional, n=15); • intermediate (intermittent and frequent, n=27); • intense (constant, n=38). No data on exposure levels presented.	Questionnaire on work-related symptoms and history of allergy (n=80). Skin prick tests to common allergens, and fungal alpha-amylase (n=83). Serum alpha-amylase-specific IgE was determined by a reverse enzyme immunoassay (positive at ≥150 OD = control + 3xSD). Bronchial and nasal provocation tests in symptomatic subjects. Oral provocation in 5 sensitised subjects. Average employment time was 9.5 years.	Prevalence of sensitisation to fungal alpha-amylase (skin prick test): • total: 26/83 (31.3%) • low: 2/15 • intermediate: 6/27 • intense: 18/38 Results questionnaire: • nasal symptoms: 47/80 (suggestive for allergic rhinitis • bronchial asthma: 24/47 Oral challenge of 5 sensitised persons provoked skin and bronchial symptoms in one worker. This person experienced no symptoms when eating bread.	Losada et al. 1992[50]

No data on exposure levels or job/task categories

89 bakery workers, 104 persons with bakers' asthma, and 43 control subjects (not working in a bakery), Germany.	No exposure data presented.	Skin prick tests to common and bakery allergens, including fungal alpha-amylase. Specific IgE antibodies to fungal alpha-amylase (n=169) were determined enzymatically (enzyme-allergo-sorbent-test). Lung function testing, including challenge testing with methacholine and alpha-amylase.	Prevalence of sensitisation to fungal alpha-amylase. Skin prick test positive: • bakery workers: 19% (17/88) • asthmatics: 24% (25/104) • control subjects: 2% (1/43) • symptomatic bakers: 27% (39/142) • asymptomatic bakers: 5% (2/44) Specific IgE positive: • bakery workers: 19% (17/89) • asthmatics: 12% (10/81) • control subjects: 10% (4/41) • symptomatic bakers: 21% (25/119) • asymptomatic bakers: 4% (2/45)	Baur et al. 1998[64]
385 workers in 19 bakeries, currently exposed to dust from bread improver, flour and other ingredients, 383 participating in the study, the UK.	No exposure data presented.	Interview on work-related symptoms by physician, and skin prick tests to common and work-related allergens, including fungal alpha-amylase. Based on these, workers were allocated to the following groups: • occupational asthma (alone or in combination with rhinitis); • occupational rhinitis; • respiratory irritation (non-specific); • asymptomatic (= no work-related symptoms).	Prevalence of sensitisation to fungal alpha-amylase: • all workers: 16% (62/383) • atopics: 31% (40/132) • non-atopics: 9% (22/257) Prevalence for diagnostic categories: • asthma: 100% (2/2) • rhinitis: 100% (10/10) • respiratory irritation: 27% (18/66) • asymptomatic: 10% (32/305) The authors explain the low prevalence of asthmatics among this population by healthy worker effect.	Smith et al. 1997[44]
36 workers in an enzyme producing factory, the USA.	No exposure data presented.	Self-administered questionnaires on medical history, and presence of symptoms; skin prick test	Results for alpha-amylase from *A. oryzae*. Skin prick test positive: 2/36 (6%, non significant increase	Biagini et al. 1996[69]

Enzymes produced included: alpha-amylase from *A. oryzae*, *B. licheniformis*, or *B. subtilis* formulations; alkaline proteases; amylo-glucosidases.		and Specific IgE to measure sensitisation; pulmonary lung function testing.	compared to controls). Authors reported that specific IgE antibodies to alpha-amylase from *A. oryzae* was significantly higher among exposed workers than among non-exposed controls. In general, the most commonly reported symptoms were: itchy, watery eyes (36%), sneezing (33%), chest tightness (31%), muscle aches (31%), cough (28%), flu-like sensation (28%), runny nose (25%).	
118 workers in bakeries, and confectioners, Germany.	No exposure data presented.	Measurement of specific IgE to fungal alpha/ amylase (enzyme immunoassay).	Prevalence: • symptomatic: 12 (34%) • asymptomatic: 0 (0%) Skin prick test confirmed positive cases. Immediate onset of rhinitis or asthma was observed in 4 bakers with symptoms, who were challenged by alpha-amylase by inhalation.	Baur et al. 1986[84]
Eight workers in enzyme producing factory. Enzymes produced: papain, alpha-amylase from *A. oryzae*, proteases, trypsin.	No exposure data presented.	Skin prick test to fungal alpha-amylase.	Five workers were positive for fungal alpha-amylase; four of them gave also a positive reaction to papain.	Flindt et al. 1979[85]

AM, average mean; 95% CI, 95% confidence interval; GM, geometric mean; OR, odds ratio; SD, standard deviation; TWA, time weighed average concentration.

Case reports

Study design and population information	Exposure information	Health information	Results	Reference
Five bakers with respiratory allergy, Spain.	Exposure to bread improvers containing fungal alpha-amylase. No exposure data presented.	Tests (skin prick test, specific IgE) for sensitisation to fungal alpha-amylase and bronchial provocation test.	One patient scored positive for all sensitisation tests to alpha-amylase. This person was also sensitised to cellulose, but not to wheat flour allergens.	Quirce et al. 1992[86]
Baker with asthma, Spain.	No exposure data presented. Alpha-amylase present as component in additive for bread making.	Tests (skin prick test, specific IgE) for sensitisation to fungal alpha-amylase and bronchial provocation test.	All tests were positive. Skin prick tests with wheat, rye and soya flour extracts were negative.	Blanco Carmona et al. 1991[87]
Baker with asthma, France.	No exposure data presented. Alpha-amylase present as component in additive for bread making.	Tests (skin prick test, specific IgE) for sensitisation to fungal alpha-amylase and bronchial provocation test.	Positive for sensitisation tests. Provocation test to the enzyme gave a delayed reaction.	Birnbaum et al. 1988[88]

Annex **F**

Exposure-response relationships

Cross-sectional studies as part of a longitudinal prospective cohort study by Cullinan et al. 1994[52]

Study design and population information	Exposure information	Health information	Results	Reference
(1) Initial cross-sectional phase of a longitudinal cohort study in 3 bakeries, 1 flour packing factory, and 3 mills where wheat was milled, UK. 264 workers without pre-employment.	Exposure assessed by self-administered questionnaire on job-title and work history, including exposure to flour in previous employment. Full-shift personal inhalable dust sampling in 11-15 workers of each task-based exposure group in each factory (collected 1990/1991): • low: < 1 mg/m^3 • medium: 1-5 mg/m^3 • high: >5 mg/m^3 Determination of fungal alpha-amylase in dust samples by sandwich-immunoassay (n=256).	Self-administered questionnaire on work-related respiratory symptoms (4-5 years retrospective) and smoking habits. Skin prick tests to common allergens, and to wheat flour and fungal alpha-amylase (n=256). Skin prick tests were considered positive if the mean wheal diameter was at least 3 mm greater than the inert control.	*Sensitisation* Prevalence, exposure at time of study: • low: 3.1% (7/225) • medium: 16.7% (3/18) • high: 15.4% (2/13) Prevalence, highest exposure ever: • low: 2.5% (5/203) • medium: 9.5% (2/21) • high: 29.4% (5/17) Prevalence ratio, highest exposure ever: • medium: 3.9 (95% CI, 0.8-20.2) • high: 9.9 (95% CI, 2.8-34.6) Prevalence ratio for sensitisation relative to atopy: 2.9 (95% CI, 0.8-9.7).	Nieuwenhuijsen et al. 1999[22] Exposure levels: Nieuwenhuijsen et al. 1994[71] Cohort design: Cullinan et al. 1994[52]

	Exposure at time of study (AM±SD): • low: 0.7±0.8 ng/m^3 • medium: 10.7±2.2 ng/m^3 • high: 46.7±16.6 ng/m^3 Highest exposure ever worked in (AM±SD): • low: 0.8±0.8 ng/m^3 • medium: 10.5±2.3 ng/m^3 • high: 48.0±16.6 ng/m^3 Mean duration of employment was 28 months (range between 1 month to maximal 4 years).		*New-work-related symptoms* Chest, at time of study: • low: 6% (14/225) • medium: 0% (0/18) • high: 15.4% (4/13) Eye and nose, at time of study: • low: 16.4% (38/225) • medium: 21% (4/18) • high: 23.1% (3/13) Skin, at time of study: • low: 5.2% (12/225) • medium: 5.3% (1/18) • high: 30.8% (4/13) Chest, at highest exposure ever: • low: 6.2% (13/203) • medium: 4.6% (1/21) • high: 11.8% (2/17) Eye and nose, at highest exposure ever: • low: 16.2% (34/203) • medium: 22.7% (5/21) • high: 17.7% (3/17) Skin, at highest exposure ever: • low: 5.7% (12/203) • medium: 4.6% (1/21) • high: 23.5% (4/17) None of the 12 workers with positive skin prick test to fungal alpha-amylase had new work-related chest symptoms, one had new work-related eye and nose symptoms and one had new work-related skin symptoms. This might be explained by workers with symptoms moving to a low-exposure environment (healthy worker effect).	
(2) Cross-sectional phase of a longitudinal cohort study in 3 bakeries, 1 flour packing factory, and 3 mills where wheat was milled, UK.	Determination of fungal alpha-amylase in dust samples by sandwich-immunoassay (n=256). Exposure at time of study (AM±SD): • low: 0.7±0.8 ng/m^3 • medium: 10.7±2.2 ng/m^3 • high: 46.7±16.6 ng/m^3	Symptoms recorded by self-administered questionnaire, skin sensitisation to fungal alpha-amylase by skin prick test. In this study, a different statistical analysis was employed (prevalence ratio)	Prevalence (ratios) (Cox regression): Prevalence sensitisation: • low: 8% (18/225) • medium: 9% (2/22) • high: 31% (4/13) Prevalence ratio, sensitisation:	Brisman et al. 2004[57] Exposure levels and categories: Nieuwen-huijsen et al. 1994[71], 1999[22]

258 workers in bakeries, follow-up 7 years.	Highest exposure ever worked in (AM±SD): • low: 0.8±0.8 ng/m^3 • medium: 10.5±2.3 ng/m^3 • high: 48.0±16.6 ng/m^3 The highest exposure ever worked in was used for classification. Forty persons were not classified due to missing information. Median duration of employment was (range): 40 (1- 91) months.	compared to previous studies on this cohort (odds ratio). Odds ratios are generally higher than prevalence ratios, but according to the authors, prevalence ratio is a better estimate of relative risk.	• medium: 3.1 (95% CI, 0.6-17) • high: 4.0 (95% CI, 0.9-18) Prevalence, chest symptoms: • low: 9.7% (22/225) • medium: 22.7% (5/22) • high: 38.5% (5/13) Prevalence ratio, chest symptoms: • medium: 1.7 (95% CI, 0.6-4.9) • high: 3.0 (95% CI, 1.1-8.1) Prevalence, eye and nose symptoms: • low: 24.9% (56/225) • medium: 45.5% (10/22) • high: 53.8% (7/13) Prevalence ratio, eye and nose symptoms: • medium: 1.9 (95% CI, 0.9-3.8) • high: 1.9 (95% CI, 0.9-4.2) Trends were observed for eye-nose symptoms, and sensitisation by increasing exposure. Authors noted that precision of the study is low, because the distribution of alpha/ amylase exposure was highly skewed, with 86% of the workers in the low exposure category.	Cohort design: Cullinan et al. 1994[52]
(3) Nested case-control within a prospective cohort study. Cohort of 300 new employees from 7 UK bakeries and flour mills, not previously exposed to flour; follow-up 1-91 months (median 40 months). Those who developed work-related symptoms or with a	Categories: • low: bread wrappers and confectioners without direct contact with flour; • medium: bread and roll makers, cleaners and other confectioners; • high: workers directly handling flour and mixing or braking doughs. Inhalable dust concentrations (GM (95% CI)): • low: 0.58 mg/m^3 (0.5-0.7 mg/m^3);	All cohort members still employed were surveyed at 6-monthly intervals for 3 years. 52 Workers were surveyed 7 times. At each survey, self-administered questionnaire on smoking habits and on work-related chest, eye/nose or skin symptoms, that had commenced after first employment at the study site. Skin prick tests to common respiratory allergens, to wheat flour and to fungal al	Prevalence of sensitisation to fungal alpha-amylase (positive skin prick test): 24/ 300 • low: 17/58 (cases/ control), OR 1 (95% CI, -) • medium: 38/12 (cases/ control), OR 13 (95% CI, 0.8-209) • high: 46/31 (cases/ control), OR 23 (95% CI, 0.3-182). Incidence rates (cases per	Cullinan et al. 2001[53] Cohort design: Cullinan et al. 1994[52]

positive skin prick test were compared with an employment-duration-matched control group.	medium: 1.17 mg/m^3 (1.0-1.4 mg/m^3); • high: 4.37 mg/m^3 (3.8-5.1 mg/m^3). No exposure data on fungal-alpha-amylase presented. Median duration of employment was (range): 40 (1-91) months.	alpha-amylase. Two controls (nonsymptomatic and negative skin prick test), matched for duration of employment, were selected for each case (symptomatic or positive skin prick test)	100 person-years): • skin prick test alpha-amylase: 2.5 • skin prick test flour: 2.2 • eye/nose symptoms: 11.8 • chest symptoms: 4.1 Approximately 50% of the cases developed within 24 months of employment. Compared to the controls, sensitised persons were more often atopics (OR 4.1, 95% CI 0.4-47, not significant). There was no difference in smoking habits. Only 25% of workers reporting chest problems were sensitised to flour or fungal alpha-amylase. Even less workers reporting eye/nose or skin symptoms were sensitised to flour of fungal alpha-amylase (17% and 18% respectively). This suggests that a high proportion of the work-related symptoms had no allergic origin but probably reflect an irritant or inflammatory response to airborne dust.

AM, average mean; 95% CI, 95% confidence interval; GM, geometric mean; OR, odds ratio; SD, standard deviation.

Other cross-sectional studies

Study design and population information	Exposure information	Health information	Results	Reference
178 workers from 14 bakeries, the Netherlands. Maintenance workers were excluded.	Full-shift personal inhalable dust and fungal alpha-amylase concentrations measured (sandwich-immunoassay). Fungal alpha-amylase exposure levels (job with highest exposure ever worked in, GM±SD (range)): • low: 0.7±4.0 ng/m^3 (0.2-8.8); • medium: 1.3±3.8 ng/m^3 (0.2-33.1) ; • high: 18.1±4.6 ng/m^3 (0.2-221.8); • indistinct/variable: 6.1±8.2 ng/m^3 (0.2-150.2). Job-title categories: • low (n=71): workers handling alpha-amylase only occasionally; • medium (n=39): doughmakers and all-round staff from wheat-bread producing bakeries and bread and mixed bakers from small bakeries; • high (n=23): doughmakers in one crispbake factory; • indistinct/variable (n=36): all-round staff in the crispbake factory. Mean years of employment in bakery industry was 10.2 ± 8.7 years (range:0.2-43 years).	Self-administered questionnaire (n=178) on work-related symptoms, smoking habits and job history; lung function measurements. Skin prick tests to work-related and common allergens, and analysis of specific IgE to fungal alpha-amylase in blood (n=169). Skin prick tests were considered positive if at the 15-min reading, the mean wheal diameter was at least 3 mm greater than the negative control. Specific IgE was determined by means of an enzyme immunoassay using monoclonal mouse antihuman IgE. Optical density readings at 492 nm exceeding the OD of the reagent blank with more than 0.05 was interpreted as a positive reaction.	Overall prevalence of sensitisation to fungal alpha-amylase: • skin prick test: 9.5% (16/169) • IgE-positive: 7.7% (13/169) Prevalence, skin prick test: • low: 1.4% (1/71) • medium:12.8% (5/39) • high: 30.4% (7/23) • indistinct: 8.3% (3/36) • reference population(laboratory animal workers): 1.7% (7/416) Prevalence ratio, skin prick test: • medium:8.6 (95% CI, 1.01-74) • high: 15.9 (95% CI, 1.95-129) • indistinct: 4.6 (95% CI, 0.48-45) • atopy: 20.8 (95% CI, 2.74-158) Prevalence among atopic workers (n=49), skin prick test: • low: 4% • medium: 35% • high: 55% Prevalence, specific IgE: • low: 2.5% (2/71) • medium: 13% (5/39) • high: 15% (4/23) Prevalence ratio, specific IgE: • medium: 4.6 (95% CI, 0.85-22) • high: 3.9 (95% CI, 0.65-24) Prevalence ratios for IgE were not statistically significant except for atopy: 8.3 (95% CI, 1.84-38)	Houba et al. 1996[14], Doekes et al. 1998[6]; Houba et al. 1996[70], 1997[89]

			Sensitisation to fungal alpha-amylase (determined by skin prick test) was moderately to strongly associated with self-reported, work-related rhinitis and asthmatic complaints (chest tightness): Prevalence ratios: All workers (n=169) • rhinitis: 4.78 (95% CI, 2.05-11.2) • chest tightness: 11.95 (95% CI, 3.21-44.5) Atopic workers (n=65) • rhinitis: 3.33 (95% CI, 1.17-9.50) • chest tightness: 4.44 (95% CI, 1.00-19.9) 60% of the sensitised workers reported symptoms, and about 30% of the symptomatic bakery workers was sensitised (IgE) to wheat flour or alpha-amylase. The authors considered a non-specific reaction to dust as a possible explanation. Smoking habits, age and years working in the baking industry were not associated with sensitisation.	
246 workers from traditional and industrial bakeries (n=74), compared with 251 workers from a petrochemical plant in the same region (controls), Belgium.	Personal inhalable dust concentrations measured (during shift 5-7 hours). Alpha-amylase in dust samples analysed by sandwich-immunoassay. Job categories: • low: industrial packers; • medium: industrial bakers and traditional pastry bakers; • high: traditional bread bakers, and traditional bread + pastry bakers. Exposure to alpha-amylase (GM±SD): • low (n=34): 0.15 ±	Self-administered questionnaire on respiratory, asthma and allergy-related symptoms, supplemented with questions on smoking habits. Skin prick testing on common and bakery-specific antigens (wheat flour, rye flour, and fungal alpha-amylase). Skin prick tests were considered positive if at the 15-min reading, the mean wheal diameter was at least 2 mm. Lung function tests performed by spirometry.	Overall prevalence of sensitisation to fungal alpha-amylase: • bakery workers: 7.5% (18/246) • controls: 0.8% (2/251) Odds ratio (adjusted for age and smoking habits) was 11.6 (95% CI, 2.4-55.1). Overall prevalence of sensitisation for any allergen: • bakery workers: 39.4% • controls: 42.6% Prevalence of sensitisation, alpha-amylase exposure: • low: 3.0% (1/33) • medium: 4.7% (4/86)	Droste et al 2003[56] Droste et al. 2005[90] Data on exposure levels: Bulat et al. 2004[20]

	1.74 ng/m^3; • medium (n=73): 0.47 ± 7 ng/m^3; • high (n=107): 0.50-0.61 ± 4 ng/m^3. Maximum exposure was 136 ng/m^3 in an industrial baker. Mean duration of employment was: 8.9 years.		• high: 12.6% (13/103) Intergroup difference not statistically significant: medium versus low, odds ratio 2.1 (95% CI, 0.2-20.2); high versus low, odds ratio 4.4 (95% CI 0.5-37.2). Overall work-related symptoms (chronic cough, shortness of breath, wheeze): Prevalence: • low: 5.9% (2/34) • medium: 18.9% (14/74) • high: 39.6% (38/96) Odds ratio (95% CI): • medium: 3.9 (0.8-18.2) • high: 10.9 (2.5-48.7) Spirometry did not reveal dose/response relationships with lung function parameters.	
186 workers in one US bakery.	Two exposure categories: • low exposure: not handling dough, e.g. office, transportation, oven areas; • high exposure: handling raw materials and/or dough: e.g., bread & bun production, distribution. Full-shift air sampling in personal breathing zone (n=83), and general area (n=19). Inhalable flour dust levels (GM and range): • low: 0.24 mg/m^3 (ND-1.4) • high: 3.01 mg/m^3 (trace-65) Fungal alpha-amylase concentrations (sandwich immunoassay; GM and range): • low: 0.12 ng/m^3 (0.019-1.2) • high: 2.10 ng/m^3 (0.095-11,000)	Self-administered questionnaire on job history and work-related symptoms and smoking habits (n=161). Specific serum IgE antibodies to fungal alpha-amylase, flour and wheat, and to common allergens to assess atopy (n=96).The method was a highly sensitive enzyme-enhanced chemiluminescent enzyme immunoassay, Immulite 2000. Traditionally, IgE levels ≥ 0.35 kU/L serum are considered positive for sensitisation. The threshold for this assay is 0.10 kU/L. There was no difference in prevalence of atopy between the two exposure groups.	Prevalence of sensitisation to fungal alpha-amylase: • low: 4% (2/51) • high: 11% (5/45) Prevalence ratio: 2.83 (95% CI, 0.65-18.84). A number of employees working in lower-exposure group reported past work in higher exposure group; prevalence of sensitisation to fungal alpha-amylase if these employees were included in the high-exposure group: • low: 0% (0/33) • high (current and past): 11% (7/63) Prevalence of sensitisation to fungal alpha-amylase (positive if IgE ≥ 0.35 kU/L): • low: 0% (0/33) • high (current and past): 6% (4/63) Atopics were significantly more likely to be sensitised to alpha-amylase at the low cut-off value (p=0.04).	Page et al. 2009[46], 2010[28]

	Inhalable flour dust and alpha-amylase concentration were positively correlated (r=0.64, p<0.01). Mean tenure was 13 years (higher exposure group), and 16 years (lower exposure group).	Prevalence, runny nose: • low: 6% (6/91-93) • high (16% (10/61-64) Prevalence ratio: • 3.81 (95% CI, 1.25-11.61) Prevalence, stuffy nose: • low: 6% (6/91-93) • high: 18% (11/61-64) Prevalence ratio: • 2.75 (95% CI, 1.07-7.05) Prevalence, frequent sneezing: • low: 8% (7/91-93) • high: 21% (13/61-64) Prevalence ratio: • 2.68 (95% CI, 1.13-6.34) Note: few symptoms were significantly related to sensitisation. Thus, a preference for the lower or higher cut-off value could not be determined.

AM, average mean; 95% CI, 95% confidence interval; GM, geometric mean; SD, standard deviation.

Annex **G**

Meta-regression analysis

In deriving a reference value, data by Nieuwenhuijsen et al. (1999) and Houba et al. (1996) were combined in a meta-regression analysis as follows.

First of all the background sensitisation rate was estimated on the basis of population studies without occupational exposure. From this data, the committee calculated the expected number of cases for each exposure category in the Nieuwenhuijsen and Houba study, on the basis of number of persons in the exposure group category. Average exposure for each exposure category was taken from the respective publications. For each exposure group the ratio of the observed and expected number of cases was calculated.

For the meta-regression analysis, the committee used a linear Poisson model $RR=\alpha(1 + K_{\text{alpha-amylse sensitisation}} * E)$, where RR is the relative risk, α is an intercept parameter representing the background rate of sensitisation (expected cases), and $K_{\text{alpha-amylse sensitisation}}$ is the slope of increase in the AR per unit exposure (E) to alpha-amylase (in ng enzyme/m^3). $K_{\text{alpha-amylase sensitisation}}$-values were obtained by fitting Poisson regression models using the statistical programme SAS. The intercept was fixed ($\alpha = 1$) to force the model to go through the odds ratio of 1 (background prevalence of the non-exposed reference groups (zero exposure)). As such, the committee derived a $K_{\text{alpha-amylase sensitisation}}$-value of 0.924 (confidence interval 0.344-1.504, p=0.008), resulting in the formula $RR=1 + 0.924 * E$. With a background rate of 1.17 per 100, an extra risk of 1 per 100 (1%) means an additional risk of 2.17, which corresponds to a RR of 1.855 (RR=additional risk divided by background risk). Using this formula an exposure level of 0.9 ng enzyme/m^3 was

calculated, which corresponds to an additional risk on sensitisation of 1% compared to the background sensitisation rate.

Exposure group	Exposure level (ng/m^3)	Number of persons in group	Number of persons sensitised
Nieuwenhuijsen et al. (1999)			
Reference	No data	No data	No data
low	0.8	203	5
medium	10.5	21	2
high	48.0	17	5
Houba et al. (1996)			
Reference	0.0	416	7
low	0.7	71	1
medium	1.3	39	5
high	18.1	23	7

Exposure level expressed as geometric mean (highest exposure ever worked in), 8 hour TWA. Reference Houba-study: laboratory animal workers not occupationally exposed to fungal alpha amylase.

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

www.healthcouncil.nl