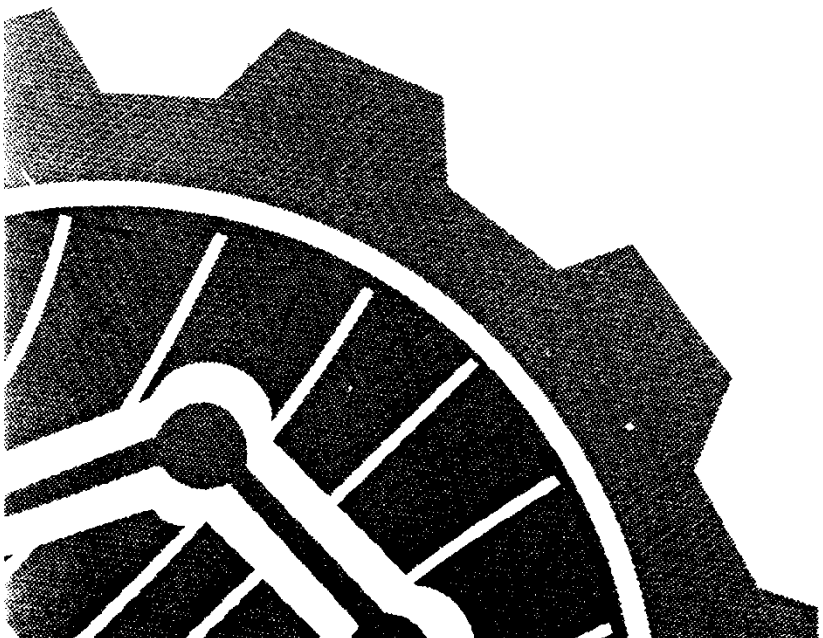
NICNAS

National Industrial Chemicals Notification & Assessment Scheme

PRIORITY EXISITING CHEMICAL

'Savinase' — Proteolytic Enzymes in Detergents

FULL PUBLIC REPORT



Preface

This assessment is made under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). NICNAS was established by the Commonwealth *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to help protect people and the environment from the harmful effects of industrial chemicals by finding out the risks to occupational health and safety, to public health and the environment.

NICNAS has two major parts: one focussing on the risks associated with new chemicals before importation or manufacture; and one focussing on existing industrial chemicals already in use in Australia. As there are many thousands of existing industrial chemicals in Australia, NICNAS has a mechanism of prioritising assessments by declaring certain existing chemicals to be Priority Existing Chemicals (PEC). This report provides the full public report of a PEC assessment. A summary report will also be publically available and will be published in the Commonwealth *Chemicals Gazette*.

NICNAS is administered by Worksafe Australia. Assessments under NICNAS are done in conjunction with the Commonwealth Department of the Arts, Sport, the Environment and Territories and the Department of Health, Housing and Community Services.

This assessment report has been prepared by the Director, Chemicals Notification and Assessment in accordance with the Act. This report has not been subject to tripartite consultation or endorsement by the National Occupational Health and Safety Commission.

In accordance with section 37 of the Act, applicants may apply to the Director for variation of this report using the approved form. A fee must be paid with the application.

On publication of the Summary Report about a PEC in the *Chemicals Gazette*, the chemical will no longer be a Priority Existing Chemical in accord with section 62 of the Act.

For the purposes of subsection 78(1) of the Act, copies of full public reports may be inspected by the public at the Library, NOHSC, Plaza level, Alan Woods Building, 25 Constitution Avenue, Canberra, ACT 2600, between 9am to 5pm Monday to Friday (except on public holidays). In addition, copies of the full public report may be obtained on request.

For further information, contact the Chemical Safety Group at:

GPO Box 58
SYDNEY NSW 2001
AUSTRALIA

OR

334-336 Illawarra Road
MARRICKVILLE, NSW 2204
AUSTRALIA

Telephone: (61) (02) 8577 8800

Facsimile: (61) (02) 8577 8888

Contents

1. Introduction

The chemical known as 'Savinase' - a proteolytic enzyme - was declared by the Minister for Industrial Relations as a Priority Existing Chemical (PEC) by notice in the *Chemical Gazette* of June 2, 1992.

The declaration was limited to the use of proteolytic enzymes in the detergent industry.

The declaration by the Minister was made on the basis that there were reasonable grounds for believing that manufacture, handling, storage, use and disposal of the chemical could give rise to a risk of adverse health effects.

In summary these grounds were:

- Past exposure of Australian workers to proteolytic enzymes in detergents had resulted in asthma-like reactions. Proteolytic enzymes are known respiratory sensitisers in humans.

- If detergents containing proteolytic enzymes are to be manufactured in Australia, potentially a significant number of workers could be exposed to this hazard.

In Australia, the use of enzymes in the detergent industry had been the subject of bans imposed by the Australian Council of Trade Unions (ACTU) since 1971. However, since then there have been significant developments overseas in the technology relating to control of enzyme dust levels.

There was substantial agreement by industry and employee organisations on the need for an objective scientific assessment of proteolytic enzymes in the detergent industry.

With this in mind the objectives of the assessment were to:

- characterise the potential health hazards presented by proteolytic enzymes; and

- to determine if these hazards could be satisfactorily controlled in the workplace.

In order to meet the assessment objectives, information was collected from a range of sources, including the information dossiers documenting toxicology, manufacturing process and data relevant to occupational exposure obtained from applicants, a literature search, site visits and information from overseas industry associations.

In beginning to assess this chemical as a priority existing chemical it was recognised that 'Savinase' was only one of the commercial names for this class of enzyme, and proteolytic enzymes used in the detergent industry fall into a general category of enzymes known as 'proteinases'. A range of Chemical Abstract Services numbers (CAS Numbers) have been assigned for proteinases and there are a multitude of common names, commercial and trade names to be found. Proteolytic enzymes are more commonly referred to as proteinases in the nomenclature considered.

Because of this confusing nomenclature it was found to be inappropriate to limit this assessment just to Savinase, and therefore other proteinases used in the detergent

industry have been considered. Accordingly, the generic name 'proteinase' is used in this report.

Following declaration of Savinase as a PEC, importers and manufacturers were required to apply for assessment of the chemical. The applications received indicated that while there is significant importation of proteinases as components in finished detergent products, there is at present no manufacture or intention to manufacture the enzymes themselves in Australia. Applications were received from manufacturers of laundry detergents and from importers/ manufacturers of enzyme preparations. This report therefore focuses on the use of proteinases in the manufacture of enzymatic detergents, not on the manufacture of proteinases themselves, and is further focussed on use in laundry detergents.

2. Background

2.1 Proteinases in detergents

A variety of enzyme products have been developed for use in so-called 'biological or enzymatic detergents' to enhance the removal of organic material from textile fibres. The most widely used of these are proteinases whose function and mode of action is to remove protein stains - such as grass, blood, egg and human sweat - by proteolytic degradation to more soluble polypeptides and amino acids.

Detergent proteinases are derived from the fermentation processes of non-pathogenic, alkalophilic strains of bacteria and, in particular, the Bacillus genus; such as Bacillus subtilis, Bacillus lentus and Bacillus licheniformis. These enzymes are particularly stable under the conditions of temperature and alkalinity found in laundering and are more specifically referred to as 'alkaline' or 'high alkaline' proteinases. It is generally regarded that the properties and health effects of all proteinases are similar.

Proteinase enzymes were originally available as dried fermentation products, known as enzyme concentrates, which were added to detergents as dusty powders. However, today enzymatic laundry detergent powders are generally manufactured with granulated or encapsulated enzymes which have been specifically formulated to reduce enzyme dust levels during manufacture and use.

2.2 Health issues

Following the introduction of enzymatic detergents in the late 1960s, it soon became apparent that occupational exposure to enzyme dusts could cause skin irritation [1+4] and allergic respiratory reactions. Respiratory sensitisation - also known as enzyme or occupational asthma - was recognised as a common occurrence in detergent workers worldwide [1, 2, 5, 6], including Australia.[7] Adverse health effects were particularly prevalent in workers in the detergent industry exposed to high enzyme dust levels. Adverse effects appeared to be reversible in most workers following removal from further exposure to enzyme-containing dust.[8] Of the many studies of workers in the detergent industry since the introduction of enzymes, only one report has suggested that there may be a longer term loss of respiratory function in some individuals.[9]

2.3 The Australian perspective

In Australia, enzyme-elicited allergic reactions in workers using enzyme powders led to an ACTU ban in 1971 on the manufacture of enzymatic detergents. This action was accompanied by a voluntary withdrawal from the use of enzymes in detergent products by the main Australian detergent producers. The ACTU ban on the use of proteolytic enzymes in the soap/ detergent industry still stands in 1992.

During 1992 a number of companies began to import laundry detergents containing proteinases into Australia. A reassessment of the use of proteinases in laundry detergents in Australia, including possibilities for domestic detergent manufacture, is therefore timely. It is generally agreed that enzymatic detergents should only be manufactured here if the risk of adverse health effects to workers is acceptably low.

In April 1992, the WorkCover Authority of New South Wales was requested to carry out a survey of a storage facility for enzyme-containing detergents and recommended that a code of practice be produced on the safe handling and storage of enzymatic detergent products. This code of practice was developed by a state level tripartite working party for safe handling and storage of enzymatic detergent powders and liquids. The code of practice will be considered for endorsement at the February 1993 meeting of the Board of the WorkCover Authority of New South Wales.

2.4 Overseas use of proteinases

Detergents-containing enzymes - or 'enzymatic detergents' - have continued to be manufactured and used in Europe, America and many Asian countries. Recognition that enzyme asthma in workers in detergent factories was due to exposure to high levels of enzyme dust led to major changes to work practices in the 1970s to reduce airborne respirable dust levels. Engineering controls were introduced to achieve stricter dust control. Guidelines for the safe handling of enzyme-containing detergents were developed and implemented, such as by the United Kingdom Soap and Detergent Industry Association (SDIA). Enzymes were encapsulated to prevent the release of fine dust particles during their manufacture and use.

In recent years liquid detergents containing enzymes have also been introduced. Similar control practices as those established for powders have been implemented for the manufacture of these liquid enzymatic detergents.

To monitor the effectiveness of the workplace changes, dust monitoring and health surveillance of workers were introduced in detergent factories.

As a result of these changes since the early 1970s, there has been a marked reduction in the levels of airborne enzymes and in the incidence of acute respiratory illness in detergent workers.[10, 11] In the past decade there have been only a few reports in the literature of sensitisation in workers handling encapsulated enzymes.[12, 13]

3. Applicants

Ajax Chemicals
291+303 Woodpark Road
Smithfield NSW 2164

Amway of Australia Pty Ltd
46 Carrington Street
Castle Hill NSW 2154

Campbell Brothers Ltd
Campbell Street
Bowen Hills QLD 4006

Colgate-Palmolive Pty Ltd
Level 15
345 George Street
Sydney NSW 2000

Cussons Pty Ltd
282+300 Hammond Road
Dandengong VIC 3175

Harcros Industrial Chemicals
3+5 Alan Street
Rydalmere NSW 2116

Kao (Australia) Marketing Pty Ltd
Suite 403
32 Walker Street
North Sydney NSW 2060

L&K: Rexona Pty Ltd
Pembroke Road
Minto NSW 2566

Novo Nordisk Bioindustrial Pty Ltd
22 Loyalty Road
North Rocks NSW 2151

Solvay Biosciences Pty Ltd
Brandon Office Park
530 Springvale Road
Glen Waverley VIC 3150

4. Chemical Identity

Proteolytic enzymes used in the detergent industry, such as Savinase, fall into the generic category known as proteinases. Savinase is the trade name for one proteinase product of Novo Nordisk A/S, Denmark.

One of the difficulties reflected in the nomenclature for these enzymes is that proteinases are fermentation products of a number of differing bacterial strains and these products have been given a range of CAS numbers. There are many common names and commercial or trade names in use for these enzymes. This confused nomenclature has been established in the literature and therefore it is appropriate to broadly assess the group of enzymes known as proteinases.

4.1 Common names and Chemical Abstract Services Numbers

Proteinase	9001-92-7
Subtilisin	9014-01-1
Proteinase, Bacillus alkaline	9073-77-2
Proteinase, (Myxobacter alpha-lytic)	12585-31-8

4.2 EC/IUB Number

The proteolytic detergent enzymes are classified by the International Union of Biochemists (IUB) as 'Microbial serine proteinases' with Enzyme Classification (EC) number 3.4.21.14.

4.3 Other names

Alkaline bacterial proteolytic enzyme	Proteolytic enzyme
Alkaline protease	Serine proteinase
Bacillus alkaline proteinase	Serine protease
Microbial serine proteinase	Serine proteolytic enzyme
Protease	Subtilisin protease

4.4 Trade names

Alcalase	Esperase	Maxatase	Optimase
Durazym	Kazusase	Opticlean	Savinase

4.5 Molecular weight

Proteinases used in detergents are alkaline proteases with a molecular weight range of 22,000 to 28,500.

4.6 Chemical composition

Proteinases are single polypeptides of variable molecular weight containing approximately 275 amino acids. Different proteinases differ in several amino acids.

4.7 Enzyme preparations

At present, proteinases are imported into Australia in commercial enzymatic laundry detergents and in small quantities for incorporation into detergents.

Detergent manufacturers wish to manufacture enzymatic detergents in Australia by importing proteinases as enzyme preparations in either granulate, liquid or slurry form. These preparations are formulated overseas, with enzyme concentrate as the basic ingredient.

The enzyme concentrate contains between 20 to 50 per cent w/w enzyme protein together with non-enzyme protein, carbohydrate/polysaccharide, and inorganic salts. Proteinase concentrate will not be imported into Australia but is used overseas to produce the enzyme preparations, ie enzyme granulates, liquids and slurries.

4.7.1 Enzyme granulate (or marume)

Enzyme granulates generally contain the following major ingredients: up to 20 per cent enzyme concentrate, sulphates, carbonates, titanium dioxide, silica/silicates and polyethylene glycol.

The granules are white to off-white in colour, with a slight odour of hydrolyzed vegetable protein. The granules contain between 1 and 5 per cent enzyme. The enzyme is embedded in a core of inorganic salts which is encapsulated in an inert protective layer, usually polyethylene glycol.

Enzyme granulate is usually included in commercial detergent powders at a concentration of 0.02 per cent to 1 per cent. The maximum concentration of enzyme in finished detergent powders is 0.05 per cent by weight.

The size distribution of proteinase granules varies with different enzyme preparations. However, based on the data supplied on three granulates the mean particle size is 500 to 700µm.

4.7.2 Enzyme liquid and slurry

Enzyme liquid and slurry forms contain between 2 to 5 per cent enzyme protein. These enzyme forms have a slight fermentation odour. The liquid is usually formulated in polyol or glycol and water. The slurry is usually formulated in ethoxylated alcohols.

The enzyme-containing liquids and slurries are included in liquid detergent products at a concentration of 0.2 to 1.0 per cent. Therefore, the maximum amount of enzyme protein in the finished liquid detergents is 0.05 per cent by weight.

A spray liquid laundry stain remover, which contains proteinases at a concentration of

0.007 per cent, is also available to the public.

5. Physical and Chemical Properties

5.1 Water solubility

Proteinase enzymes are readily soluble in water. Under conditions of high relative humidity and in water the enzymes will slowly hydrolyse and lose their activity.

5.2 Vapour pressure

The liquid and slurry forms of the enzyme have low vapour pressure. The enzyme granulates have negligible vapour pressure.

5.3 pH stability

Proteinases used in laundry detergents are alkaline proteinases which exhibit optimum activity in the pH range of 8 to 11. Proteinases have a pH dependent loss of enzyme activity in aqueous solution. The enzymes are immediately inactivated at pH <4.

5.4 Thermal stability

Proteinases, in the form of granulate, liquid and slurry, are stable at room temperature for several months. The optimum temperature for enzyme activity is 40 to 60°C. Proteinases are inactivated or denatured at temperatures above 80°C.

5.5 Enzyme activity

The activities of proteinases are generally dependent on their source; that is, the strain of bacteria, purity, and conditions of the assay. It is more practical to measure the amount of enzyme present by measuring enzyme activity rather than weight. Therefore a knowledge of enzyme activities and how this is determined is central to many of the monitoring techniques discussed in this report.

Particular enzymes will have varying specific activities for the pure crystalline proteolytic enzymes. The enzyme activity is described in units which relate to the method of enzyme analysis used. The units include:

- Anson Units (AU);
- Durazym Protease Units (DPU);
- Delft Units (DU);
- Glycine Units (GU);

- Novo Protease Units (NPU);
- Showa Denko Protease Units (PU); and
- Showa Denko Units (SU).

As these different methods measure activity in respect to a specific protein substrate, one unit of measurement does not necessarily convert to another unit of measurement.

The enzyme activity of the proteinase granulates varies from 1,200 to 1,800 kGU/g. The enzyme activity of liquids and slurries range from 1800 to 4200 kGU/g (1 kGU = 1,000 GU). The relationship between enzyme activity and the exposure standard which must be considered for air monitoring is set out in Table 4 (Section 12.6).

6. Methods of Detection and Analysis

6.1 Structural analysis

Proteinase enzymes can be characterised using:

- Fast Protein Liquid Chromatography (FPLC);
- High Performance Liquid Chromatography (HPLC); or
- Raman spectrum in deuterium oxide.

6.2 Analytical methods for measuring enzyme levels

Most analytical techniques used to determine enzyme levels involve the measurement of either enzyme activity or antigenic activity. These methods can be used to quantitate levels of enzyme in:

- dust collected from air sampling;
- dust collected from elutriation tests;
- enzyme granules and liquids; and
- finished detergent products.

6.2.1 Measurement of enzyme activity

Enzyme activity is a measure of the proteolytic capacity of the enzyme sample as determined by reaction with a suitable protein substrate, usually a substituted casein or haemoglobin. There are four main steps involved in this analysis:

- enzymic digestion of the substrate to form amino acids and peptides;
- quenching and separation to stop digestion and precipitate undigested substrate and enzyme;
- formation of an amino acid-complex with a chemical indicator; followed by
- colorimetric analysis of the indicator complex.[14, 15]

Detection limits for the various reported techniques range from 0.002ug/ml to 0.02ug/ml of pure enzyme.

6.2.2 Measurement of antigenic activity

Since allergic reactions to microbial proteinases are mediated by specific IgE

antibodies in serum, the quantitation of antigenic activity of air monitoring samples by immunoassay provides an *in vitro* correlation to the allergic response in sensitised workers. The main advantage of immunoassay techniques is that in addition to intact enzyme they detect inactive forms, such as proteinase fragments, which can also be allergenic.[16]

Enzyme antigenic activity has been determined by Two-Site Immunoradiometric Assay (TSIRA). This method is based on the binding of enzyme antigen to radiolabelled (^{125}I) rabbit anti-proteinase antibodies and quantitation by scintillation counting, and has been used to measure enzyme (Esperase) dust from personal sampling with a detection limit of 0.004 µg/ m^3 air.[17]

A similar method - Inhibition Enzyme Immunoassay (IEIA) - utilises a chromogenic substrate, p-nitrophenylphosphate, in the determination of an enzyme linked antibody-antigen complex which is then quantified colorimetrically. Detection limits for two different proteinases, Savinase and Alcalase, in dust collected from a high volume air sampler have been reported as low as 0.0002 and 0.0005 µg/ m^3 respectively.[16]

6.3 Elutriation

The elutriation test is used to analyse the dustiness of enzyme granulates and hence the quality of encapsulation. The test provides a measurement for use in manufacturing quality control procedures.

In the elutriation test a known mass of encapsulated material (granulate) is placed on a porous plate in a glass tube and fluidised by a flow of air from below the plate. The air passes through the bed of granulate and carries with it any dust in the original sample plus any dust generated as the grains move and collide with each other and the container walls under the influence of the air stream. The dust is then subjected to a determination for enzyme activity.

Elutriation tests are routinely performed on each batch of encapsulated proteinase by the enzyme manufacturers. The results of routine elutriation tests for proteinases now supplied by manufacturers, as determined by Unilver in their elutriation apparatus, is of the order of 100 to 200 GUs per 60g of encapsulate material, with a target of 165 GU. Most major detergent manufacturers also carry out randomised elutriation tests on receipt of enzyme granulate.

The target level of 165 GU per 60g of granulate is equivalent to between 3 and 5 x 10^{-6} per cent of granulate. This is an extremely low percentage of fine dust in granulate.

6.4 Air monitoring

Air monitoring techniques are used to measure levels of total dust and enzyme in the workplace environment. These measurements provide a good measure of where worker exposure to enzyme and total dust may be occurring and the degree of exposure. Air monitoring data also provides a background against which to assess future changes implemented to improve the control of exposure.

There are two types of samplers used in air monitoring; high volume samplers and personal samplers. Both types of samplers provide an estimate of the average amount of airborne enzyme over a sampling period.

6.4.1 High volume samplers

The most common air sampler used in detergent factories is the Galley sampler. The Galley sampler is a high volume static sampler and was developed by the detergent industry in 1969. High volume sampling ensures that sufficient dust is collected for a determination of enzyme levels.

The collection period for a Galley sampler is a minimum of 1 hour but in practice is usually 4 or 8 hours. The flow rate of the samplers varies from 500 to 850 L/ min for dust monitoring and a lower rate of approximately 300 L/ min for aerosol monitoring. At the end of this period the filter disk is weighed and the total dust concentration calculated. The collected dust is then analysed for enzyme activity. The results can be converted to airborne concentrations of proteinase (g/ m^3) by using the specific activity - that is, activity/ unit weight - of the enzyme (Table 4).

Galley samplers are usually in a fixed position and located in areas where the highest levels of exposure are likely.

6.4.2 Personal samplers

Increased sensitivity of analytical methods for proteinase have increased the feasibility of using personal samplers.[17] Personal samplers are worn by workers and measure exposure in the personal breathing-zone air. They indicate an individual's exposure to enzyme during their working day. For sufficient sensitivity, personal monitors are worn for 8 hours and usually with a flow rate of 1 to 2 L/ min. As for Galley samplers, the filter disk is weighed and total dust concentration calculated. The collected dust is then analysed for enzyme activity. To date, no personal sampler has been found suitable for adoption by the detergent industry.

7. Use

Proteinases are a group of alkaline proteolytic enzymes. The major use of these enzymes in Australia is in laundry detergents. Proteinases are currently being imported into Australia in commercial enzymatic laundry detergents which have been formulated overseas. For the manufacture of enzymatic detergents in Australia, proteinases will be imported in enzyme preparations either in granulate form for mixing in powder laundry detergents or in liquid or slurry forms for inclusion in liquid laundry detergents.

The concentration of the enzyme in the granulate, liquid and slurry is expected to be less than 5 per cent. During formulation of the laundry detergents, the enzyme granulate, liquid or slurry will be added to other detergent ingredients, at up to 1 per cent concentration. Therefore, the final concentration of enzyme protein in finished laundry detergents will be less than 0.05 per cent.

The precise volume of import of proteinases will depend on the commercial success of the enzymes in the Australian market. Initially, proteinases are expected to be included in about 10 per cent of Australian laundry detergents.

In Australia, approximately 130,000 tonnes of laundry detergents are sold each year.[18] Therefore, if the trend in the use of proteinases in Australia follows the overseas pattern then potentially 80 per cent of all laundry detergents will contain these enzymes within this decade.

The estimated total volume of import of granulate, liquid and slurry could increase from approximately 100 tonnes in the first year of manufacture to 1,000 tonnes per year within a decade. This would be equivalent to increasing the import from 5 to 50 tonnes of proteinases per year.

8. Manufacture of Proteinase-containing Detergents

8.1 Manufacturing processes for powder laundry detergents

The two major processes for the manufacture of powder laundry detergents are spray drying and dry blending.

Spray drying process

The three main steps in the spray drying process include crutching, spray drying and post addition. Crutching involves mixing detergent raw materials together to form a thick slurry. The slurry is then pumped as a stream of droplets into a spray drying tower and dried to form hollow powder beads. In the post-addition step, the spray dried beads are mixed with relatively low proportions of other dry ingredients, such as perfume and enzyme granulate, and conveyed to the packing area.

Dry blending process

Dry blending, the other major detergent powder production process, involves mixing ingredients in their dried forms in a blender or fluid bed. The enzyme granulate is added after this mixing step.

Handling of enzyme preparations

The encapsulated proteinases (granulate) are commonly transported in returnable or one way "big bags" with an inner lining, each containing 700 to 1000 kg. Upon arrival the big bags are unloaded from the trucks and transported to storage area. As needed, the bags are moved to the enzyme feed area and hoisted onto the enzyme hopper. An operator unties the outlet spout and the contents are released into the hopper. After emptying, the outlet spout is tied by the operator and the empty bag is removed.

Usually the enzyme is automatically weighed and carried in a conveyor or pipe and dosed into the detergent after the other detergent ingredients have been mixed. The finished detergent product will then be conveyed to the filling machines. The filling and sealing of the cartons is automated. Packaged detergent product is packed onto pallets and the pallets are transferred to the warehouse and stored until distribution to retail outlets.

Rejected packs of enzymatic detergent from the filling area will be collected and the detergent recycled or disposed of.

8.2 Manufacturing process for liquid detergents

Handling of enzyme preparations

The enzyme liquid and slurry are transported in large, sealed Schutz containers. Schutz containers have a rigid polyethylene inner container, an outer metal casing, a vent and a double valve safety tap for connecting to plant equipment. Upon arrival the containers are unloaded from the delivery truck and taken to a storage area. As needed, the containers are transferred to the dosing area. The container is then connected to the dosing system supply piping. A vent at the top of the container is opened to facilitate drainage of the contents.

The liquid enzyme is fed through pipes to the liquid detergent process. The enzyme may be added either batchwise to the cooled liquid detergent in an intermediate mixing tank or injected continuously into the product en route to storage tanks prior to filling. The bottles are automatically filled with detergent product and capped. The bottles are placed in cartons and transferred to the warehouse and stored until distribution to retail outlets.

9. Occupational Exposure

The major routes of exposure to proteinases are inhalational and dermal. Any activity that will cause dust or aerosol formation will increase the potential for worker exposure.

The potential for individual exposure depends on the type of work and the duration of the work activity. The major work areas and when exposure may occur are detailed below. Some workers - such as supervisors - move around a manufacturing plant and are therefore likely to enter all work areas for a limited duration.

Transport and storage

Transport and storage workers transfer both enzyme preparations and enzymatic detergents in containers.

Spillage from big bags and Schutz containers is the most significant potential source of exposure under normal transport and storage conditions. As enzyme levels are relatively low in finished enzymatic detergents, spillages from finished products would present a less significant source of exposure.

Enzyme feed area

Workers in the enzyme feed room may be exposed to the enzyme preparations, which contain 2 to 5 per cent enzyme.

Workers may be exposed to the encapsulated enzyme during the transfer of the enzyme from the big bag to the hopper. Worker exposure to the liquid enzyme may occur during the connection of the Schutz containers to pipes. Enzyme containers will usually only need replacing once per shift. The replacement process involves one operator and is usually completed within 30 minutes.

Worker exposure to enzyme could also occur in dosing the enzyme into the detergent if the dosing process is not automated and enclosed.

Filling line

Operators on the filling line work in the filling and packing areas for up to 8 hours per day. These workers may be exposed to enzymatic detergents which contain up to 0.05 per cent enzyme. The potential for worker exposure to enzyme-containing detergents will be greatest at the head of the packing machine during the filling operation. Malfunction of the filling lines may create clean-up tasks resulting in higher worker exposure.

Reclaim

Emptying the contents of damaged product containers for reclaim purposes could lead to dust or aerosol formation.

Maintenance

Exposure to the enzyme is also likely to occur during regular cleaning and maintenance of plant equipment or ventilation filters or when rectifying faults.

Clean up

Exposure may also occur during the clean up of accidental spills.

Quality assurance

Workers in quality assurance may be exposed during routine tests on enzyme preparations, such as the elutriation test.

Summary

The greatest potential for exposure to proteinases is during any work which involves the enzyme preparations; that is, liquid, slurry or granulate forms. In particular, this will include:

- connecting and emptying big bags and Schutz containers;
- routine maintenance and cleaning of the enzyme feed area, hoppers and dosing piping and conveyors; and
- clean up of any accidental spills in transporting big bags and Schutz containers, and in the enzyme feed areas.

10. Evaluation of Animal Toxicological Data

10.1 Acute Toxicity

Table 1
Summary of acute toxicity tests

Test	*Species*	*Dose**	*Outcome*
Oral	rat	0-4.44g/kg Savinase powder	LD_{50}=2.9g/kg (F) LD_{50}=3.0g/kg (M)
	rat	5g/kg ground Opticlean-M	LD_{50}>5g/kg
Inhalation toxicity	rat	0-157mg/m^3 Savinase powder	LC_{50}=130mg/m^3
	rat	0-298mg/m^3 Opticlean P	LC_{50}=229mg/m^3
	rabbit	25% Savinase powder in water (pH 7)	slight irritant
		25% Savinase powder in borate buffer (pH 9.1)	slight irritant
	rabbit	0.5g Opticlean-M	slight irritant
Eye irritation	rabbit	5% Savinase powder in water	moderate irritant
Skin sensitisation	guinea pig	Savinase Liquid SP 240	non sensitiser
	guinea pig	Opticlean-M in water	sensitiser

* *Savinase powder: powder, Novo Nordisk Bioindustrial.*

Savinase Liquid SP 240: Savinase in a mixture of propylene glycol and water, Novo Nordisk Bioindustrial.

Opticlean P: powder, Solvay Biosciences.

Opticlean-M: enzyme granulate, Solvay Biosciences.

10.1.1 Oral toxicity

A. Rat; Savinase powder [19]

Savinase powder, 15.4 AU/ g, 20 g suspended in 80 ml water, was administered by gavage to groups of 10 male and 10 female Wistar rats at doses of 0, 1.48, 1.6, 2.0, 2.65, 3.65, 4.0 and 4.44 g/ kg. The animals were observed for 14 days. Gross pathology was performed at necropsy. Individual animal data were not provided. All deaths occurred within the first 24 hours after treatment. No treatment-related histopathological changes were observed. LD_{50} values calculated were 2.9 g/ kg for female rats and 3.0 g/ kg (or 46 AU/ kg) for male rats.

Under the conditions of the study, Savinase powder had low acute oral toxicity in rats. These results are consistent with the enzyme being degraded at low pH, such as pH 2 of the stomach.

B. Rat; Opticlean-M [20]

This study was carried out according to the OECD *Guidelines for Testing of Chemicals Number 401*.

Opticlean-M encapsulated granules (proteinase activity of 990000 GU/ g) were finely ground and suspended in 1 per cent methylcellulose solution at 50 per cent w/ v. This preparation was administered by gavage to Sprague-Dawley rats (5/ sex) at a dose of 5 g/ kg bodyweight. The animals were observed for 14 days after dosing. There were no deaths. The only clinical sign was piloerection which was observed in all rats shortly after dosing and was no longer evident on day two. Macroscopic examination at necropsy did not reveal any adverse effects. The oral LD_{50} was found to be >5 g/ kg bodyweight.

Under the conditions of the study, Opticlean-M was found to be of low acute oral toxicity.

10.1.2 Inhalation toxicity

A. Rat; Savinase powder [21]

In an inhalation study, groups of 7 male and 7 female rats were snout only exposed to Savinase powder at doses of 0, 58, 70, 132 and 157 mg/ m^3 for 4 hours. The rats were observed for 14 days and weighed immediately prior to exposure (day 0), the day following exposure (day 1) and on days 3, 7, 10 and 14 post-exposure. The surviving animals were sacrificed at the end of 14 days. All animals, whether they died during the study or sacrificed at the end, were subjected to macroscopic examination of the abdominal, thoracic and cranial cavities. The lungs were weighed and the lung to bodyweight ratio was calculated for each animal.

Eighteen animals died during the study, all within 24 hours of exposure, including one animal in the 70 mg group, six in the 132 mg group and eleven in the 157 mg group. In the 157 mg group, 5 of the 11 animals which died did so during exposure. Most treated animals had rapid and shallow respiration and blood around their snouts during exposure.

Bodyweight and food consumption were lower on day 1 following exposure in the treated animals. However, bodyweight gain and food consumption had returned to

normal by day three.

Macroscopic examination of the lungs of all animals which died during the study revealed massive haemorrhage, congestion and oedema. The lung to bodyweight ratios for these animal were high, mean value 2.0, when compared with the controls, mean value 0.39. This was considered to be a result of the massive haemorrhage and oedema together with congestion in the lungs.

Brown areas on the lungs of surviving treated animals killed at the end of the 14 days were considered to be due to accumulation of haemosiderin pigment within lung alveolar macrophages and the accumulation of these macrophages and pigment near the surface. These animals had slightly higher lung to bodyweight ratios when compared to the controls.

Estimation of the particle size distribution for each treated group showed that between 65 and 83 per cent of the enzyme powder was below 5.5µm diameter, and therefore respirable.

LC_{50} value calculated for the particulate aerosol generated from Savinase powder was 130 mg/ m^3, indicating Savinase has high acute inhalational toxicity. The adverse effects observed in this inhalational study are consistent with the proteolytic activity of the enzyme; that is, breakdown of the lung tissue.

B. Rat; Opticlean P [22]

Groups of 5 female and 5 male Wistar albino rats were exposed by snout-only to Opticlean P Conc. (a proteinase powder) at doses of 0, 108, 196 and 298 mg/ m^3 for 4 hours. The rats were observed for 14 days. The powder was ground before packing into the dust generator. All rats were weighed daily and food and water consumed per cage of rats was also measured daily. The surviving animals were sacrificed at the end of 14 days. All animals, whether they died during the study or sacrificed at the end were subjected to macroscopic examination. The lungs were weighed and the lung to bodyweight ratio was calculated for each animal.

During the study, 10 animals died. One animal in the 196 mg/ m^3 dose group died on day 1 - that is, the day after exposure. In the 298 mg/ m^3 dose group, 2 animals died during exposure and 6 animals died on day 1. Exaggerated respiratory movements were seen in most animals exposed to the test material, but the behaviour of all surviving rats had returned to normal by day 7. Brown staining was observed around the snout, jaws and urogenital areas of some of the animals in each of the treatment group, but all animals were normal by day 10.

Bodyweight gain was lower in the treated animals up to 2 days following exposure and food and water consumption was lower for up to 3 days.

Macroscopic examination of all animals which died during the study revealed congestion of the lungs and the presence of frothy fluid in the trachea. The lung to bodyweight ratios were high for all animals that died as a result of exposure and slightly higher for most rats that survived exposure when compared to the controls. This was due to a high lung weight considered to be a result of congestion in the lungs. Grey areas seen on the lungs of some of the surviving treated animals were believed to be treatment related.

Estimation of the particle size distribution for each treated group showed that between 70 and 82 per cent of the enzyme powder was below 5.5µm diameter.

LC_{50} value calculated for the particulate aerosol generated from Opticlean P was 229 mg/ m^3, indicating Opticlean powder has high acute inhalational toxicity. The adverse effects observed in this inhalational study are consistent with the proteolytic activity of the enzyme; that is, breakdown of the lung tissue.

10.1.3 Skin irritation

A. Rabbit; Savinase powder [23]

Two groups of 6 New Zealand white rabbits were used to assess the skin irritancy of a single dermal application of Savinase. One group of rabbits was tested with Savinase as a 25 per cent w/ v aqueous solution (pH 7.0) and the other group was tested with Savinase as a 25 per cent solution in Sorensen's borate buffer (pH 9.1). The test solution (0.5 ml) was applied on a patch to both intact and abraded skin on the clipped dorsal and flank areas. After patch application the trunk was covered with an occlusive wrap for 24 hours. The skin reaction was assessed immediately after removal of the wrap (24 hours post application) and at 72 hours post application. The skin reactions were scored according to Draize.[24]

Similar skin reactions were noted for abraded and intact skin and for Savinase solutions at different pHs (7.0 and 9.1). All animals showed mild to moderate erythema at 24 hours in intact and abraded skin, and at 72 hours only 5 animals showed very slight erythema. Very slight oedema was noted in 2 animals at 24 hours but was not present at 72 hours.

Under the conditions of this study, Savinase powder was considered to be slightly irritating to rabbit skin.

B. Rabbit; Opticlean-M [25]

This study was carried out according to the OECD *Guideline for Testing of Chemicals Number 404*.

Opticlean-M (0.5 g, 990000 GU/ g) was moistened with 0.5 ml distilled water and applied under a semi-occlusive bandage to the clipped dorso-lumbar region of each of three male New Zealand white rabbits. The bandage was removed after 4 hours. The site was washed and scored for oedema and erythema. The animals were observed for 4 days. There was very slight erythema in all three animals on day 1 only.

Under the conditions of this study, Opticlean-M was considered to be very slightly irritating to rabbit skin.

10.1.4 Eye irritation

A. Rabbit Savinase powder [26]

Eye irritancy was measured by instilling 0.1 ml of a 5 per cent w/ v solution of Savinase powder into the conjunctival sac of the left eye of 8 Danish Landstrain albino female rabbits. The untreated eye of each rabbit served as a control. Savinase powder, with an enzyme activity of 30.1 KNPU/ g, was prepared as a 5 per cent w/ v concentration in water. The treated eyes of 5 rabbits were washed with tap water 5 minutes post application, and the treated eyes of the other 3 rabbits were washed 24 hours post application. The eyes were examined at 1, 24, 48 and 72 hours, and 7 days post application. The eyes were also tested with fluorescein dye at 1 (only the eyes washed at 5 minutes), 24, and 72 hours and at 7 days, and the reactions were graded.

Of the 5 treated eyes washed at 5 minutes, two had corneal opacity and slight chemosis and redness of the conjunctivae and 2 had slight chemosis and redness, at 1 hour. At 24 hours only one eye had slight redness of the conjunctivae and all eyes were free of reaction at 48 hours.

Of the 3 treated eyes washed at 24 hours, one eye was slightly red at 1 hour, one eye had signs of corneal opacity and slight redness up to 48 hours, and the third eye had signs of corneal opacity to 24 hours, oedema up to 72 hours and slight redness up to 7 days. Washing the treated eyes appeared to assist the eye to return to normal.

The results of this study indicate that Savinase powder is a moderate eye irritant in rabbits at the concentration tested.

10.1.5 Skin sensitisation

A. Guinea pig; Savinase liquid SP 240 [27]

A Buehler test for skin sensitisation was carried out using Dunkin Hartley female guinea pigs. This study was carried out to determine the allergenic potential of a Savinase in a liquid detergent base.

Preliminary study

A dose finding test, using closed patch application and 2 animals for each test material, determined the maximum non-irritant concentrations.

Savinase powder, concentrate PBCT 005, has an enzyme activity of 76 KNPU/ g. Savinase Liquid SP 240 is a liquid product containing Savinase enzyme in propylene glycol, with a 9.0 KNPU/ g enzyme activity.

Induction and challenge study

In the induction phase, a patch wetted with 0.5 ml of one of the test solutions was placed on the shaved back of each animal and covered with an occlusive wrap. The test solutions included 5 per cent Savinase SP 240 in water, 0.5 per cent Savinase SP 240 in dilute detergent, 80 per cent glycol in water and 5 per cent formalin in water as a positive control. The patch was removed at 6 hours. This application was repeated 3 times per week for 3 weeks. A control group of 30 animals were left untreated in this phase.

Two weeks after the final induction both flanks of each animal were shaved. A challenge patch was applied to each flank, one patch with the same test solution as the induction and the other flank with one other test solution. A test solution of 2 per cent Savinase powder in water was also used at challenge. The 30 control animals were treated with the test solutions. The patches were kept in place under an occlusive wrap for 6 hours. Challenge sites were evaluated 24 and 48 hours after removal of the patches.

Positive sensitisation responses were observed in 7/10 of the animals positive control group. All other test animals did not show positive sensitisation responses or signs of irritation. One animal died during the study.

Under the conditions of the study, Savinase was considered not to be a skin sensitiser in guinea pigs.

B. Guinea pig; Opticlean-M [28]

A delayed contact hypersensitivity test was carried out according to the OECD *Guideline for Testing of Chemicals Number 406*.

A preliminary study was carried out to select Opticlean-M concentrations to be used in the main induction and challenge study. A 10 per cent induction concentration, to produce a slight irritant effect, and a 5 per cent non-irritant concentration for the challenge phase, for Opticlean-M, were selected.

In the induction phase, a gauze patch wetted with 0.5 ml of 10 per cent Opticlean-M in water was placed on the clipped backs of 10 female Hartley Dunkin albino guinea pigs and covered with an occlusive wrap. The patch was removed at 6 hours and skin reactions were assessed 24 hours later. This application was repeated 3 times per week for 3 weeks. A control group of 10 animals were similarly treated with water only.

Two weeks after the final induction the opposite flank of each animal was clipped. A challenge patch with 0.5 ml of 5 per cent Opticlean-M was applied to both control and test animals under an occlusive wrap for 6 hours. Challenge sites were evaluated 24, 48 hours and 72 hours after removal of the patch.

All animals induced and challenged with Opticlean-M showed well-defined erythema up to 72 hours post-challenge. Slight oedema was observed in most of these animals post-challenge. Only slight, localised erythema was noted in three control animals when challenged with Opticlean-M. Therefore, Opticlean-M elicited a more marked and persistent dermal reaction in the test guinea pigs compared to the controls.

Under the conditions of the study, Opticlean-M produced delayed contact hypersensitivity in guinea pigs.

10.2 Repeated-dose toxicity

A. Rat; Savinase powder [29]

A short-term, repeated-dose study with Savinase was conducted in rats. Groups of SPF Wistar rats (10 males and 10 females) received doses of 0, 170, 490 and 1210 mg of Savinase per day. The Savinase powder, enzyme strength of 15.4 Anson Units/ g, was suspended in 10 ml of water and administered by gavage daily for 28 days. The 1210 mg dose is equivalent to 18.7 AU/ kg bodyweight.

The rats disliked the taste and smell of the enzyme suspension and in the first days of the test tried to avoid the gavage. One animal in the 1210 mg group was accidentally rubbed with enzyme suspension causing irritation and hair loss. The animal recovered within 18 days. Five animals died as a result of enzyme aspiration. One of these animals, which died in the early days of treatment, was replaced with a new rat of the same age and weight. During the study, one animal was sacrificed due to dehydration and diarrhoea on day 4 and was replaced with a new rat.

Bodyweight was measured weekly and food and water intake were recorded twice weekly. Bodyweight gain was significantly reduced for both sexes receiving 1210 mg and for females receiving 490 mg. Food intake per gram bodyweight gain (food utilisation) was reduced for both sexes receiving 1210 and 490 mg. In males, increasing water intake was found with increasing dose of the enzyme. A 24 hour urine specimen was collected for urine analysis on one of the ultimate days of treatment. Males administered 1210 mg had a slightly increased urine volume but no change in the specific gravity was noted.

Samples of blood were taken for haematology analysis from each animal on day 0 before dosing and on day 29. At the end of the study, haemoglobin was significantly decreased for males and females in the 1210 and 490 mg groups and females in the 170 mg group. Group mean values of lymphocytes were reduced in the 1210 mg dose group and in all female dose groups. All animals were sacrificed on day 30 and blood samples were collected from each animal for measurement of biochemical parameters. Group mean values of serum urea (BUN) for both sexes in the 1210 and 490 mg groups were increased two-fold when compared to control values.

At necropsy, gross pathology and organ weight analysis were carried out. A slightly mottled liver was seen in most of the females in the 1210 and 490 mg groups and in one male in the 170 mg group. Organ weight changes did not indicate any adverse effects.

Histology was carried out on the organs of only the 1210 mg group and the controls. Iron pigments were observed in liver, kidney, spleen and lung of test animals as well as in controls and therefore considered to be without significance.

Adverse effects were observed at and above 490 mg (7.6 Anson Units) per kg seen as significant decreases in weight gain, food utilisation and blood haemoglobin concentration. At 170 mg (2.6 Anson Units) per kg Savinase was well tolerated.

B. Rat; Opticlean P [30]

This study was carried out according to the OECD *Guidelines for Testing of Chemicals Number 407*.

Opticlean-P dissolved in water was given by gavage to Charles River CD rats (5/ sex/ dose) at a dose level of 62.5, 250, or 1000 mg/ kg/ day for 28 days. Animals in the control group received distilled water.

All animals survived the treatment period. Clinical signs were a hunched posture and ungroomed appearance with a greasy and brown stained fur in all the high dose animals in the first week of treatment. There was a slight decrease in the rate of bodyweight gain in all the high dose animals. Food consumption was similar in all groups. Haematology conducted during week 4 showed a decrease in the white blood cell count (WBC) which consisted of a decrease in neutrophils and lymphocytes. The decrease in WBC was observed in both sexes and was dose-dependent in the females. However, the decrease was statistically significant only in the high dose males. Clinical chemistry showed a slight increase in albumin (A) levels in the mid dose and high dose males and a decrease in globulin (G) levels in all treated males. Consequently, there was an increase in the A/ G ratio. There was also a dose-dependent decrease in phosphorus levels in the males. An increase in cholesterol levels was observed in the mid dose and high dose males and females. At necropsy there was an increase in absolute liver weight in the high dose males and a dose-dependent increase in absolute kidney weight in the males. In contrast, in the females absolute liver and kidney weights were lower than control values in all treated groups. There were no treatment related adverse macroscopic or histological findings.

10.3 Genotoxicity

Table 2
Summary of mutagenicity

Test	*Species*	*Proteinase*	*Dose*	*Outcome*
Reverse Mutation	*S. typhim*.	Savinase powder	33-3300 µg/plate	negative
		Opticlean P	15-1500 µg/plate	negative
In vivo Chromosome Aberration	Chinese Hamster	Savinase	60-1500 mg/kg	negative
Dominant Lethal	Mouse	Savinase	100-1000 mg/kg	negative

10.3.1 *Salmonella typhimurium* reverse mutation assay

A. Savinase powder [31]

Savinase at concentrations of 33, 100, 333, 1000, 3,300 and 10,000 µg was tested for gene mutation using *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98, both in the presence and absence of microsomal enzymes (S9 mix).

Savinase powder (SP 88), 15.4 AU/ g, was dissolved in water. The positive control for this study was 2-aminoanthracene dissolved in dimethylsulphoxide. Water was used as the vehicle control. When compared to the vehicle control, Savinase at the concentrations tested did not produce any statistically significant dose-response increase in the number of revertant colonies. On the other hand, the positive controls showed marked increases.

In *Salmonella typhimurium*, Savinase was not mutagenic under the test conditions reported.

B. Opticlean-P [32]

Opticlean-P dissolved in water at concentrations of 15, 50, 150, 500, and 1500 µg/ plate was tested for gene mutation using Salmonella typhimurium strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98, both in the presence and absence of microsomal enzymes (S9 mix). The positive controls for this study were 2-aminoanthracene, N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, and 2-nitrofluorene dissolved in dimethylsulphoxide. Water was used as the vehicle control. When compared to the vehicle control, Opticlean-P at the concentrations tested did not produce any statistically significant dose-response increase in the number of revertant colonies. On the other hand, the positive controls showed marked increases.

In *Salmonella typhimurium*, Opticlean-P was not mutagenic under the test conditions reported.

10.3.2 *In vivo* Chromosome Aberration Assay

A. Savinase powder [33]

Preliminary toxicity test

Groups of 5 male Chinese hamsters were administered by gavage Savinase (SP 88) at dosages of 750, 1000 and 1500 mg/ day for five days. During treatment one animal in each group died as a result of gavage error. Bone marrow cells in the 1500 mg group were examined and cell division was not significantly depressed.

Main cytogenetic study

Savinase was administered by gavage at 0, 60, 300 and 1500 mg/ kg concentrations to groups of 5 male hamsters for five days. Savinase powder was prepared as a suspension in water and given at a dose volume of 10 ml/ kg bodyweight. Cyclophosphamide, the positive control, was administered on two consecutive days at 55 mg/ kg/ day. Animals were weighed and observed daily during the study. Three hours after the last treatment cell division was arrested by administration of colchicine (4 mg/ kg) by intraperitoneal injection. Animals were sacrificed three hours after colchicine administration. Bone marrow cells were collected from each animal for chromosome aberration analysis.

When this dosing program was first implemented, four animals given 1500 mg and one in each of the other Savinase treated groups died during treatment. The study was repeated with additional animals being included in the 1500 mg and 300 mg groups to ensure sufficient animal survival during treatment. Two animals in the 1500 mg group died. Five surviving animals from each group were used for bone marrow collection and analysis. Bodyweight gain was not affected by Savinase treatment and no adverse clinical signs were observed during the study.

The percentage of metaphases with one or more aberrations was similar for control, 1500 mg group and 60 mg group. The percentage was slightly increased in the 300 mg group. In contrast, cyclophosphamide induced significant chromosomal damage, predominantly as single chromatid breaks or gaps.

Under the conditions of this study, Savinase did not increase the damage to chromosomal structure in bone marrow cells in Chinese hamsters.

10.3.3 Dominant lethal study in mice

A. Savinase powder [34]

In this study, groups of 10 male CD^{-1} mice received 0, 100 or 1000 mg/ kg/ day Savinase by oral gavage for five consecutive days. Savinase powder (SP 88), 15.4 AU/ g, was prepared daily in distilled water. Trimethyl phosphate (1.0 per cent in water) was used as the positive control in 10 mice. The males were mated weekly thereafter with 3 female mice each time for eight weeks. The males were sacrificed at the end of the mating period and their reproductive tracts examined. Bodyweights of male mice were recorded daily during the dosing period and weekly thereafter. The females from each batch of weekly matings were allocated into one of three groups and killed on either day 4, 9 or 18 of gestation, and their reproductive tracts were examined. Females were examined on days 1, 7 and 14 post-coitum and all females that appeared not to be pregnant were killed approximately 18 days after pairing and their reproductive tracts were examined.

No deaths occurred during the study and no adverse clinical signs due to Savinase treatment were observed. Bodyweight gain in Savinase treated males was slightly greater than that of controls. Mating performance of the males was comparable in all groups. Slight differences in conception rate and fertility in female mice showed no consistent relationship with Savinase treatment. The conception rate and fertility were significantly decreased in the positive controls in week 1 of mating. Male reproductive organ weights were similar in all groups.

The rate and normality of zygote development in the Savinase treated groups was similar to the control group. In contrast, the positive controls treated with Trimethyl phosphate showed a significant decrease in the rate of zygote development during the first week of mating, with a corresponding increase in the number of abnormal zygotes. Pre-implantation and post-implantation losses in Savinase treated females killed on days 9 and 18 post coitum were within normal limits. A significant increase in post-implantation losses was observed in the positive control group in the second week of mating. The number of viable foetuses was similar in all groups except for the positive control group in which there were no viable foetuses resulting from the first mating. The number of foetal abnormalities were similar in all groups and no effects were attributable to treatment with Savinase or trimethyl phosphate.

Under the conditions of the study, Savinase did not result in dominant lethal mutations in the sperm cells of male mice.

10.4 Respiratory sensitisation

A number of animal studies - in guinea pigs and monkeys - have been conducted to evaluate the effects of proteinase dust and aerosol on the respiratory system.[35+37] These studies have specifically focused on the clinical effects and the immunological responses to inhalational exposure of animals to proteinase enzymes.

In these inhalation studies:

- immunological responses were detected in animals exposed to very low

concentrations of proteinases, such as 0.0083 mg/ m^3; [37]

- not all animals exposed to the same concentration of enzyme demonstrated an immunological response;
- antibody titers were measurable at levels of exposure below which clinical signs were observable; and
- a concentration-related antibody response was observed.

10.5 Overall assessment of animal toxicological data

Proteinases have low acute oral toxicity but high acute inhalational toxicity and cause slight skin irritation and moderate eye irritation. The animal inhalation toxicity studies were done on enzyme powder, or in some cases crushed granules. As the powdered enzyme has a much greater proportion of respirable dust than the enzyme granulate, the inhalation toxicity results present a worst case result. No skin sensitisation was observed in guinea pigs exposed to an enzyme-containing liquid but skin sensitisation was noted in guinea pigs exposed to crushed granules containing proteinase. Evidence of skin sensitisation in animals is therefore equivocal. Two 28 day repeated-dose gavage studies in rats using two different powder detergents showed no specific target organ toxicity. Proteinases tested negative in *in vitro* bacterial mutation assay and *in vivo* mammalian genotoxicity studies.

Respiratory sensitisation to proteinases has been documented in guinea pigs and monkeys.

11. Human Health Effects

11.1 Health effects

Soon after the introduction of proteinases in detergent powders it was recognised that proteinases caused skin irritation[1+4] and allergic respiratory reactions.[1, 2, 5, 6, 7]

Respiratory sensitisation is noted as an increased responsiveness of the respiratory system upon inhalation of a respiratory sensitiser. Once this increased responsiveness has developed, any further exposure to the respiratory sensitisers, even at much lower levels, may cause symptoms. Sensitisation is indicated by positive reactions in immunological tests, such as skin prick testing and ELISA or RAST blood tests. Individuals found by immunological tests as being sensitised will not necessarily develop respiratory symptoms on exposure. Care needs to be taken when reading and interpreting case reports in the literature with regard to the terms 'sensitisation' and 'respiratory sensitisation'.

During the 1960s - the first years in which enzymatic detergents were manufactured - proteinases were used as dusty, fine powders and workers were exposed to very high levels of enzyme dust. This high level of exposure led to enzyme-induced respiratory diseases, known as respiratory sensitisation.

Respiratory sensitisation was usually identified with the onset of overt symptoms involving the upper and lower respiratory tract and occurred after repeated exposure over a period of several days, weeks or more frequently a month or more. Reported symptoms included sneezing, nasal congestion and increased nasal secretions, sore throat, tightness in the throat or chest, wheezing and shortness of breath. Respiratory sensitisation was often referred to as "occupational-induced asthma" or "enzyme-induced asthma". Severity of symptoms were usually related to the degree of exposure and generally lasted for a few hours to several days although certain clinical effects have been reported to persist much longer.[9, 38] Adverse health effects were particularly prevalent in detergent workers exposed to high enzyme dust levels.

Most reports indicated complete recovery of lung function on cessation of exposure, although one report suggested that there may be a longer term loss of function, such as loss of pulmonary elastic recoil.[9]

One study has shown that respiratory symptoms were present in some individuals who had negative skin prick tests to proteinases.[7] This could have been a result of irritant respiratory reactions rather than allergic respiratory reactions occurring upon exposure to high levels of enzymes.

Atopy is a pre-existing constitutional propensity for allergic conditions. Atopy is diagnosed as a personal or family history of asthma, skin problems, hay fever and/ or a positive skin prick test to common allergens.

Earlier studies, especially those of employees exposed to very high levels of enzyme, indicated that atopic individuals have a greater risk of becoming immunologically sensitised.[10, 11, 39, 40] The studies also demonstrated that the majority of sensitised individuals do not develop respiratory symptoms. These studies and many others have shown that non-atopics can also become sensitised to proteinases.

Two studies make particular mention of atopy interacting with people's exposure to enzyme dust.[10, 11] In one study it was found that over 80 per cent of those who originally worked in the industry before 1969 and who were atopic, became positively sensitised on skin prick testing to the enzyme dust, compared with a level of 40 per cent in those who were non-atopic.[10] Both studies showed that even though a greater percentage of atopics become sensitised, a significant percentage of non-atopics also become sensitised.

These UK studies[10, 11] were large studies, longitudinal in nature and therefore theoretically have greater power to pick up significant differences in highly and less exposed employees over a long period of time. The studies concluded that with the control of atmospheric enzyme dust levels in the workplace the incidence of enzyme-induced respiratory symptoms had dropped dramatically. Flood et al.[11] concluded that such a low occurrence of respiratory disease related to enzyme dust compares more than favourably with the level of disease in other industries which use common occupational allergens.

Reports in the literature have shown a decline in the percentage of detergent workers becoming immunologically sensitised and a great reduction in the number of workers developing respiratory symptoms as working conditions improved. [10, 11, 41] For example, in the 1970s it was estimated that in the United States of America 1,000 (25 per cent) of 4,000 workers exposed to detergent enzymes may have developed clinical respiratory sensitisation.[42] However, by 1985 the number of estimated cases had decreased to 40, or 1 per cent of exposed workers. Positive immunological tests in detergent workers have continued to be reported through the 1980s, but the majority of cases have been asymptomatic.[12, 13, 38, 43]

A more recent and cross sectional study[12] was only able to review those employees who were still present in the detergent industry (a survivor effect). In this study, 3 of the 12 employees working with enzyme materials in a dry bleach plant became sensitised to proteinases as demonstrated by positive RAST results. A health questionnaire survey showed that the frequency of respiratory symptoms was similar in exposed and non-exposed groups. This was a much smaller study than the UK studies and cross sectional in nature, and had inherent methodological limitations. However, it does demonstrate that it is possible for workers to become sensitised to enzymes in a working environment with supposedly good dust control technology in place and low levels of airborne enzymes and which uses encapsulated enzymes. Details of the dust controls measures in the factory were not provided. Methods for encapsulation of enzymes has improved since this study was undertaken.

Skin irritation was also noted in workers exposed to high concentrations of enzyme powders during the late 1960s.[1, 3, 4, 44, 45] The incidence of skin irritation amongst workers was not nearly as widespread compared with incidence of respiratory symptoms. Proteinases appear to act as primary skin irritants. The data indicates that skin irritation is more likely to develop at high enzyme concentrations, when in prolonged contact with enzymes, in moist conditions and when abrasion is present.

There is no evidence that proteinases cause skin sensitisation. Studies have shown that skin sensitisation does not occur.[3, 45] Evaluation of primary data, summarised below, also supports this.

11.2 Human Toxicity Test Data

Skin irritation, Savinase powder [46]

The skin irritancy of Savinase was assessed in a group of ten humans (4 females and 6 males). A 1 per cent solution of Savinase powder suspended in water was applied once daily for 24 hours for 10 days to the volar forearms of the subjects. The material was held in place under an occlusive wrap and the application sites were examined when the applications were renewed. There were no signs of erythema or oedema. The chemical was considered to be a non-irritant to the skin of humans under the conditions of this study.

Skin sensitisation, Savinase liquid SP 240 [47]

A human repeat insult patch test was carried out on 22 volunteers using Savinase Liquid SP 240, which contained Savinase in a mixture of propylene glycol and water. The enzyme activity of the liquid product was 9.0 KNPU/ g. A Savinase powder with activity of 76 KNPU/ g was suspended in water and used at rechallenge in one subject.

Preliminary study

A dose finding test was performed with four volunteers exposed to SP 240 at concentrations of 2.5, 14, 25, 36, 47, 58, 69, and 80 per cent (v/ v in water). The test material was applied on two parallel patches, as described in the main study, to the left upper arm of each volunteer. All volunteers reported uncomfortable burning sensations and 3 out of 4 removed the patches before the end of the 24 hours exposure period. The reactions were scored at 48 and 96 hours post application. No reactions were seen at 2.5 per cent. However, most patches were removed early. At the higher concentrations vesiculation was often present with oedema spreading outside the patch area. The highest concentration of SP 240 was selected as 2.0 per cent because the induction patches were to remain in place for 48 hours in the main study.

Induction and challenge study

At the first induction, SP 240 at concentrations of 0.5, 1.0, 1.5, and 2.0 per cent was applied to small pads on adhesive tape. The tape was applied (not occluded) for 48 hours to the dorsal surface of the upper arm of 22 volunteers. Skin reactions were assessed at 24 or 48 hours after patch removal. Due to the unacceptable level of irritation at these concentrations on the first application, further induction concentrations were at 0.1, 0.2, 0.3 and 0.5 per cent, and the patches were applied to a new site. The total number of induction applications was five in 15 days. The degree of irritation increased with the concentration of Savinase liquid.

Twenty days after the application of the final induction, challenge patches at 0.1, 0.2, 0.3 and 0.5 per cent concentrations were applied to both arms of each subject, one at the original site and one at a new site. The patches were held in place for 47 hours and the reactions assessed 48 and 96 hours post application. Irritant reactions at challenge were similar to those seen during induction. A rechallenge was conducted in one subject because the reactions were papular at all concentrations, indicative of possible sensitisation. This subject was rechallenged with 0.3 per cent and 0.1 per cent SP 240, 0.24 per cent Propylene glycol and 0.036 per cent Savinase powder. The concentrations of Propylene glycol and 0.036 per cent Savinase powder were equivalent to that which is present in 0.3 per cent SP 240. The patches were held in place for 48 hours and examined at 55 and 96 hours post application. Signs of sensitisation were not present.

Under the conditions of the study, Savinase Liquid SP 240 was not a skin sensitiser in humans.

11.3 Diagnosis of sensitisation

Skin tests, such as prick or scratch tests, are considered to be a reliable method of identifying sensitised individuals.[8] Sensitisation has also been demonstrated by immunological tests, such as enzyme-specific IgE Radioallergosorbent Tests (RAST) on blood.[6, 10, 43] More recently ELISA assays has been used to measure antibodies and have been reported to be more sensitive than RAST.[13] However, as mentioned previously a diagnosis of sensitisation does not necessarily mean clinical signs of respiratory sensitisation will follow.

11.4 Diagnosis of respiratory sensitisation

Pulmonary function tests often reveal impairment of flow rates and include one second Forced Expiratory Volume (FEV_1) and Forced Vital Capacity (FVC) measured during the symptomatic phase of the condition.[8] Chest x-rays are not an effective diagnostic tool as they have been shown to have a limited role in evaluating workers with possible occupational asthma.

12. Assessment of Occupational Health and Safety

12.1 Toxicity

Human experience has shown that exposure to proteinases can lead to respiratory sensitisation and, to a lesser extent, skin irritation. Similar effects have also been observed in animals.

Proteinases can degrade skin by proteolytic action. Data show that skin irritation in humans occur only at high enzyme concentrations, with prolonged enzyme contact and under moist conditions.

There are no data to suggest that proteinases are human skin sensitisers. As the enzymes have a large molecular weight they are unlikely to cross the skin and are therefore unlikely to come in contact with immune system through dermal exposure.

Proteinases are known human respiratory sensitisers.

Characteristics of respiratory sensitisation include:

- Only some exposed individuals become sensitised.
- Symptoms do not occur on first exposure.
- The risk of sensitisation appears to be related to concentration inhaled.
- Sensitisation is believed to be irreversible.
- Sensitised individuals may never develop symptoms.
- Symptoms usually disappear when the individual is removed from the sensitiser.
- Once a person is sensitised, further exposure may lead to symptoms at very low concentrations.

These characteristics of respiratory sensitisers highlight the importance of preventing worker exposure to proteinases.

While detergent workers have been shown to be sensitised to proteinases, very few workers sensitised to proteinases develop respiratory symptoms.

Data indicate that atopic individuals may be at a greater risk of becoming sensitised to proteinases. However, the data does not demonstrate that atopics are more likely to develop respiratory symptoms. Non-atopics can also become sensitised to proteinases. Therefore, a blanket exclusion of atopics from work with enzymatic detergents cannot be justified.

As proteinases are irritants and respiratory sensitisers, inhalational and dermal exposure to these enzymes should be avoided.

Encapsulation of proteinases reduces the hazardous nature of the enzymes. The mean particle size of the enzyme granules - 500 to 700 μm - is well above the inspirable and respirable particle size. Inspirable particles are those that are small enough to be inhaled - that is, <185 μm - and thereby enter the respiratory tract. The inspirable fraction may further be divided into 'respirable' and 'non-respirable' fractions. The respirable fraction is composed of the very fine particles - that is, <7 μm - which are able to reach the lower bronchioles and alveolar regions of the lungs. The non-respirable fraction is deposited in the upper respiratory tract, including the nose, pharynx and larynx. As proteinases are potent sensitisers, particles containing enzyme should not be inspirable and, more importantly, should not be respirable.

Elutriation test results indicate that the amount of fine dust, and therefore inspirable enzyme, in granulates is extremely low. However, mechanical damage to the granules could release smaller particles containing enzyme.

Proteinases, as human respiratory sensitisers, are classified as hazardous substances against National Occupational Health and Safety Commission's *Guidance Note for Determining and Classifying a Hazardous Substance*.[48] Proteinases are also classified as hazardous substances because they have an exposure standard set by the National Occupational Health and Safety Commission (NOHSC). As defined in the *Guidance Note for Determining and Classifying a Hazardous Substance*, if a sensitiser is included in a mixture above the concentration cut-off of 1 per cent then the mixture is considered to be a hazardous substance. As enzyme preparations - that is, granulate, liquid and slurry forms of the enzymes - contain up to 5 per cent of enzyme, they are above this concentration cut-off and are therefore classified as hazardous substances..

The information required for the labelling of hazardous substances is detailed in the National Commission's *Guidance Note for the Labelling of Workplace Substances*.[49] The label on a hazardous substance should draw the attention of an employee who is handling or using the substance to the significant hazards involved. Therefore, enzyme preparations should be labelled with the following phrases:

- **Risk Phrase 42**
  - May cause sensitisation by inhalation.
- **Safety Phrase 38**
  - In case of insufficient ventilation, wear suitable respiratory equipment.

Detergents contain a maximum of 0.05 per cent of proteinases and are therefore not classified as hazardous. Detergent manufacturers could increase the concentration of enzyme above 0.05 per cent in future products, especially as the market for concentrated detergent products is increasing. However, it appears very unlikely that the concentration will increase 20-fold and thereby exceed the 1 per cent concentration cut-off for a hazardous substance.

12.2 Occupational exposure

The most likely routes of worker exposure to proteinases are inhalational and dermal. Hence, any activity that leads to dust or aerosol formation will increase the potential for worker exposure.

During the manufacture of enzymatic detergents the highest levels of exposure are most likely to occur in work involving enzyme preparations; that is, transport, filling head, enzyme dosing, maintenance and clean up of enzyme spills. The areas for enzymatic detergent filling and reclaim, and wherever spills occur, present a lesser but significant exposure source.

As proteinases are respiratory sensitisers, worker exposure to proteinases must be minimised.

Some of the circumstances which could increase worker exposure to proteinases include:

- open processes, such as mixing and conveying;
- leakages in pipes containing liquid enzyme under pressure;
- sweeping up powder spills;
- using high pressure water to clean up liquid spills;
- damage to granulate, such as treading on granules or driving over granules in a forklift truck;
- reclaiming enzymatic detergents;
- excessive or rough handling of granulate;
- opening vessels - such as storage or mixing vessels - which are not under negative pressure;
- breakdowns in the filling line where enzymatic detergents are exposed; and
- malfunctions in the manufacturing process of equipment which handle enzyme preparations or enzymatic detergents and which require maintenance.

These circumstances should be avoided or minimised by engineering controls and safe work practices. Personal protective equipment should be worn if there is the potential for greater exposure, such as for maintenance work.

Although the toxicities of other enzymes used in laundry detergents have not been assessed in this report, the control measures outlined in this report should also be appropriate for controlling exposure to these enzymes.

12.3 The United Kingdom experience

12.3.1 History

Enzymatic detergents were first manufactured in large quantities in the United Kingdom in 1968. As in other countries people soon realised that the handling of the fine enzyme powder was causing occupational asthma. In response to this problem the United Kingdom Soap and Detergent Industry Association (SDIA) set up the Standing Committee on Enzymatic Washing Products in 1969. The Committee published in 1973 recommendations for the maintenance of safe operating conditions in a factory handling enzymatic detergents. A sub-committee of the SDIA made recommendations for the medical supervision of workers in the enzymatic detergent industry. The guidelines were adopted by many United Kingdom detergent companies.

Since these first guidelines were published, the detergent manufacturers have been supplied with both encapsulated enzyme and liquid enzyme forms. Also, extensive monitoring of atmospheric total dust and enzymes and health surveillance of employees has been carried out. The changes to the physical form and the information collected on occupational exposure were taken into account by the SDIA's *Revised Operating Guidelines* which were published in June 1991 [50] and the most recent SDIA Medical Recommendations of May 1992. [51] The purpose of the SDIA guidelines is to detail safe operating conditions, define how to determine the effectiveness of the controls and recommend how to monitor the health aspects of exposure.

The UK occupational exposure standard (OES) is 0.06 µg/ m^3 for pure crystalline subtilisin in air, which is equivalent to 1.92 GU/ m^3. Members of the SDIA have agreed on an occupational exposure guideline (OEG) of 1 GU/ m^3, half the current UK OES. The industry have also set a working target level of 0.5 GU/ m^3 to ensure that the OEG is rarely exceeded. The occupational exposure standard for subtilisins in Australia is also 0.06 µg/ m^3. [52]

Since the implementation of the SDIA guidelines in United Kingdom detergent factories, there have been very few reports of occupational asthma.

The SDIA provided some brief data on air monitoring in several factories. L&K:Rexona supplied air monitoring and health surveillance data on one United Kingdom detergent factory. The data highlight the fact that with the adoption of SDIA guidelines enzyme levels can be maintained at and well below the exposure standard and occupational asthma can be controlled.

12.3.2 SDIA air monitoring data

The SDIA has collated monitoring data from several detergent manufacturing factories in England for 1991/92. The monitoring data is provided at Appendix 1. The data includes results on atmospheric enzyme and total dust levels in six powder detergent manufacturing factories and enzyme levels in air were also measured in two liquid detergent factories. The SDIA working target level for atmospheric enzyme levels is 0.5 GU/ m^3 and was exceeded in less than 2 per cent of the readings. The highest monthly average for all factories was well below 0.5 GU/ m^3. The atmospheric dust levels exceeded 1000 µg/ m^3 in only a few percent of the measurements.

The levels of atmospheric enzymes were similar in powder and liquid factories. The data indicate that enzyme levels are usually kept well below the working target of 0.5 GU/ m^3.

12.3.3 L&K:Rexona monitoring data

L&K: Rexona supplied atmospheric monitoring data and health surveillance data from one of their United Kingdom powder detergent plants. This plant reintroduced proteinases in 1984. SDIA operating guidelines and medical recommendations were implemented at this time.

The air monitoring data, for the years 1985 to 1991, includes the monthly average total dust levels and enzyme activity of the air samples. Sampling stations were located at the enzyme dosing point, packing room and waste recovery area. The target monitoring value for enzymes was < 0.5 GU/ m^3 in all areas, and for total dust was < 500 µg/ m^3 for the packing room and < 1000 µg/ m^3 for all other areas.

The air monitoring data show that the enzyme levels in the three areas of the factory were maintained well below the maximum target of 0.5 GU/ m^3, which is four-fold less than the Australian occupational exposure standard. Of 15,000 samples, none were above 0.5 GU/ m^3. The average monthly values for enzyme activity were above 0.05 GU/ m^3 in only 9 out of 84 results, demonstrating how low all the measurements were.

Data for the total dust levels is summarised at Table 3.

The total dust levels were greatest in the enzyme dosing and waste recovery rooms, with levels often exceeding the maximum target of 1000 µg/ m^3. The enzyme dosing and product reclaim rooms were areas where potentially exposure to higher levels of total dust could have occurred. However, these rooms were enclosed, access restricted and safety procedures followed, as detailed by the SDIA guidelines. Total dust in the packing area, where less strict guidelines apply, was well controlled.

Table 3
Total dust levels

Room	*Target Level (µg/m³)*	*Number of Samples*	*Samples >1000 µg/m³*	*Samples >500µg/m³*
Enzyme dosing	<1000	1,982	12%	50%
Packing	<500	11,425	0.2%	3.2%
Waste recovery	<1000	2,214	4.6%	17%

Health monitoring was carried out according to the medical recommendations of the SDIA. During health monitoring of the workforce from 1982 to 1991 no worker was identified as having developed occupational asthma to enzymes.

The monitoring data from this factory demonstrates that by implementing and adhering to control measures, such as the SDIA guidelines, enzyme levels can be controlled at levels well below 0.06 µg/ m^3 - the Australian exposure standard. It also demonstrates that occupational asthma in the workforce can be controlled.

As proteinases are respiratory sensitisers, worker exposure to proteinases must be minimised.

12.4 Assessment of control measures

Overseas detergent factories have implemented many measures to control worker exposure to proteinases. For example, in the United Kingdom the SDIA guidelines provide detailed information on the measures necessary to control exposure and have been adopted by many detergent manufacturers. As the data in section 12.3 demonstrates, adoption of the SDIA guidelines has successfully controlled the atmospheric levels of proteinases and therefore worker exposure in UK detergent factories. The value of agreed industry guidelines is recognised as important in achieving safe and healthy workplaces.

Industry best practice will also be an important way to reduce the level of worker exposure. For example, fewer spills and improved practices to reduce product reclaim will reduce the number of workers who would need to carry out higher risk clean up and recovery operations.

Potential manufacturers of enzymatic detergents in Australia have indicated in their information dosiers that they would be following similar control measures in their detergent factories.

These measures, such as encapsulation of enzymes, engineering controls, safe work practices and personal protective equipment include the following:

Encapsulation of enzymes

Use of good quality encapsulates.

Engineering controls

Enclosure of:

- enzyme feed and detergent reclaim areas; and
- enzyme handling equipment - such as storage vessels and filling heads - and conveyors of enzyme preparations and enzymatic detergents.

Ventilation:

- General ventilation of the factory.
- Dilution ventilation of the enzyme feed room and powder reclaim room. Dilution ventilation usually ensures approximately 10 changes of air per hour.
- Local exhaust extraction of the enzyme hopper, the equipment used for reclaiming detergents, the filling head and enzyme handling equipment, such as mixers and conveyors. Local extraction is usually at a velocity of 1 m/ sec.

In the powder detergent process extracted air from the enzyme feed room is ventilated to a dedicated high efficiency wet scrubber or high efficiency filter. Dust is removed from the wet scrubbers with the assistance of cold water and the water is then heated to deactivate the enzyme before emptying and prior to maintenance work. Extracted air from the areas involving detergent powders is exhausted to centralised dust collectors, as excessive foaming can occur if wet scrubbers are used.

In the liquid detergent process, extracted air is ducted to a coalescing filter. The

enzyme is removed from the filter with the assistance of cold water and the water is then heated to deactivate the enzyme before emptying.

Automation

Automation of as much of the detergent manufacturing process as possible, and in particular enzyme dosing.

Safe work practices

- Restricted access to enzyme feed room.
- Storage of enzyme preparations in a designated area.
- Addition of enzymes into the detergent process at the latest possible stage and with minimal mixing.
- Immediate clean up of spills.
- Vacuuming or removal by gentle shovelling of enzyme granulate or enzymatic powder detergent spills.
- Clean up of liquid spills containing enzymes using low pressure water.
- Provision of accessible washing facilities, especially near enzyme feed rooms.
- Placement of inner big bag liners into a dedicated bin in the enzyme feed room and later removal for disposal by a licensed operator or contractor.
- Recycling of big bags and Schutz containers.
- Good personal hygiene practices.

Personal protective equipment

To be worn by:

- workers replacing big bags or when connecting enzyme liquid containers to pipes;
- maintenance workers;
- workers cleaning up enzyme spills; and
- workers cleaning up major spills of enzymatic detergents.

Personal protective equipment includes overalls, gloves, safety glasses and a P1 respirator.

Experience, air monitoring data and health surveillance data can be used to assess the effectiveness of the measures taken to control occupational exposure.

Overseas experience has shown that control measures, such as those described above, can effectively control worker exposure to proteinases. The United Kingdom SDIA

guidelines provide details on how occupational exposure has been controlled in the United Kingdom for many years.

12.5 Encapsulation

Encapsulation has been one of the measures taken to control the level of exposure to proteinases. In the first years of manufacturing enzymatic laundry detergents, proteinases were added as fine powders. To prevent the release of fine enzyme dusts manufacturers of enzymes began encapsulating proteinases. Early encapsulation involved binding the enzymes to the surface of sodium tripolyphosphate particles and spraying with a tacky non-ionic detergent.

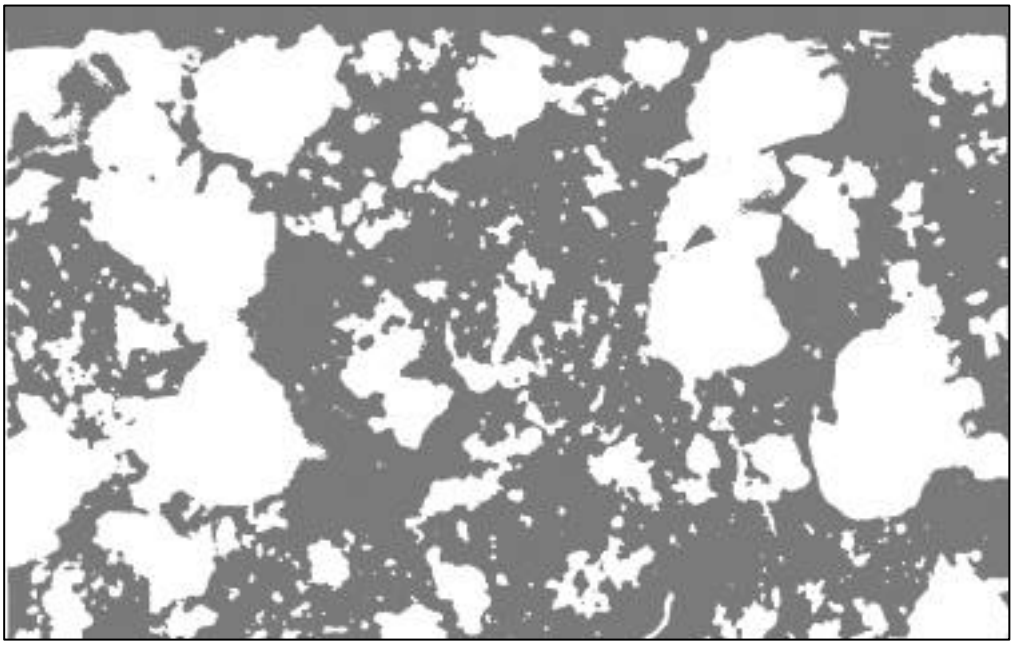
In recent years the quality of encapsulation has greatly improved, such as the introduction of tougher protective coatings for granules. Current encapsulates consist of enzyme embedded in an internal core which is coated with an inert layer. The mean particle size of enzyme granules is 500 to 700 µm. This particle size is well above the respirable (< 7 µm) and inspirable (< 185 µm) sizes. This indicates that granules are very unlikely to be taken into either the upper or lower respiratory tract.

Current encapsulated enzymes are generally supplied as granulates which produce total fine enzyme dust emissions of between 100 to 200 GU under standard Unilever elutriation test conditions. Enzyme manufacturers generally aim to supply encapsulated enzyme with a maximum of 165 GU. The results of the elutriation test can be converted to the amount of enzyme dust in an encapsulated sample using the specific activities of proteinases.

The maximum target of 165 GU is approximately 3 to 5×10^{-6} per cent of enzyme in granulate and is equivalent to 3 to 5 µg enzyme/ 100g of granulate. This is an extremely low level of dustiness and is indicative of good quality encapsulation. This order of enzyme dust is well below that for the first encapsulates used in the early 1970s and many orders of magnitude less than measurements for the original enzyme powders.

Photograph 1

View showing a collection of encapsulated enzyme granules with thick outer layers. Note the smooth, clean, dust free surface of the 'as supplied' particles. In this view, the rectangular grain in the top right field has a full scale size of approximately 1 mm x 0.5 mm.



Photograph 2

Enzyme and granule fragments left after a few grains were crushed between two glass slides (scale as for photograph 1).

As noted earlier, one study [12] reported that 3 out of 12 workers were found to be RAST positive in a dry bleach factory using encapsulated proteinases. The exposed workers

did not exhibit any more respiratory symptoms compared to non-exposed workers. This study demonstrates that it is possible for employees to become sensitised in workplaces which use encapsulated enzymes. Since this 1985 study was undertaken encapsulation properties of proteinases have improved, such as the introduction of tougher protective coatings. The report states that the factory used a dust-control engineering system but no details of the system were provided. Workpractices were also not detailed. However, it was noted that the encapsulate was added during the detergent blending stage, a practice not recommended by this report. Mixing could lead to damage of the granules, thereby releasing small particles containing enzymes. The study suggests that good quality encapsulated material and careful handling are important steps in controlling worker exposure.

An independent assessment of the integrity of current encapsulation of proteinase enzymes was carried out and the findings are provided at Appendix 2. The findings of this assessment showed that encapsulated enzymes are coated in an off-white outer layer below which the active enzyme is embedded in an yellow-brown core. The beads are irregularly shaped with approximately 0.1 to 1mm length along the major axis, as shown in Photograph 1. The majority of the beads have a smooth, clean dust free surface. However, some granules have a thin outer layer through which the core material can be seen. Visual examination and preliminary tests indicate that encapsulated enzyme in the 'as supplied' condition has extremely low fine dust content.

However, the assessment found that the surface coating can be readily removed by brushing or rubbing with a moist cloth or sponge. On testing under harsh conditions, the internal core material was found to be quite friable and when crushed it shattered into many small and medium sized particles, as shown in Photograph 2. These smaller particles could be inspirable and present more of a hazard.

Enzyme manufacturers state that the elutriation test aims to simulate the effects of attrition on the granules. However, the test does not simulate the potentially harsher in-plant granulate attrition and dust generation conditions. The test should therefore only be used as a method for assessing the quality of encapsulation and not as an indication of the effects of handling conditions.

The major dust hazard relating to the use of encapsulated enzymes will therefore be associated with events in which the individual grains may be damaged or worn as a result of mechanical action. Such events would include; accidental spills, machinery failures or maintenance operations, and disposal of used enzyme containers.

In summary, encapsulation is one tool for controlling worker exposure to proteinases. Intact granules are not inspirable and granulate in the 'as supplied' condition contains very low amounts of respirable enzyme. However, as the core material is friable, damage to the granules could result in the release of enzyme particles. Normal handling and storage conditions should not cause such damage to the granules.

Encapsulation should not be seen as the only measure necessary for the control of occupational exposure but as an essential part of an overall control strategy. It would therefore be good practice if industry implemented good plant design and enforced housekeeping strategies in order to eliminate the possibility of granulate damage.

Only good quality encapsulates should be used. The elutriation test provides a measure of the quality of encapsulation and is routinely carried out by enzyme manufactures on each enzyme batch and the results recorded. It would be further good practice if detergent manufacturers were provided with the results of the

elutriation tests to check the quality of encapsulation before use. To ensure that the granules are arriving undamaged at the detergent factories, detergent manufacturers could carry out further elutriation tests on randomised batches of granulate.

12.6 Air monitoring

Air monitoring of total dust and enzyme levels provides a good measure of where exposure may be occurring and a background against which to assess future control improvements. In detergent factories, monitoring is usually carried out with high volume samplers, in particular Galley samplers. However, the use of personal samplers is becoming more feasible.

A number of studies have compared the monitoring results from Galley and personal samplers and found that personal samplers produce higher results.[12,17,53] Bruce et al. [53] concluded that the higher results were due to inaccuracies arising from either the small sample size or due to a dilution effect. In contrast, both Agarwal[17] and Liss[12] see the higher results as a true reflection of worker exposure.

Both Galley samplers and personal samplers provide an estimate of the average amount of airborne enzyme over a sampling period and do not provide data on peak enzyme levels. Galley samplers are not located in the breathing zone of the employees and do not account for the movement of workers nor for any occasional acute high exposure which is limited to a small area. Galley samplers provide measurements in a lesser time frame due to their greater sampling volume. While personal samplers may provide a more accurate measurement for individual exposure, this sampling should be performed in conjunction with high volume sampling - such as Galley sampling - until their accuracy is validated and their appropriateness determined. At present, Galley samplers are the preferred sampler used by the detergent industry.

Enzyme concentrations are measured and reported as proteolytic activity per cubic metre of air. To convert the exposure standard from enzyme weight to enzyme activity, the specific activity of the proteinase must be used. The exposure standard for various commercial proteinases has been converted to units of enzyme activity/ m^3 and the results are provided in Table 4.

Table 4
Conversion of the exposure standard (0.06 μg/m³) to enzyme activity/m³.

Alcalase	3.4×10^{-6} AU/m³	(2.5 GU/m³)
Durazym	17×10^{-6} DPU/m³	(1.9 GU/m³)
Esperase	16×10^{-3} NPU/m³	(3.95 GU/m³)
Kazusase	26.4×10^{-2} PU/m³	(4.15 GU/m³)
Opticlean	0.79 DU/m³	(1.92 GU/m³)
Optimase	0.79 DU/m³	(1.92 GU/m³)
Savinase	24×10^{-3} NPU/m³	(5.94 GU/m³)

12.7 Exposure standard

NOHSC has adopted an exposure standard for pure crystalline subtilisins, which includes proteinases, of 0.06μg/ m³. [52] This exposure standard was not based on the health effects of proteinases.

The same exposure standard (0.06 μg/ m³) is given in the American Conference of Governmental Industrial Hygienist's Threshold Limit Values and the United Kingdom's Occupational Exposure Standards.

The Australian Exposure Standard represents a maximum or peak concentration to which workers may be exposed to atmospheric proteinases. According to the National Commission's *Exposure Standards for Atmospheric Contaminants in the Occupational Environment*, '...compliance with these peak limitation exposure standards should be determined over the shortest analytically practicable period of time, but under no circumstances should a single determination exceed 15 minutes'. However, measuring for proteinases for 15 minutes or less is at present not possible. For a Galley sampler - the most common sampler - the shortest practical time for monitoring is 2 to 4 hours, and is usually 8 hours in practice. This presents a problem in ensuring compliance with the exposure standard. It is recommended that the NOHSC give consideration to reviewing the peak limitation exposure standard and replacing the peak value with a time weighted average value. Such a revision would facilitate compliance with the exposure standard.

As proteinases are sensitisers, the goal should be to maintain the lowest level of airborne enzymes. To reflect peak enzyme levels the shortest time practicable for monitoring enzyme levels should be used.

In practice it is possible to maintain the levels of airborne enzyme levels well below the exposure standard in well controlled workplaces. This has been shown to be the case in a L&K:Rexona factory in the United Kingdom (Section 12.3.3) where the enzyme levels were always maintained four-fold less than the exposure standard and, in the majority of measurements, forty-fold less than the exposure standard. This level of control prevented enzyme-induced occupational asthma occurring in workers.

A study by Liss[12] demonstrated that sensitisation, as determined by immunological tests, occurred in 3 of 12 workers exposed to encapsulated proteinases (Esperase) in a dry bleach factory. Air sampling for proteinases was carried out using high volume samplers. The highest recorded level of airborne enzyme was 1.57 µg/ m^3. The occupational exposure standard of 0.06 µg/ m^3 for pure crystalline subtilisin is equivalent to 3.9 µg/ m^3 for Esperase. The study demonstrates that it is possible for workers to become sensitised in an environment where proteinase levels are maintained at less than half of the exposure standard. A health questionnaire survey showed that the frequency of respiratory symptoms was similar in exposed and non-exposed workers.

Developments in analytical method - specifically much lower limits of detection - since the present exposure standard value was established now mean that a lower exposure standard is technically feasible. Overseas data has shown that atmospheric levels of proteinases can be maintained well below the exposure standard and that sensitisation can occur in individuals working in an environment where the level of airborne proteinase was maintained at less than half of the exposure standard. Accordingly, it is also recommended that NOHSC give consideration to reducing the exposure standard. Consideration could be given to developing monitoring methods which allow measurement of peak enzyme levels.

12.8 Health surveillance

The effects of occupational exposure can be monitored by health surveillance. Health surveillance for proteinases includes assessing the development of clinical allergic disease and the determination of the sensitisation status in workers in the detergent industry.[51,17]

The determination of the development of clinical symptoms of occupational asthma involves taking the patient's history, physical examination and assessment of lung function - such as using a Vitalograph or other equivalent spirometer to estimate FEV 1.0 and FVC. Immunological tests, including skin prick tests, RAST and ELISA, can be used to assess the sensitisation status of the subjects. Chest x-rays should only be carried out where there has been a positive clinical test, as they have been shown to have a limited role in evaluating workers with possible occupational asthma.

Australia is in a unique situation in being able to implement health surveillance in all detergent factories before enzymes are introduced into the workplace. This should include:

- taking a detailed history;
- completing a questionnaire (including smoking habits);
- a physical examination; and
- lung function test for each employee who will be working with enzymes or enzymatic detergents.

The history should include; allergic, atopic and chronic chest conditions, and medication. This information should be used as a baseline and a means of comparison for identifying the development of occupational asthma.

A routine health review for each employee should be regularly carried out. This

review should include a history since the previous examination and re-assessment of lung function. Immunological tests should be performed at the discretion of the medical adviser.

Pre-placement medical examinations, together with taking a medical history, are often recommended in order to identify workers at greater risk of becoming sensitised, such as atopics and those with chronic chest disease(s). The SDIA Medical Recommendations [51] state that on the basis of the pre-placement results the medical adviser will inform management as to whether atopic individuals should work with enzyme products.

The association between atopy and the development of respiratory diseases has not been clearly defined. The association between atopy and sensitisation has been shown, however the majority of sensitised individuals do not develop respiratory symptoms. Non-atopics have also been shown to become sensitised to proteinases. In Australia, there is a high percentage - 15 to 25 per cent - of atopics in the general population. The data does not support a blanket exclusion of atopics from working with enzymatic materials. Atopics should be informed that there is a greater risk of them becoming sensitised if exposure should occur and what are the implications of becoming sensitised.

The details above are generic requirements for health surveillance. It is recommended that the NOHSC give consideration to including proteinases in the schedule of substances requiring health surveillance required under the National Commission's *Control of Workplace Hazardous Substances: National Model Regulations.* Detailed consideration also needs to be given to defining a specific health surveillance program for proteinases.

It is also recommended that the development of guidelines for occupational physicians be undertaken. These guidelines should include a clear statement of the importance of early diagnoses, a definition of occupational asthma and management of such cases, and appropriate use of the specified health surveillance program.

13. Public Health Assessment

13.1 Assessment of Toxicological Hazards

In humans, proteinases are known respiratory sensitisers and potential skin and eye irritants at high concentrations.

There have been a limited number of published papers on the public health aspects of enzymatic laundry detergents. Two large studies indicated that the addition of enzymes to laundry powders did not increase the incidence of primary irritation among users.[5, 54] Two small studies indicated that early domestic use of these products was associated with dermatitis.[55, 56] As dermatitis may also occur in some individuals when using non-enzymatic detergents[57], it is difficult to identify which factor(s) causes the dermatitis. Exposure of consumers, including infants, to fabrics washed in the enzyme containing detergents or presoak agents did not increase the risk of primary skin irritation.[5, 58]

Enzymatic detergents do not appear to increase the potential for skin sensitisation or respiratory sensitisation in consumers, including atopic individuals.[5, 6, 59]

Recently, an air propelled aerosol (spray) stain remover containing proteinase was introduced to the Australian domestic market. The product, NEON, is water based and contains 0.007 per cent proteinase. Data provided on the product show that the smallest droplet size is 13.4 µm and therefore the spray has no respirable fraction. Company laboratory tests indicate that when NEON is sprayed on to a fabric from a distance of 150 mm, 98.5 per cent of the expelled product is deposited on the fabric. Therefore, due to its manner of use and large droplet size the spray, is unlikely to be inhaled under normal conditions of use.

13.2 Assessment of Public Exposure

Proteinases will be included in domestic laundry detergents, powders and liquids, at a maximum concentration of 0.05 per cent by weight. Amounts of proteinases imported into Australia could increase from 5 tonnes in the first year to 50 tonnes per year. Depending on the acceptability of laundry products containing proteinases, public exposure to the proteinases may be widespread but only at very low levels.

Accidental spillage during transport of the imported enzymes or spillage of damaged detergent packages would add little to public exposure. The enzyme is biodegradable and discharge of enzyme containing detergents in effluents to the municipal water is unlikely to present adverse effects to human health.

The results of an in-home study published in 1970 indicated that very low levels of dust were generated from laundry products containing an 'agglomerated enzyme complex'.[60] A maximum of only 0.2 per cent of the dust was in the respirable range (< 5 µm). It was estimated that consumers were exposed to 0.0113 µg enzyme (1.5 AU Equivalents) per week during laundering. Since this study was carried out, improved encapsulation of the enzyme has greatly reduced the level of dustiness of enzymes. Therefore, the level of consumer exposure to proteinases using today's enzymatic detergent powders is expected to be much lower than the levels measured in the

study.

Data provided for NEON, a spray stain remover, indicate that proteinases are present at low concentrations and inhalational exposure to proteinases in the product under normal conditions of use will be very low. Therefore, this spray product is unlikely to present a hazard to public health when used correctly. However, any spray presents a potential inhalational hazard if misused. As proteinases are respiratory sensitisers, it is recommended that an appropriate warning, such as;

'CAUTION: AVOID BREATHING SPRAY',

should be included on the label of spray laundry products containing proteinases to ensure that the spray is not inhaled.

Therefore, due to low level of exposure under normal use conditions, proteinases in laundry products are unlikely to pose a significant health and safety hazard to the public.

14. Environmental Assessment

14.1 Environmental exposure

Proteinases will be formulated into powder and liquid laundry detergents at a number of manufacturing sites located in Sydney, Melbourne and Brisbane. It is envisaged that formulation wastes will amount to approximately 30 g/ day proteinases at each site. This waste is heat treated to denature the protein before release to the effluent with an approximate denatured enzyme concentration of less than 1 ppm. Empty import containers are heat treated and either recycled or disposed of to landfill.

All proteinases used in laundry detergents will ultimately be released to the aquatic environment via domestic discharge of used washing solution into the sewerage system.

Biodegradation

Biodegradation was observed when Savinase was tested in activated sludge using the closed bottle manometric respirometry test according to EEC Director 79/ 831. These values are 20 per cent after 5 days, 38 per cent after 15 days and 41 per cent after 22 days, and are within the limits observed for readily biodegradable compounds.[61] Further, these results were supported by monitoring the loss of dissolved organic carbon throughout the test period (85 per cent after 22 days) which, according to the above guidelines, is indicative of ready biodegradabiltiy.

Bioaccumulation

Although no studies were provided, bioaccumulation of the enzyme is not expected given its high water solubility, high molecular weight and high biodegradation potential.

14.2 Environmental effects

The acute aquatic toxicity (semi-static) study of Savinase was conducted according to the Danish National Testing Board and OECD TG-203 guidelines using zebra fish, Brachydanio rerio, as the test species with the following results.

Savinase	zebra fish	LC_{50} (96h) = 200-400mg L^{-1}
Savinase (90˚C inactivated)	zebra fish	LC_{50} (96h) >1000mg L^{-1}

This study indicates that Savinase is practically non-toxic to fish, while Savinase which has been inactivated by 90˚C heat treatment is considered still less toxic to the test species.

14.3 Environmental hazard

Proteinases are unlikely to present a hazard to the environment at any stage of their use, whether it be when reformulated into laundry detergents - resulting in an estimated 30 g/ day per site of inactivated reformulation waste disposed of to sewer - or when consumers drain the laundry detergent from washing machines to the sewer.

A predicted environmental concentration for proteinases in sewage water throughout Australia can be estimated from the following assumptions: 50 tonne maximum annual use, an Australian population of 17 million and daily per capita water usage volume of 150L. This provides a predicted environmental concentration of 0.05 ppm in sewage water which would be swiftly reduced to insignificant levels (likely to be in the low ppb range) by biodegradation in sewerage treatment plants and dilution in rivers, lakes and oceans which act as receiving waters to nearly all treatment plants in Australia. The likely environmental concentrations of proteinases are at least 3 orders of magnitude lower than acute aquatic toxicity levels.

Proteinases are not expected to exhibit ecotoxic characteristics because they have a high molecular weight, high biodegradation potential and high water solubility and, as such, are not likely to be bioavailable. As proteinases are unlikely to present a risk to the environment, there are no specific recommendations for control in this area.

15. Conclusion

From the assessment of the hazards of proteinases, overseas experience, SDIA guidelines, air monitoring data and health surveillance data we have concluded that proteinases can be used safely in Australia if the appropriate control measures are implemented and adhered to.

16. Recommendations

Proteolytic enzymes used in the detergent industry, such as Savinase, fall into the generic category known as proteinases. Proteinases are the fermentation product of a number of differing bacterial strains and these products have been given different common and trade names. However, the properties and health effects of proteinases are similar. This assessment report has considered proteinases used in laundry detergents.

Proteinases, which include Savinase, are proven human respiratory sensitisers and are classified as hazardous substances against the National Commission's *Guidance Note for Determining and Classifying a Hazardous Substance*.[48] As hazardous substances employers should be aware of their obligations to provide information, eg MSDS and labels, about the hazards of proteinases and to assess and control the risks to health. Further details of these obligations, consistent with employers general duty of care, are provided in the National Commission's *Control of Workplace Hazardous Substances: National Model Regulations and National Code of Practice*.[62]

As all States and Territories have made a commitment to enact uniform regulations consistent with the national model regulations in 1993, employers should read the recommendations of this report in conjunction with the obligations set out in these regulations.

It is not possible to identify a minimum concentration at which exposure to proteinases does not cause sensitisation. There is evidence that once a person has become sensitised they are more likely to develop symptoms at concentrations far below levels typically seen as hazardous. However, experience has shown that in well controlled environments adverse health effects such as respiratory symptoms are unlikely to develop in sensitised individuals. Exposure to sensitisers, such as proteinases, must be minimised.

16.1 Safe Use

The availability of operating guidelines developed overseas, for example by the Soap and Detergent Industry Association, and work being completed in Australia by the NSW Workcover Authority, such as the *Code of Practice for Safe Handling and Storage of Enzymatic Detergent Powders and Liquids*, together with the findings and recommendations of this assessment report provide a sound basis by which proteinases can be used safely by the Australian detergent industry.

16.2 Code of Practice

It is recognised that there is a need for a Code of Practice which addresses the use of proteinases in detergent manufacturing processes in Australia. It is recommended therefore that consideration be given by the NOHSC to develop a Code of Practice for proteinases to give specific guidance to employers in this area. Because difficulties in providing control for proteinases are shared with other sensitisers the need for a generic Code of Practice for sensitisers which would encompass proteinases as well as other occupational sensitisers, such as isocyanates, is further recommended.

16.3 Exposure Standard

It is recommended that the NOHSC give consideration to reviewing the peak limitation exposure standard and replacing the peak value with a time weighted average value. Such a revision would facilitate compliance with the exposure standard.

It is also recommended that NOHSC review the current exposure standard of 0.06 $\mu g/m^3$, with a view to reducing the standard based on considerations such as achievable levels of atmospheric proteinases and levels at which sensitisation has occurred.

16.4 Health Surveillance

Workers involved in the manufacture of enzyme-containing detergents should undergo health surveillance.

A pre-placement medical check should be carried out and used as a base line to assist in identifying future signs of occupational asthma. Atopics should be informed of their increased risk of becoming sensitised and what the implications are. The data do not support a blanket exclusion of atopics from working with enzyme-containing materials.

Workers who experience any asthma-like symptoms, skin or eye irritation after exposure to the enzyme should inform their employer and consult a doctor.

It is recommended that NOHSC give consideration to including proteinases in the schedule of substances requiring health surveillance required under the National Commission's *Model Regulations to Control Workplace Hazardous Substances*.[62] While this report has identified some generic requirements for health surveillance, detailed consideration needs to be given to defining a specific health surveillance program. Development of guidelines for occupational physicians which include a clear statement of the importance of early diagnoses, a definition of occupational asthma, and management of such cases and appropriate use of the specified health surveillance program is also recommended.

16.5 Spray Laundry Products

Proteinases are sold to the public in spray laundry products. It is recommended that an appropriate warning, such as;

'CAUTION: AVOID BREATHING SPRAY',

should be included on the label of spray laundry products containing proteinases to ensure that the spray is not inhaled.

It is also recommended that the droplet size of spray laundry products containing proteinases should not be in the respirable range, that is $<7 \mu m$.

16.6 Control of Occupational Exposure

To minimise worker exposure to proteinases, detergent manufacturers should aim to control atmospheric proteinases at the lowest practicable level. The level should not exceed the current exposure standard. Experience has shown that levels can be maintained well below this standard.

To minimise and monitor worker exposure to proteinases the following guidelines and precautions should be observed.

16.6.1 Encapsulation

Good quality encapsulate and careful handling of the granulates are necessary in order to avoid the generation and dispersion of enzyme dust.

Detergent manufacturers should obtain a copy of the elutriation test results for each batch of enzymes to ensure that they are using good quality encapsulated material.

Batches of enzyme should be randomly checked by the detergent manufacturer using the elutriation test to ascertain whether damage to the encapsulate is occurring during transportation.

16.6.2 Engineering Controls

The detergent manufacturing process should be as automated as possible.

In the liquid detergent plant gravity feed should be used where possible to reduce pressure in the system.

Enclosure

The enzyme feed area should be enclosed.

The reclaim area should be enclosed.

Enzyme handling equipment, which includes conveyors, storage tanks and mixing tanks should be enclosed.

The filling machine should be enclosed.

Any pump and pipe flanges carrying enzymatic detergents should be encased.

Ventilation

Ventilation should ensure that the level of airborne enzyme does not exceed the exposure standard, which is currently 0.06 µg/ m^3.

General ventilation of the plant is recommended.

For powder and liquid processes the enzyme feed room and powder reclaim room should also be under general ventilation.

Local ventilation is required at specific points of the process.

a) For the powder process:

- enclosed conveyors carrying the enzyme and the dosed product, the filling machine, and the operation for powder reclaim should be under a local exhaust system connected to an efficient dust collecting unit; and

- detergent powder dust should not be exhausted to a wet scrubber as excessive foaming may occur and this reduces the efficiency of the unit.

b) For the liquid process:

- all storage and mixing tanks, and filling machines should be under a local exhaust system connected to an efficient coalescing filter.

All ventilation equipment should be regularly checked and maintained.

16.6.3 Safe Practices

When working with enzymes or enzyme-containing detergents workers should avoid the generation of dust clouds and aerosols.

Industry best practice should be introduced or maintained to reduce the level of worker exposure. For example, fewer spills and improved practices to reduce product reclaim will reduce the number of workers who would need to carry out these higher risk operations.

The dosing of enzymes should occur after the major detergent ingredients have been mixed, as late in the process as possible and with minimal mixing.

Enzyme preparations, ie granulate, liquid and slurry, should be stored in a designated area.

Good personal hygiene practices should be implemented.

Equipment and surrounding area should be cleaned prior to any major maintenance work.

All spills should be cleaned up promptly, to reduce the spread of enzyme and limit mechanical damage to the encapsulated material.

Adequate ventilation should be provided during clean up of spills.

Do not sweep, brush or use air blowing or high pressure water jets to clean up spills.

Clean all floors and equipment by washing, gentle shovelling, vacuuming or mopping.

To avoid the generation of dust, enzyme granulate and enzymatic detergent spills should be vacuumed or gently shovelled up.

To avoid the generation of aerosols, liquid enzyme and detergent spills should be cleaned up using low pressure water.

Big bags should be returned to the suppliers of the enzymes where possible or disposed of carefully, such as by a licensed operator or contractor.

Schutz containers should be cleaned with low pressure hot water and reused if possible.

All enzyme-containing material should be denatured by using heat or hot water (> 80°C) before disposal.

Plant equipment, including ventilation equipment, should be regularly checked and maintained.

Workers involved in the manufacture of the proteinase-containing detergents should receive induction and training with respect to the safe handling of enzymes and products containing enzymes.

Operators of the enzyme feed room, maintenance and reclaim workers should receive specialised training, such as for the wearing and use of respirators.

16.6.4 Personal Protective Equipment

Workers who may come into direct contact with proteinases include:

- workers undertaking normal operations in the enzyme feed rooms, such as replacing big bags or connecting pipes to Schutz containers;
- workers cleaning and maintaining equipment, including ventilation equipment, which has contained proteinases; and
- workers who clean up enzyme spills or clean up major spills of enzymatic detergents.

These workers should:

- wear suitable personal protective equipment, including;
  - protective clothing, such as overalls, in accordance with AS 3765.1,[63]
  - impervious gloves, such as PVC or nitrile gloves, in accordance with AS 2161,[64]
  - goggles or safety glasses complying AS 1337[65] and chosen and used in accordance with AS 1336[66]; and
  - particulate respirator, Class P1 filter, which is complying with AS 1716[67] and should be used in accordance with AS 1715;[68] and
- observe good personal hygiene practices at work, such as washing after any contact and wearing clean overalls.

16.6.5 Air Monitoring

Atmospheric enzyme and total dust levels should be monitored routinely. Atmospheric monitoring provides a measure of worker exposure, identifies areas where high levels are found and provides a basis for measuring the effectiveness of control improvements.

Sampling time should be the minimum time practicable to indicate peak levels of airborne enzymes.

Air samplers should be located in the enzyme feed room, the enzyme dosing area, filling area and the reclaim room. Records of enzyme and dust levels should be kept and made accessible to employees.

Consideration should be given to developing monitoring methods which allow for the measurement of peak enzyme levels in air.

17. Requirements for Secondary Notification

Under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), secondary notification of Savinase shall be required if any of the circumstances stipulated under Subsection 64(2) of the Act arise.

In particular, a secondary notification will be required if enzyme preparations are to be manufactured in Australia.

18. References

1. Flindt, M.L.L., 'Pulmonary disease due to inhalation of derivatives of Bacillus subtilis containing proteolytic enzyme', *Lancet*, vol 1, p1177, 1969.

2. Gothe, C.J., Nilzen, A., Holmgren, A., Szamosi, A., Werner, M. and Wide, L. 'Medical Problems in the detergent industry caused by proteolytic enzymes from Bacillus subtilis', *Acta Allergologica*, vol 27, pp63+86, 1972.

3. Zachariae, H., Thomsen, K. and Rasmussen, O.G., 'Occupational enzyme dermatitis: results of patch testing with Alcalase', *Acta Dermatovener*, vol 53, p145, 1973.

4. Little, D.C. and Dolovich, J., 'Respiratory disease in industry due to B. subtilis enzyme preparations', *Canadian Medical Association Journal*, vol 108, p1120, 1973.

5. Griffith, J.F., Weaver, J.E., Whitehouse, H., Poole, R.L., Newmann, A. and Nixon, G.A., 'Safety evaluation of enzyme detergents', *Oral and Cutaneous Toxicity, Irritancy and Skin Sensitization Studies*. Federal Cosmet Toxicology, vol 7, p581, 1969.

6. Pepys, J., Hargreave, F.E., Longbottom, J.L. and Faux, J. 'Allergic reactions of the lungs to enzymes of Bacillus subtilis', *Lancet*, vol 1, p1181, 1969.

7. Mitchell, C.A. and Gandevia, B., 'Respiratory symptoms and skin reactivity in workers exposed to proteolytic enzymes in the detergent industry', *American Review of Respiratory Disease*, vol 104, p1, 1971.

8. Little, D.C., 'Allergic Disease in Detergent Workers', *Occupational Asthma*. Ed: Frazier, C.A., Van Nostrand Reinhold Company, New York, 1980.

9. Musk, A.W. and Gandevia B., 'Loss of pulmonary elastic recoil in workers formerly exposed to proteolytic enzyme (alcalase) in the detergent industry', *British Journal of Industrial Medicine*, vol 33, pp158+165, 1976.

10. Juniper, C.P, How, M.J., Goodwin, B.F.J. and Kinshott, A.K., 'Bacillus subtilis enzymes: a 7-year clinical, epidemiological and immunological study of an industrial allergen', *Journal of the Society of Occupational Medicine*, vol 27, p3, 1977.

11. Flood, D.F.S., Blofeld, R.E., Bruce, C.F., Hewitt, J.I., Juniper, C.P. and Roberts, D.M., 'Lung function, atopy, specific hypersensitivity and smoking of workers in the enzyme detergent industry over 11 years', *British Journal of Industrial Medicine*, vol 42, p43, 1985.

12. Liss, G.M., Kominsky, J.R., Gallagher, J.S., Melius, J., Brooks, S.M., and Bernstein, I.L., 'Failure of enzyme encapsulation to prevent sensitization of workers in the dry bleach industry', *Journal of Allergy and Clinical Immunology*, vol 73, pp348+355, 1984.

13. Sarlo, K., Clark, E.D., Ryan, B.S. and Bernstein, M.D., 'ELISA for human IgE antibody to Subtilisin A (Alcalase): Correlation with RAST and skin test results with occupationally exposed individuals', *Journal of Allergy and Clinical Immunology*, vol 86, pp393+9, 1990.

14. Chien, P.T., 'The development of a fluorometric method for the assay of subtilisins', *American Industrial Hygiene Association Journal*, vol 39, pp808+816, 1978.

15. Dunn, E. and Brotherton, R., 'The use of N,N-dimethyl casein in the determination of proteolytic enzymes in washing products and airborne dust samples', *Analyst*, vol 96, pp159+163, 1971.

16. Miller, L.S., Moore, V.S., Wardwell, A.L. and Smith, L.A., 'Inhibition Enzyme Immunoassay for the Detection of Airborne Detergent Enzymes Causing Occupational Allergy', *Journal of Industrial Microbiology* Suppl No. 5, pp213+219, 1990.

17. Agarwal, M.K., Ingram, J.W., Dunnette, S. and Gleich, G.J., 'Immunochemical Quantitation of an Airborne Proteolytic Enzyme - Esperase, in a Consumer Products Factory', *American Industrial Hygiene Association Journal*, vol 47, no 2, pp138+143, 1986.

18. Retail World, *Annual Report*, December 1991.

19. 'Acute Oral Toxicity of Savinase given to rats', Report No. 1978+01+03 LM+H/ PNi, *Novo Industri*, 1978. Data on file, Novo Industri, Denmark.

20. 'Acute Oral Toxicity to Rats of Opticlean-M', Huntingdon Research Centre Report No. 87634D/ KLC 111/ AC, England, 1987.

21. 'Savinase Acute Inhalation Toxicity In Rats, 4 hour LC_{50}', Report No. NV064/ 7818, Huntingdon Research Centre, England, 1978. Data on file, *Novo Industri*, Denmark.

22. 'Opticlean P Conc. Acute Inhalation Toxicity in Rats, 4 Hour Exposure', Huntingdon Research Centre Report No. KIC 119/ 871041, England, 1987.

23. 'Savinase (SP 88): Irritance to Rabbit Skin (Single Application)', LSR Report No. 77/ Ntl25/ 178, 1977. Data on file, *Novo Industri*, Denmark.

24. Draize, J.H., *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*, p47, Association of Food and Drug Officials of the United States, Austin, Texas.

25. 'Irritant Effects on Rabbit Skin of Opticlean-M', Huntingdon Research Centre Report No. 8747OD/ KIC 112/ SE, England, 1987.

26. 'Rabbit eye irritation test; Savinase enzyme activity 30.1KNPU/ g', Test Report No. 1978+12+13 RKH/ PNi, *Novo Industri*, 1978. Data on file, Novo Industri, Denmark.

27. 'Savinase SP 240 Buehler Tests for Delayed Contact Hypersensitivity', IRI Report No. 2132, Inveresk Research International, Scotland, 1981. Data on file, *Novo Industri*, Denmark.

28. 'Delayed Contact Hypersensitivity in the Guinea Pig with Opticlean-M', Huntingdon Research Centre Report No. 87876D/ KIC 114/ SS, England, 1987.

29. 'Twenty-eight day Subacute Oral Toxicity Study in Rats', Study No. 1576, *Novo Industri* A/ S, 1982. Data on file, Novo Industri, Denmark.

30. 'Twenty-eight day Oral Toxicity Study in Rats with Opticlean P+Conc', Huntingdon Research Centre Report No. KIC 120/ 871531/ ST, England, 1987.

31. 'Testing for Mutagenicity Activity in Savinase (SP 88)', IRI Project No. 408494, Inveresk Research International, Scotland, 1977. Data on file, *Novo Industri*, Denmark.

32. 'Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Opticlean-

P Conc', Huntingdon Research Centre Report No. KIC 115/ 87617, England, 1987.

33. 'Savinase (SP 88): Assessment of the Action on Bone Marrow Cell Chromosomes Following Sub-acute Oral Administration in the Chinese Hamster', LSR Report No. 77/ NTL24/ 344, Life Science Research, England, 1977. Data on file, *Novo Industri*, Denmark.

34. 'Savinase (SP 88): Examination for Dominant Lethal Action on Oral Administration to Male Mice', LSR Report No. 77/ NTL23/ 407, Life Science Research, England, 1977. Data on file, *Novo Industri*, Denmark.

35. Hillebrand, J.A., Thorne, P.S. and Karol, M.H., 'Experimental sensitization to Subtilisin', *Toxicology and Applied Pharmacology* 89, 449-456, 1987.

36. Coate, W.B., Busey, W.M., Schoenfisch, W.H., Brown, N.M. and Newmann, E.A., 'Respiratory toxicity of enzyme detergent dust'. *Toxicology and Applied Pharmacology*, vol 45, pp477+496, 1978.

37. Cashner, F., Schuyler, M., Fletcher, R., Ritz, H. and Salvaggio, J., 'Immunologic responses of Cynomolgus monkeys after repeated inhalation exposures to enzyme and enzyme-detergent mixtures', *Toxicology and Applied Pharmacology*, vol 52, pp62+68, 1980.

38. Zachariae, H., Hoegh-Thomsen, J., Witmeur, O., Wide, L., 'Detergent enzymes and occupational safety', *Allergy*, vol 36, pp513+516, 1981.

39. Newhouse, M.L., Tagg, B., Pocock, S.J., and McEwan, A.C., 'An epidemiological study of workers producing enzyme washing powders', *Lancet*, vol 1, p689, 1970.

40. Pepys, J., Wells, I.D., D'Souza, M.F., and Greenberg, M., 'Clinical and immunological responses to enzymes of *Bacillus subtilus* in factory workers and consumers', *Clinical Allergy*, vol 3, pp143-160, 1973.

41. Weill, H., Waggenspack, C., DeRouen, T., and Ziskind, M., 'Follow-up observations of workers exposed to enzyme detergents', *Annual of the New York Academy of Sciences*, vol 221, pp76+85, 1974.

42. Bernstein, I.L., 'Occupational asthma', in: Brooks, S.M., Lockley J.E., Harber, P., (Eds), *Clinics in chest medicine*. pp255+272, Philadelphia, WB Saunders Co. 1981.

43. Dor, P.J., Agarwal, M.K., Gleich, M.C., Welsh, P.W., Dunnette S.L., Adolphson C.R., and Gleich G.J., 'Detection of antibodies to proteases used in laundry detergents by the radioallergosorbent test', *Journal of Allergy and Clinical Immunology*, vol 78, pp877+86, 1986.

44. McMurrain, K.D., 'Dermatologic and pulmonary responses in the manufacturing of detergent enzyme products', *Journal of Occupational Medicine*, vol 12, pp416+420, 1970.

45. Goethe, C-J., Nilzen, A., Holmgren, A., Szamosi, A., Werner, M. and Wide, L., 'Medical problems in the detergent industry caused by proteolytic enzymes from Bacillus subtilis', *Acta Allergologica*, vol 27, pp63+86, 1972.

46. 'Skin Irritation Study in Man', Study No. 3708, 1978. Data on file, *Novo Industri*, Denmark.

47. 'Skin Sensitisation Evaluation of Savinase Liquid in a Human Volunteer Trial', IRI Report No. 2185, Inveresk Research, Scotland. Data on file, *Novo Industri*, Denmark, 1981.

48. National Occupational Health and Safety Commission, *Guidance Note for Determining and Classifying a Hazardous Substance*. Australian Government Publishing Service, Canberra, 1991.

49. National Occupational Health and Safety Commission, *Guidance Note for the Labelling of Workplace Substances*. Australian Government Publishing Service, Canberra, 1991.

50. Soap and Detergent Industry Association, *The Standing Committee on Enzymatic Washing Products, Fifth Report*. The Soap and Detergent Industry Association, June 1991.

51. Soap and Detergent Industry Association, 'Medical supervision of enzyme workers', in: *The Standing Committee on Enzymatic Washing Products, Fifth Report*. The Soap and Detergent Industry Association, November 1991.

52. National Occupational Health and Safety Commission, *Exposure Standards for Atmospheric Contaminants in the Occupational Environment*, Australian Government Publishing Service, Canberra, 1991.

53. Bruce, C.F., Dunn, E. and Brotherton, R. 'Methods of measuring biologically active enzyme dust in the environmental air of detergent factories', *Annual of Occupational Hygiene*, vol 21, pp1+20, 1978.

54. Mason Bolam R.M., Hepworth, R. and Bowerman, L.T., 'In-use evaluation of safety to skin of enzyme-containing washing products', *British Medical Journal*. vol 2, pp499+501, 1971.

55. Jensen, N.E., 'Severe Dermatitis and Biological Detergents', *British Medical Journal*. vol 1, p299, 1970.

56. Ducksbury, C.F.J. and Dave, V. K., 'Contact dermatitis in Home Helps following the use of enzyme detergents', *British Medical Journal*, vol 2, p499, 1970.

57. Adams, R.M., *Occupational Skin Disease*. Grune and Stratton, N.Y., 1983.

58. White, I.R., Lewis, J. and El Alami, A. *Possible Adverse Reactions to an Enzyme-containing Washing Powder*. Contact Dermatitis, vol 13, pp175+179, 1985.

59. Pepys, J., Mitchell, J., Hawkins, R., and Malo, J.L. 'A longitudinal study of possible allergy to enzyme detergents', *Clinical Allergy*, vol 15, pp101+115, 1985.

60. Hendricks, M.H., 'Measurement of enzyme laundry product dust levels and characteristics in consumer use', JAOCS, June: 207, 1970.

61. EEC-Directive 79/831. Annex 5. Methods for the Determination of Ecotoxicity. 5.2. Degradation. Manometric Respirometry. - DGXI/283/82, Rev.6.

62. National Occupational Health and Safety Commission, *Control of Workplace Hazardous Substances: National Model Regulations to Control Hazardous Substances and National Code of Practice to Control Workplace Hazardous Substances*. Australian Government Publishing Service, Canberra, 1991.

63. Australian Standard, AS 3765.1 - *Clothing for Protection Against Hazardous Chemicals*. Standards Australia, Sydney, 1990.

64. Australian Standard, AS 2161 - *Industrial Safety Gloves and Mittens*. Standards Australia, Sydney.

65. Australian Standard, AS 1337 - *Eye Protection for Industrial Applications*. Standards Australia, Sydney.

66. Australian Standard, AS 1336 - *Recommended Practices for Eye Protection in the Industrial Environment.* Standards Australia, Sydney.

67. Australian Standard, AS 1716 - *Respiratory Protective Devices*, Standards Australia, Sydney, 1991.

68. Australian Standard, AS 1715 - *Selection, Use and Maintenance of Respiratory Protective Devices*, Standards Australia, Sydney, 1991.

Appendix 1

SDIA Monitoring Data

Table 5
Levels of Enzymes in Atmosphere (GU/m³)

Factory		*Number of Readings*	*Overall Average*	*Highest Monthly Average*	*% Individual Readings Above Limit*
A+	1990	2659	0.050	0.070	0.9
	1991 (3/4)	1480	0.049	0.099	1.3
B+	1990	2536	0.023	0.033	-
	1991 (3/4)	1326	0.030	0.035	-
C+	1990	1358	0.013	0.028	-
	1991 (3/4)	1112	0.026	0.044	-
D+	1990	3752	0.080	0.200	0.5
	1991 (3/4)	8145	0.050	0.110	0.5
E+	1990+91		<0.107	Range <0.034 - 0.445	
F+	1990+91		<0.054	Range <0.034 - 0.150	
G*	1990	2916	0.040	0.066	-
	1991 (3/4)	3773	0.053	0.305	1.5
H*	1990	390	0.070	0.150	1.3
	1991 (3/4)	1254	0.050	0.070	1.3

\+ *Factories producing powder detergents containing enzymes.*

* *Factories producing liquid detergents containing enzymes.*

Table 6
Levels of Dust in Atmosphere (μg/m³)

Factory		*Number of Readings*	*Overall Average*	*Highest Monthly Average*	*% Individual Readings Above Limit*
A	1990	3060	195	271	0.8
	1991 (3/4)	2336	272	379	2.5
B	1990	2539	327	1370	4.0
	1991 (3/4)	1325	332	1260	5.6
C	1990	1358	262	767	3.4
	1991 (3/4)	1112	311	448	3.7
D	1990	3752	217	289	4.1
	1991 (3/4)	9185	170	200	0.8
E	1990-91		700		
F	1990-91		550		

The limit referred to is 1000 μg/m³ for dust

Appendix 2

MAEWO PTY LTD

AIR QUALITY, ENGINEERING AND SAFETY CONSULTANTS

ACN: 003 764 190

P.O. BOX 207 BALMAIN, NSW, 2041, AUSTRALIA
TELEPHONE (02) 555 8306, FACSIMILIE (02) 555 8393
MOBILE (02) 018 613156, INTERNATIONAL +61 (2) 555 8306

ASSESSMENT OF ENCAPSULATIION OF PROTEINASE DETERGENT ENZYMES

Prepared for Worksafe Australia

By Dr. Keith Post

September, 1992

Contents

1. INTRODUCTION

1.1 Scope of Work

On the 22nd of September, 1992, samples of four proteinase detergent enzyme materials in granular form and supporting documents were provided by Worksafe Australia for examination.

These materials are currently under review within the National Industrial Chemicals Notification and Assessment Scheme and it is the purpose of this investigation to provide an assessment of the encapsulation or dust minimisation features of the granular materials and in particular to:

1. Review the company data provided by Worksafe Australia;

2. Carry out tests if necessary in order to determine the integrity of the encapsulation of the enzyme;

3. Report on the integrity of the encapsulation of proteinase enzyme under occupational use conditions and;

4. Advise on appropriate tests and identify information gaps, if any.

1.2.1 Background Information

Enzyme additives have been used in detergents and in the late 1960's it became clear that airborne dusts containing these compounds could affect exposed workers and lead to occupational asthma in susceptible individuals. Since that time the original dusty enzyme powder concentrates have been altered by various encapsulation procedures which aim to prevent the release of fine dusts.

Early encapsulation involved fixing the enzymes onto the surface of sodium tripolyphosphate particles and rendering the resulting granules essentially dust-free by spraying on a tacky non-ionic detergent. Nowdays, the enzyme materials are provided in the form of smooth surfaced granular beads (marooms, prills) which are produced in a spray drying-spray cooling tower.

These new materials have extremely low fine dust content in their original state but they may generate respirable dust, in significant amounts, if crushed or subjected to mechanical damage in storage or handling.

1.3 Samples and Documents Examined

Approximately 1kg of each of the following materials was examined:

- OPTICLEAN M375.
  Manufactured by Solvay Enzymes and supplied by Solvay Biosciences of 530 Springvale Road, Glen Waverley, Victoria, 3150.

- SAVINASE 4.OT
  Manufactured by Novo - Nordisk and supplied by Novo - Nordisk Bioindustrial Pty Ltd, P.O. Box 46, Parramatta, NSW, 2124.

Novo - Nordisk also supplied small samples of two other grades of their Savinase product. These were similar in form to Savinase 4.OT but had different active enzyme contents.

The documents accompanying the above samples were as follows:

- Fifth Report of the Standing Committee on Enzymatic Washing Products, UK Soap and Detergent Industry Association (SDIA), June 1991 (82 pages).

- Book 2 of 6 Savinase. Notification to Worksafe Australia Parts A,B, and C. Author and date unknown (98 pages).

- Extract from L and K, Rexona Notification headed 'Summary of Health and Environmental Effects'. Author and date unknown (10 pages).

- Answers to Questions from Worksafe (L and K). Author and date unknown (6 pages).

- Attrition Dust in Granulates and Prills, Proctor and Gamble - External Methods. Supplied by Solvay Enzymes (9 pages).

- Elutriation Method for Dustiness Estimation. Supplied by Solvay Enzymes (5 pages).

In the course of this investigation, additional published documents were consulted and full details of these papers can be found in Appendix 1.

2. COMMENT ON COMPANY DATA

2.1 Review of Company Data on Enzyme Dust Generation and Control Methods

The literature provided by the enzyme manufacturers and the potential users of the materials clearly states that the enzymes have in the past, and still can, induce occupational asthma in susceptible individuals if workplace enzyme dust levels are not well controlled.

The level of control required is quite high and in order to achieve enzyme dust levels below an Occupational Exposure Standard of 0.06 JJ.g/ m^3 of pure crystalline substilisin (equivalent to 1.9 Glycine Units/ in^3) it would be necessary to maintain total enzyme dust levels below about 1 or 2±g/ m^3. This is a very low airborne dust concentration. Typical indoor office environments served with conditioned air may have between 20 and 80.Lg/ m^3 of fine airborne particulate matter, and some industrial workzones may have dust levels of milligrams per cubic meter or more.

Clearly, if workplace airborne enzyme dust levels are to be controlled so that their concentration remains below 1 or 2 p.g/ m^3, then comprehensive dust minimisation and dust control procedures will be requlred at all stages of the enzyme handling and blending operations. The technical literature, and the documents provided for review, indicate that in order to achieve such a high level of enzyme dust control it is essential to:

1. Use high quality encapsulated enzyme materials which have an extremely low fine dust content;

2. Adopt automated enzyme handling and manufacturing methods with efficient local exhaust or ventilated endosure systems;

3. Supplement local exhaust systems with general dilution ventilation;

4. Provide operator training and support to ensure that effective general housekeeping and maintenance routines are in place and;

5. Manage all steps of the enzyme handling and blending operations in such a way as to avoid spills or others events which may allow the encapsulated material to be damaged or crushed.

In summary, it appears from the information submitted for review and from the technical literature, that well-equipped and properly managed detergent blending plants have been able to achieve the necessary levels of dust control and to a large extent this has been a consequence of the use of high quality encapsulated materials.

2.2 Review of Company Data on Encapsulation Integrity and Test Methods

Unfortunately, the information provided for review contains no detailed description of the chemical, physical or mechanical properties of the encapsulant. One document states that the "enzyme products are supplied as granulates, encased in an inert material" and another suggests that the "encapsulate is fully stable at air temperatures below 50 °C and that "above this temperature, the coating of the encapsulate might be damaged by melting". The documents also indicate that Novo - Nordisk who manufacture the Savinase product see no need to perform toxicological studies on the final encapsulate as such tests would in their opinion, not add any significant information for risk assessment.

Although little information was found regarding the nature of the encapsulant, the documents provided for review did contain details of a number of test procedures designed to monitor the dustiness of the granulates.

Both manufacturers gave details of an elutriation test in which a known mass of the encapsulated material is placed on a porous plate in a glass tube and fluidised by a flow of air from below the plate. The air passes through the bed of granulate and carries with it any dust in the original sample plus any dust generated as the grains move and collide with each other and the container walls under the influence of the air stream.

Both elutriation tests subject the granulate sample to the same degree of air movement through the bed but they differ in other respects in that:

- One method includes the use of a vibrator to dislodge dust from the elutriation tube walls;

- One method uses air dried by passing it through a container filled with silica gel, whereas the Novo - Nordisk procedure appears to use air conditioned to a relative humidity to between 40 to 50%;

- One method collects the airborne dust on a pre-weighed filter to allow the total dust weight to be determined in addition to enzyme activity level, whereas the other method collects the dust by passing the air stream through a liquid medium contained in gas washing bottles.

At no time do the documents relating to the above tests suggest that the procedures are meant to simulate real in plant granulate attrition and dust generation conditions, and it appears that the main purpose of the elutriation test is to provide a measurement for use in product quality control procedures.

At present, manufacturers and users of these products appear to accept batches of these encapsulated enzyme materials if the above tests produce a total fine dust emission of less than 165 Glycine Units under standard test conditions. Such an emission rate corresponds to an enzyme activity level in the airstream leaving the fluidised bed of about 92 Glycine Units per in^3.

In one of the documents provided, a second dustiness test method is described in which the granulates are subjected to the action of rolling steel balls in a dust pot. This test may give a better indication of how the encapsulated materials might perform under rough handling conditions, but no test results, or acceptance criteria were provided for review.

To conclude this section, it should be noted that the whole topic of the dustiness of industrial materials is an area of current interest in the occupational hygiene literature and further information about existing test methods and their shortcomings, can be found in the papers listed in Appendix 1.

3. EXAMINATION OF SAMPLES

All four enzyme granulate samples were examined visually and with the aid of an incident light microscope at magnifications of xlO, x40 and xlOO. One of the samples was also used to assess the fine dust properties of damaged (crushed) granulate material. The results of these two assessments are presented below.

3.1 Visual Appearance

All four samples were visually similar and it was found that the material consisted of irregularly shaped beads which were between - 0.1 and 1 mm in length along their major axis. The beads were off white in colour and the bulk material in its 'as supplied' condition contained little, if any, fine dust. An example of one of these materials is shown in Figure 1 and the smooth, dean surface of each grain is clearly evident.

The surface coating was found to be made of a white compound which could be readily removed from the grains by brushing or rubbing with a moist cloth or sponge. Below the surface coating the grains consisted of the yellow - brown material shown in Figure 2, which presumably contained the active components. This internal material was found to be quite friable and when crushed it would shatter into many small and medium sized particles as shown in Figure 3.

In some samples the outer layer of white material, surrounding the yellow - brown core was of varying thickness and the inner core protruded through it as can be seen in Figure 4.

3.2 Dustiness and Integrity of the Encapsulation

The materials supplied for examination were all similar in that their fine dust content in the 'as supplied' condition was extremely low. In plant operations however, the dustiness of these materials will depend on the extent to which each grain is damaged or worn as a result of mechanical action.

To illustrate this, a sample of Savinase 4.0T was placed in a gas washing bottle and flushed with a stream of dry air. The bottle was shaken manually and the effluent airstreain was sampled for particulate matter. The test conditions (sample size, air flow rate, humidity etc) were selected at random, but were held constant from test to test. Two samples were evaluated. The first sample was in the 'as supplied' condition and the second sample was the same material returned to the gas washing bottle after about 0.04% of all the beads were crushed gently between two metal plates.

For the undamaged material, the effluent airstreain contained about 22 μg/ in^3 of airborne dust with about 17 μg/ m^3 in the sub 7μm size range.

For the damaged material, the effluent airstreain contained about 13 mg/ m^3 of airborne dust of which about 1.6 mg/ in^3 was less than 7 μm in size with the following size distribution.

SIZE FRACTION (μm)	PERCENT OF TOTAL MASS OF SUB 7 μm SIZED MATERIAL
6.5 - 3.2	46
3.2-1.6	30
1.6-0.8	13
0.8-0.4	5
0.4-0.2	3
0.2-0.1	2
0.1- 0.05	1

The above test results should not be taken to have any direct relationship with in plant operational conditions or other elutriation or attrition test procedures. Their purpose here is simply to illustrate that the major dust hazard relating to the use of these encapsulated materials will be associated with events in which the individual grains may be damaged. Such events would include accidental spills, machinery failures or maintenance operations, disposal of used enzyme containers and the like.

4. ENCAPSULATION INTEGRITY UNDER OCCUPATIONAL USE CONDITIONS

As noted previously, the encapsulated enzyme materials appear to rely on an outer coating and their own mechanical toughness in order to avoid the generation and dispersion of enzyme dust. Either of these two features may fail to provide adequate containment of the entrapped enzyme if, for example, excessive mechanical agitation leads to abrasion of the outer layer, or if individual grains are fractured during rough handling.

In an occupational use environment, the most likely form of encapsulant damage would be that resulting from non - standard events such as spills, breakdowns, maintenance or container disposal.

Under normal processing conditions, with the material in an 'as supplied' condition, and confined within well - exhausted plant within well- ventilated rooms, it is likely that the encapsulant would perform well and that acceptable dust control could be achieved.

5. ADVICE REGARDING APPROPRIATE TESTS AND INFORMATION GAPS (IF ANY)

5.1 Appropriate Tests

At this stage no further tests are recommended. It is clear that the encapsulated material has a low initial fine dust content and it is also clear that with good plant management, low airborne enzyme dust levels can be achieved in the workplace.

It is considered to be unlikely that high dust emission events would occur as a result of encapsulation failure, where this is taken to mean a failure of the outer coating layer of each bead. High dust emission events may however, follow major dainage or crushing of the individual granules if they are subjected to excessive compression, impaction or shearing loads. There is little to be gained by testing the materials against such phenomena, and much more to be gained by ensuring that such events do not occur.

5.2 Information Gaps

No significant information gaps relating to the matter of encapsulation integrity were found in this investigation. Further information relating to the nature of the encapsulant and the justification for the elutriation test procedure may be of interest, as would comment on alternative dustiness test methods. However, the lack of these details has not constrained the progress of this investigation, and it is not considered that such information would alter the conclusions contained in this report.

6. REVIEW

6.1 Four samples of proteinase detergent enzyme materials in granular form and supporting documents were examined in this investigation as part of Worksafe Australia's National Industrial Chemicals Notification and Assessment Scheme.

6.2 The main objective of this work was to assess the quality of the encapsulation used to prevent the generation and dispersion of airborne enzyme dusts.

6.3 A collection of industry supplied papers was reviewed and some preliminary tests were performed on the sample materials provided.

6.4 It was found that the granulates provided by different manufacturers were all similar in form and that they were coated with a white outer layer below which the active enzyme components were held within a yellow - brown friable core material.

6.5 It was noted that for safety, an extremely low workplace airborne enzyme dust concentration would be required and that such levels could only be achieved in well designed and well run plants using high quality encapsulated enzyme materials.

6.6 Visual examination and preliminary tests indicated that although the granulates had extremely low fine dust content in their 'as supplied' condition, they could readily generate high enzyme dust levels if the individual grains were mechanically damaged.

6.7 It was concluded that provided such mechanical damage was avoided, it would be possible for a detergent blending plant to incorporate these enzyme materials into their products without generating hazardous airborne enzyme dust levels in the workplace if the UK SDIA guidelines (1991) were followed.

6.8 It was concluded that there was no need to request that the enzyme manufacturers or users provide additional information or results regarding encapsulation materials or test procedures, and that attention should be directed towards ensuring that good plant design and housekeeping strategies were enforced in order to eliminate the possibility of granulate damage.

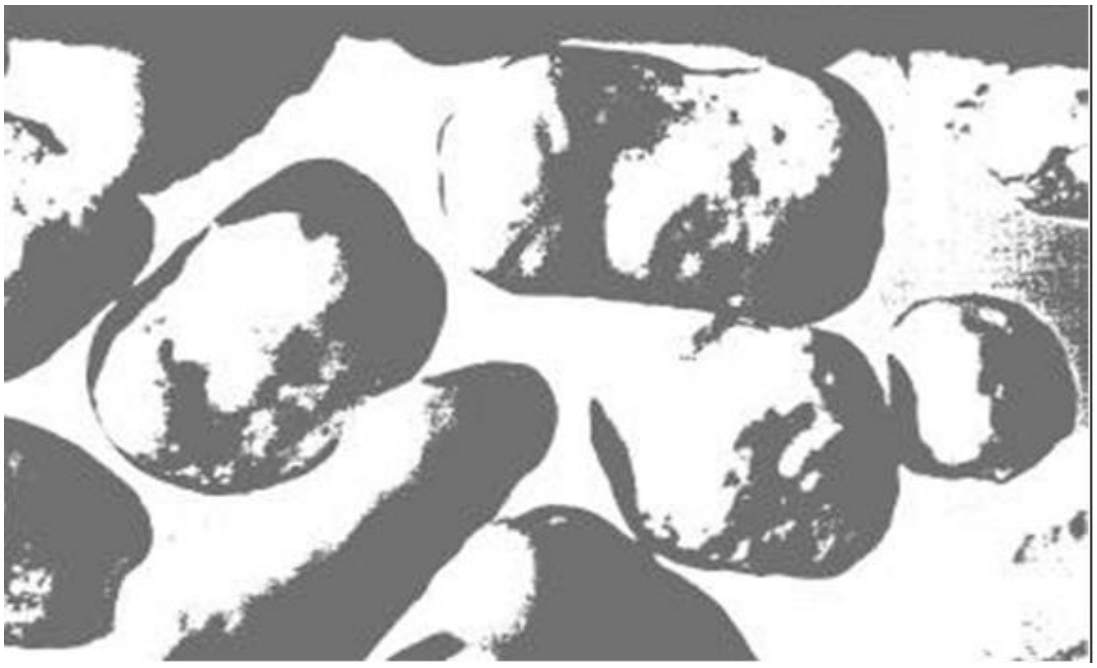


Figure 1

View showing a collection of encapsulated enzyme granules with thick outer layers. Note the smooth, clean, dust free surface of the 'as supplied' particles. In this view, the rectangular grain in the top right field has a full scale size of approximately 1 mm x0.5 mm.

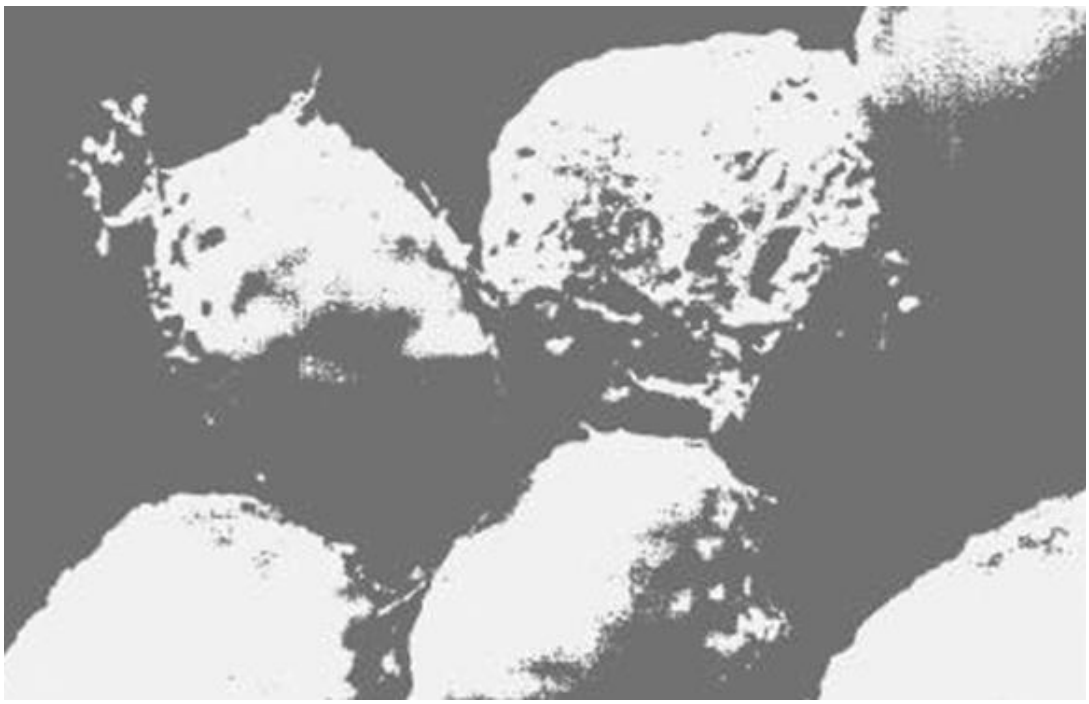


Figure 2

View showing the enzyme granules after their outer layer of white material has been removed by rolling them over a moist cloth (scale as for Figure 1).

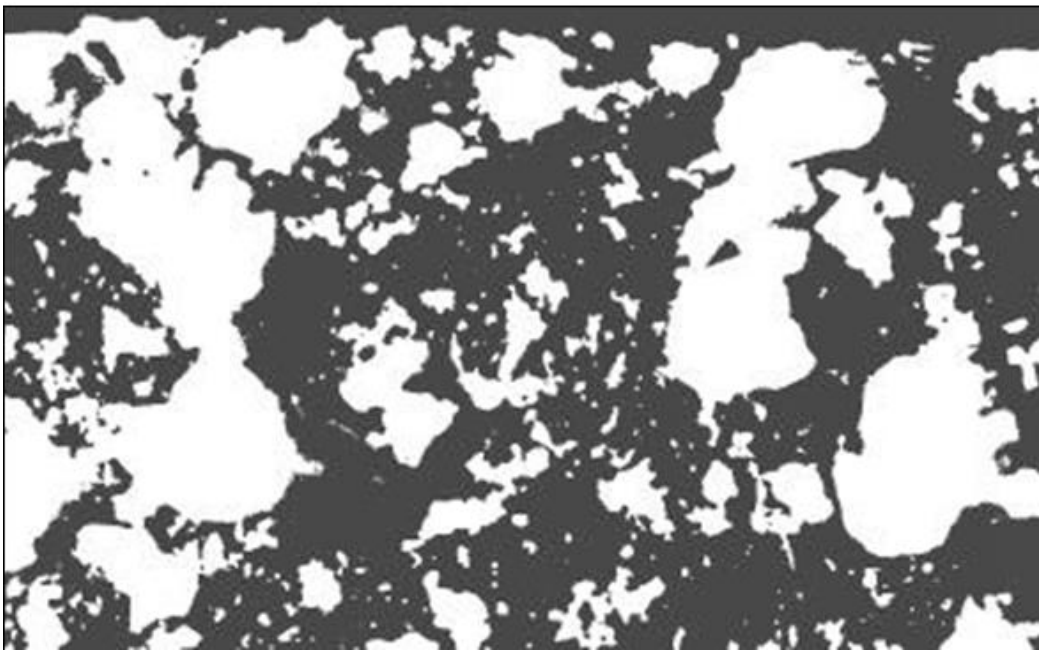


Figure 3

Enzyme and granule fragments left after a few grains were crushed between two glass slides (scale as for Figure 1).

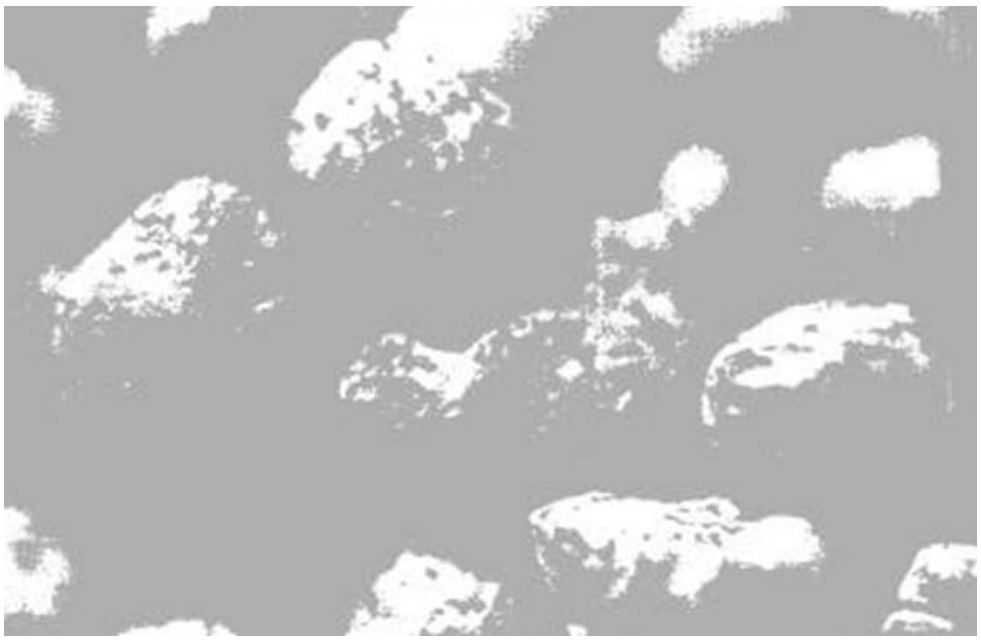


Figure 4

View showing a selection of encapsulated enzyme granules with thin outer layers of white material through which the core material can be seen (scale as for Figure 1).

Attachment 1

List of additional papers examined in the course of this investigation (in reverse chronological order).

1. Plinke, MAE; Leith, D; Holstein, DB and Boundy, MG (1991). Experimental examination of factors that affect dust generation. Am. Ind. Hyg. Assoc. J. 52 (12): 521-526.

2. Heitbrink, WA; Todd, WF; Cooper, TC and O'Brien, DM (1990). The application of dustiness tests to the prediction of worker dust exposure. Am. Ind. Hyg. Assoc. J. 51(4): 217-223.

3. Heitbrink, WA (1990). Factors affecting the Heubach and MRI dustiness tests. Am. kid. Hyg. Assoc. J. 51(4): 210-216.

4. Davies, KM; Hammond, CM; Higman, RW and Wells, AB (1988). Progress in dustiness estimation. British Occupational Hygiene Society Technology Committee Working Party on Dustiness Estimation. Ann. Occup. Hyg. 32(4): 35-544.

5. Goodfellow, HD and Smith, JW (1988). Dustiness testing - A new design approach for dust control. Proc. Ventilation '88 (ed. JH Vincent). pp 175-182. Pergamon Press.

6. Higman, RW (1985). Dustiness testing: A useful tool. Proc. Ventilation'85 (ed. HD Goodfellow). pp 693-701. Elsevier Science Publishers.

7. Duffus, JH and Brown, CM (1985). Health aspects of biotechnology. Ann. Occup. Hyg. 29(1): 1-11.

8. Bruce, CF; Dunn, E; Brotherton, R; Davies, DR; Hall, F and Potts, SCM (1978). Methods of measuring biologically active enzyme dust in the environmental air of detergent factories. A Working Party under the auspices of the Soap and Detergent Industry Association. Ann. Occup. Hyg. 21: 1-20.

9. Gilson, JC; Juniper, CP; Martin, RB and Weill, H (1976). Biological effects of proteolytic enzyme detergents. Thorax, 31: 621-634.

10. Fulwiler, RD (1971). Detergent enzymes - An industrial hygiene challenge. Am. kid. Hyg. Assoc. J. 32: 73-81.

11. The Soap & Detergent Industry Association. (1971). Recommended operating procedures for U.K. factories handling enzyme materials. Ann. Occup. Hyg. 14: 71-87.