File No: NA/510

March 1998

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Laccase

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Director
Chemicals Notification and Assessment

Laccase

1. APPLICANT

Novo Nordisk Bioindustrial Pty Ltd of Unit 3 22 Loyalty Road NORTH ROCKS NSW 2151 has applied for an assessment certificate for the enzyme 'Laccase' to be used in the product DeniLite^{TM} No application for exempt information was lodged, hence the report is published here in its entirety.

2. IDENTITY OF THE CHEMICAL

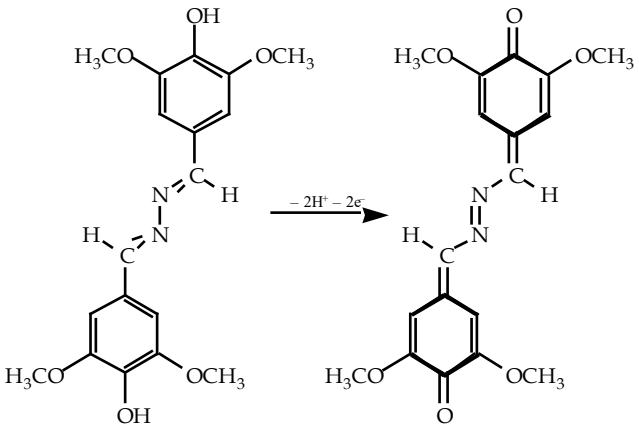
Chemical Name:	Laccase
Chemical Abstracts Service (CAS) Registry No.:	80498-15-3
Trade Name:	DeniLite^{TM}
Molecular Formula:	not applicable (enzyme)
Structural Formula	not applicable
Molecular Weight:	61 kDa
Enzyme activity:	254 LACU.g^{-1} (where LACU is the amount of enzyme which under the given analytical conditions catalyses the conversion of 1 mmol syringaldazine per minute; the laccase specific activity is 100 LACU.mg^{-1} pure active enzyme protein)
Method of Detection and Determination:	the enzyme product Laccase was analysed for the presence of the production strain using bacteriological analytical techniques; characterisation of the enzyme activity of the Laccase used in the toxicological studies was carried out according to methods outlined in the test report supplied by the notifier
Spectral Data:	not applicable

• Comments on Chemical Identity

The notified substance is an enzyme. It is isolated from a strain of *Aspergillus oryzae* which has been genetically modified to host the laccase gene from *Polyporus pinsitus*. Laccase is a phenol-oxidising enzyme converting phenols into phenoxy radicals which then undergo a series of non-enzymic reactions.

The notifier has submitted several analytical reports for batches of the enzyme in which the host organism was not detected. Samples (10 g or 10 mL) are diluted into 90 mL of Butterfield's dilution water. After plating on 5-20 YPG agar plates (typically 10) containing tetracycline, plates are incubated at 32°C ± 2°C for 4 days, and colonies on the test plates are morphologically compared to the reference strain grown on similar plates. Suspicious colonies and the reference strain are then plated on the same agar medium (YPG agar with tetracycline) and Cove T-2 agar. They may also be plated on YPSS with tetracycline and DG 18 agar. Should verification be necessary, this may be achieved by testing for Laccase production by measuring the enzyme activity (see below).

In one test batch analysed, the active enzyme protein made up 3.5% of the total organic substance (TOS, defined as 100 % -(W + A), where W = % water and A = % ash). The TOS made up 6.6% of the test batch which contained 93% water and 0.4% ash. One laccase unit (LACU) is defined as the amount of enzyme that catalyses the oxidation of 1 µmol of syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine) to tetramethoxy-azo bis methylene quinone per minute as follows at a pH of 5.50, a temperature of 30°C and a reaction time of 60 seconds.



Microbiological analysis of the test batch found low levels of coliforms, yeast, fungi, *Bacillus cereus*, streptococcus and sulphite-reducing clostridia.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	pure laccase protein is a crystalline powder; the product DeniLite™ containing the enzyme is a viscous brown liquid
Boiling Point:	not determined

Density:	1.024 g.mL^{-1} (test batch)
Vapour Pressure:	not determined
Water Solubility:	> 30% w/w @ pH ~5
Partition Co-efficient (n-octanol/water):	not determined (see notes below)
Hydrolysis as a Function of pH:	not determined (see notes below)
Adsorption/Desorption:	not determined (see notes below)
Dissociation Constant:	not determined (see notes below)
Flash Point:	not flammable
Flammability Limits:	not flammable
Autoignition Temperature:	not applicable
Explosive Properties:	not explosive
Reactivity/Stability:	not oxidising

Comments on Physico-Chemical Properties

The notified substance is an enzyme and as such, many of the standard methods of physico-chemical characterisation of chemical substances are not valid.

Purification of the enzyme is not possible, hence the enzyme (active) and impurities are expressed together as the total organic solids (TOS).

No indication of how the water solubility was estimated is given in the submission.

4. PURITY OF THE CHEMICAL

Purity Index (active enzyme/TOS)	3.5%
Toxic or Hazardous Impurities:	none
Non-hazardous Impurities (> 1% by weight):	proteins, peptides, amino acids, carbohydrates and inorganic salts (to 100%)
Additives/Adjuvants:	none

5. USE, VOLUME AND FORMULATION

The notified substance will not be manufactured in Australia. It will be imported into Australia as a component of DeniLite™ (approximately 2% Laccase) in 25 kg jerry cans. Annual imports of the notified substance are projected to remain below 1 tonne per year for the next five years.

The notified substance is intended for use in the textile industry as an enzyme catalysing the oxidation of phenolic substrates by oxygen to quinones. The proposed enzyme-based process will allow for the bleaching of textile dyes (e.g. indigo used in denim) without the use of chlorine-based chemicals such as hypochlorite. The notifier estimates that the notified substance may be used by one major customer in Adelaide and six to ten minor companies in Sydney and Melbourne.

6. OCCUPATIONAL EXPOSURE

Worker exposure to the notified enzyme during handling and storage is only likely to occur in the event of an accidental spill. No re-packing of the DeniLite™ product will take place in Australia.

Typically the DeniLite™ product containing Laccase will be poured from 25 kg jerry cans to a dosing container and then into washing machines, a procedure that takes only a few minutes each day. As the mixture is a liquid, no worker exposure to enzyme dusts is possible. However, the product may create inhalable aerosols if splashed or vigorously stirred. Skin and eye exposure may also occur during these operations.

7. PUBLIC EXPOSURE

DeniLite™ which contains the notified enzyme, is packaged in 25 kg jerry cans and in 200 kg polydrums. In normal circumstances, there will be no public exposure to DeniLite™ as it is only used in industrial settings. Public exposure to the notified enzyme through exposure to textiles treated with the DeniLite™ product is unlikely as laccase is removed during rinsing procedures. The most significant avenue for exposure of the public to the notified enzyme is via an accidental spill during transportation to the various dyeing facilities.

8. ENVIRONMENTAL EXPOSURE

Release

No release or exposure to the environment is expected from this chemical during transportation, with the exception of accidental spillage. Instructions on the Material Safety Data Sheet (MSDS) for the products containing the notified substance is sufficient for relevant authorities to cope with accidental spillage.

Empty jerry cans containing residues of the notified substance will be rinsed and the rinsings will also be poured into industrial washing machines, leaving trace amounts in cans. Empty cans will be disposed of to landfill.

Almost all the imported notified substance will be discharged to the sewer after dilution in on-site sewerage treatment plants.

Fate

Most proteins lose their tertiary structure and biological activity under a multitude of conditions (1). Being an enzyme, laccase is expected to behave in a similar fashion to other proteins. Exposure to non-aqueous environments is likely to result in physico-chemical degradation. Proteins are susceptible to ionising and non-ionising radiation, denaturation by high temperatures, oxidation, high ionic strength (salt), surfactants and extremes of pH which may all result in degradation of the molecule or inactivation of protein function. Acid and alkaline hydrolysis readily occur under strong conditions or under milder conditions at elevated temperatures.

Additionally, naturally occurring microorganisms are likely to readily biodegrade the laccase by exogenous proteases to constituent amino acids. Although no biodegradation data need to be provided for chemicals imported at rates less than 1 000 kg per annum according to the Act, the notifier has provided biodegradation data for laccase. Laccase was examined for biodegradation potential using EEC Directive 92/69, Part C.4-C (Modified Sturm Test), and OECD Test Guideline 301B. At levels of 11.5 and 23 $mg.L^{-1}$ laccase showed cumulative CO_2 production values of 89% and 91% of theoretical values, respectively. In excess of 60% this degradation occurred within the first 10 days of the test. These results indicate that laccase was readily biodegradable under the conditions of the test.

Biological membranes are not permeable to substances of very large molecular size and therefore bioaccumulation of the notified enzyme is not expected (2).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Two batches of the enzyme were used in the toxicological studies. Batch PPX 5326, a dark brown liquid (6.6 % total organic solid (TOS)) was used for most of the studies, while Batch PPX 5660 the freeze-dried version (91.7 % TOS) of the former, a light brown powder, was used for the irritation and skin sensitisation studies.

Summary of the acute toxicity of Laccase

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 1 350 $mg.kg^{-1}$ TOS	(3)
acute inhalational toxicity	rat	LC_{50} > 5.42 $mg.L^{-1}$ i.e 0.36 mg $TOS.L^{-1}$	(4)
skin irritation	rabbit	slight irritant	(5, 6)
eye irritation	rabbit	slight irritant	(7, 8)
skin sensitisation	guinea pig	not sensitising	(9)

9.1.1 Oral Toxicity (3)

Species/strain:	rat/Wistar
Number/sex of animals:	10/sex
Observation period:	14 days
Method of administration:	20 mL of batch PPX 5326 was administered orally by gavage
Clinical observations:	nil
Mortality:	nil
Morphological findings:	one male and one female had watery contents in the stomach; the liver of the male was also slightly lighter in colour
Test method:	similar to OECD guidelines (10)
LD_{50}:	> 1 350 $mg.kg^{-1}$ (TOS) ie. 47.25 $mg.kg^{-1}$ active enzyme
Comments:	clearly the level of active enzyme tested is insufficient to comment on its toxicity; however, given that the enzyme is never isolated in pure form it is appropriate that tests were conducted using TOS
Result:	the active notified enzyme in association with other organic solids was of low acute oral toxicity in rats

9.1.3 Inhalation Toxicity (4)

Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	nose only
Clinical observations:	during observation rats were observed to be wet and unkempt; all rats recovered one hour after treatment; no other treatment-related findings
Mortality:	nil
Morphological findings:	no treatment-related findings
Test method:	similar to OECD guidelines (10)
LC_{50}*:*	> 5.42 $mg.L^{-1}$ (> 0.36 $mg.L^{-1}$ TOS or > 0.01 $mg.L^{-1}$ of active enzyme)
Comments:	clearly the level of active enzyme tested is insufficient to comment on its toxicity; however, given that the enzyme is never isolated in pure form it is appropriate that tests were conducted using TOS
Result:	the notified enzyme in association with other organic solids was of slight acute inhalational toxicity in rats

9.1.4 Skin Irritation (5, 6)

Species/strain:	rabbit/New Zealand White
Number:	3 animals per test
Observation period:	6 days for Batch PPX 5660; 72 hours for Batch 5326
Method of administration:	0.5 mL of Batch PPX 5326 (6.6% TOS, 0.23 % active enzyme) was applied to the intact skin of each rabbit using semi-occlusive wrap 0.5 g of Batch PPX 5660 (91.7% TOS, 3.21% active enzyme) was moistened with 0.9% w/v NaCl solution and applied to the intact skin of

	each rabbit using semi-occlusive wrap
Comments:	Draize scores for all rabbits were zero over the entire observation period with Batch PPX 5326; with Batch PPX 5660, two rabbits developed slight erythema/eschar; this was reversed by the 72-hour period in one animal, and the 96-hour period for the second
Test method:	similar to OECD guidelines (10)
Result:	the notified enzyme (at 3.21%) in association with other organic solids was a slight irritant to the skin of rabbits

9.1.5 Eye Irritation (7, 8)

Species/strain:	rabbit/New Zealand White
Number:	3 animals per test
Observation period:	72 hours
Method of administration:	0.1 mL of Batch PPX 5326 (6.6% TOS, 0.23 % active enzyme) was applied to the conjunctival sac of one eye of each of the 3 rabbits 0.1 g of Batch PPX 5660 (91.7% TOS, 3.21% active enzyme) was applied to the conjunctival sac of one eye of each of the 3 rabbits
Comments:	no eye irritation noted for Batch PPX 5326 over entire test slight irritation of the conjuctivae noted for all 3 animals treated with Batch PPX 5660; this had disappeared by the 48-hour observation period; no effects on the cornea or iris were recorded
Test method:	similar to OECD guidelines (10)
Result:	the notified enzyme when administered as a solid in association with other organic solids was a slight irritant to the eyes of rabbits

9.1.6 Skin Sensitisation (9)

Species/strain: guinea pig/Dunkin Hartley

Number of animals: 20 test, 10 control

Induction procedure: Batch PPX 5660 (91.7% TOS, 3.21% active enzyme) - 75% (w/v) of the enzyme in distilled water was applied to the right flank of each animal using semi-occlusive wrap; patches were removed after 6 hours and residual cleaned from the site; the procedure was repeated once each week for 3 consecutive weeks

Challenge procedure: 13 days after the final induction each animal treated as described above was treated in the following way:

Batch PPX 5326 (100%) - 0.5 mL of the solution containing the notified enzyme (0.23%) was applied to the upper right flank of each animal using semi-occlusive wrap

Batch PPX 5660 - 75% (w/v) of the enzyme (3.21%) in association with other organic solids in distilled water was applied to the lower right flank of each animal using semi-occlusive wrap

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
75% Laccase PPX 5660	0/20**	0/20	0/10	0/10
100% Laccase PPX 5326	0/20	0/20	0/10	0/10

* time after patch removal
** number of animals exhibiting positive response

Test method: similar to OECD guidelines (10)

Result: the notified enzyme under the test conditions was not a skin sensitiser in guinea pigs

9.2 Repeated Dose Toxicity (11)

Species/strain:	rat/Sprague Dawley
Number/sex of animals:	5/sex/group
Method of administration:	oral gavage with dose levels at 54 (low), 270 (mid) and 1 352 (high) mg TOS.kg^{-1}.day^{-1}; ie equivalent to ~ 49 mg enzyme .kg^{-1}.day^{-1}
Dose/Study duration::	28 days
Clinical observations:	no treatment-related changes
Clinical chemistry/Haematology	no treatment-related changes in haematological parameters; urea levels were slightly increased in all treated female groups
Histopathology:	no treatment-related changes
Test method:	similar to OECD guidelines (10)
Result:	the notified enzyme in association with other organic substances exhibited no organ toxicity in a 28-day oral repeat-dose study at the concentrations tested

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation (liquid culture) Assay (12)

Strains:	TA 98, TA 100, TA 1535 and TA 1537
Concentration range:	bacteria were, exposed to 6 doses 30 - 10 000 μg.mL^{-1}) of test substance (ie 660 μg.L^{-1} of TOS or ~ 24 μg.L^{-1} of active enzyme for the highest dose) in a phosphate buffered nutrient broth either with or without S9 metabolic activation form a period of 3 hours
Test method:	similar to OECD guidelines (10)
Result:	Batch PPX 5326 containing 0.23% active enzyme was not mutagenic in the *Salmonella typhimurium* strains tested with or without metabolic activation

9.3.1 *Salmonella typhimurium*/*Escherichia coli* Plate Incorporation Assay (13)

Strains:	TA 98, TA 100, TA 1535 TA 1537 and *Escherichia coli*
Concentration range:	bacteria were exposed to 6 doses (1 667 - 5000 µg per plate) of batch 5326 (ie 330 $\mu g.L^{-1}$ of TOS or ~ 12 $\mu g.plate^{-1}$ of active enzyme for the highest dose) with or without S9 metabolic activation
Test method:	similar to OECD guidelines (10)
Comments	weak increases in revertant levels of exposed bacteria compared to the solvent controls were observed in most of the tests with all salmonella strains; these weak responses have been tentatively attributed to the presence of trace amounts of histidine in the test substance
Result:	Laccase in batch PPX 5326 was not mutagenic in *Salmonella typhimurium* or *Escherichia coli* with or without metabolic activation under the conditions of the test

9.3.2 Chromosomal Aberrations Assay with Human Peripheral Lymphocyte Cultures (14)

Cell type:	human blood lymphocyte
Doses:	5 and 10% (v/v) solutions of Batch PPX 5326 (ie 6.6 mg $TOS.mL^{-1}$ with or without S9 metabolic activation
Test method:	similar to OECD guidelines (10)
Result:	Laccase in batch PPX 5326 was not clastogenic toward human lymphocyte cells under the conditions of this study; however cultures treated with 10% v/v Laccase in the absence of S9 mix caused an increase in the frequency of polyploid cells

9.4 Overall Assessment of Toxicological Data

Laccase was administered orally to rats at a maximum dose of 1 350 $mg.kg^{-1}$. (TOS) and no signs of systemic toxicity were observed. This is equivalent to approximately 47 $mg.kg^{-1}$ of the active enzyme. Clearly this is insufficient to establish the oral toxicity of the pure enzyme. However, given that the

enzyme is never isolated in the pure form, it is more appropriate to express dosage in terms of the total organic solid (TOS) content. No signs of toxicity were observed at 1 350 $mg.kg^{-1}$ TOS, however it is not possible to dismiss effects below the 2 000 $mg.kg^{-1}$ cut-off concentration for a harmful substance as determined by the National Occupational Health and Safety Commission.

Inhalation toxicity studies with rats indicated an LC_{50} above a nominal concentration greater than 5.42 $mg.L^{-1}$. This is equivalent to 0.36 $mg.L^{-1}$ TOS or 0.01 $mg.L^{-1}$ of active enzyme. The level of enzyme is again too low for comment on its toxicity via this route. The fact that the test animals showed signs of toxicity within the first hour of treatment, suggests that higher dose levels of the enzyme may result in adverse effects in animals and possibly in animal deaths.

Batch PPX 5326 (6.6% TOS, 0.23 % active enzyme) was not irritating to the skin of rabbits, however Batch PPX 5660 (91.7% TOS, 3.21% active enzyme) induced slight erythema and eschar of the skin. These differences are likely to be attributable to the greater enzyme concentration in the latter and suggests that the enzyme could be classified as a skin irritant in its pure form.

Batch PPX 5326 (6.6% TOS, 0.23 % active enzyme) was not irritating to the eyes of rabbits, however Batch PPX 5660 (91.7% TOS, 3.21% active enzyme) induced slight conjunctival reddening. Whether these differences are a product of the differences in enzyme concentrations or differences in the physical nature of the two test batches is uncertain.

The results of the skin sensitisation studies indicate that the enzyme preparation is not a skin sensitiser in guinea pigs.

Daily oral administration of up to 1 352 mg total organic (49 $mg.kg^{-1}.day^{-1}$ pure enzyme) substance per kilogram bodyweight per day to rats over a 28-day period did not cause any deaths or systemic effects in rats. Hence the pure enzyme is not harmful in repeat dose tests.

The notified enzyme is not mutagenic in strains of salmonella or escherichia in a plate incorporation assay. However it should be noted that the maximum concentration of TOS was 330 $\mu g.plate^{-1}$ which is equivalent to approximately 12 $\mu g.plate^{-1}$ of active enzyme. This concentration is well below standard test concentrations of 5 000 $\mu g.plate^{-1}$. In addition, the notified enzyme was not mutagenic in salmonella in a liquid culture assay. However, as was the case in the plate incorporation assays, the dose levels were well below standard dose concentrations (ie 660 $\mu g.L^{-1}$ of TOS or approximately 24 $\mu g.L^{-1}$ of active enzyme for the highest dose).

The notified enzyme was not clastogenic in blood lymphocytes, however, the enzyme preparation caused polyploidy in human blood lymphocytes.

By virtue of the enzyme preparation, toxicological tests have in general employed quite low test concentrations. As a consequence classification of enzyme according to the criteria of the National Occupational Health and

Safety Commission (15) is not possible. On the basis of the above toxicological studies the major concern seems to lie in the skin and eye irritation potential of the enzyme preparation. Finally, it should be noted that there is concern over the potential for dusts and aerosols of enzymes to induce pulmonary sensitisation. Accordingly, the notifier has provided a MSDS where the enzyme is classified as hazardous on this basis.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

While not required by the Act, the following ecotoxicity studies have been supplied by the notifier. The tests were carried out according to OECD Test Methods.

Species	Test	Results (Nominal)
Rainbow Trout *Oncorthynchus mykiss*	Acute Toxicity (OECD Method 203)	LC_{50} > 100 mg.L^{-1} NOEC > 100 mg.L^{-1}
Daphnia magna	Acute Immobilisation (OECD Method 202, Part 1)	EC_{50} > 100 mg.L^{-1}
Algae *Selenastrum capricornutum*	Growth Inhibition (OECD Method 201)	NOEC < 100 mg.L^{-1} (72 h) E_bC_{50} > 100 mg.L^{-1} (72 h) E_rC_{50} > 100 mg.L^{-1} (0-72 h)
Aerobic Waste Water Bacteria	Respiration Inhibition (OECD Method 209)	EC_{50} > 3 200 mg.L^{-1} (3 h)

Limit tests for each species were conducted using a nominal concentration of 100 mg(dry material).L^{-1}. The measured concentrations in the ecotoxicity studies were around 75% of the nominal.

The duration of the algal study was 71.5 hours. During the test a 23% reduction in the growth rate of the test organisms was observed compared to the control. Microscopic investigation of the test media at the end of the test revealed no abnormalities in the algal cells. No adverse effects were observed in either the fish or daphnia studies.
In the fish study a slight turbidity was observed after 24 hours (test solution was replaced after 24 hours) and at the end of the test. A bit of foaming was noted at the start of the test and after replacement.

The ecotoxicity data indicate that the notified substance is practically non-toxic to aquatic organisms (fish, daphnia, algae and sewage micro-organisms).

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notifier has estimated a predicted environmental concentration (PEC) of 2.9 ppb of laccase TOS as a result of discharge from a large textile plant to the sewer (based on the use of 2.34 kg of laccase TOS, with a waste water flow of 4 000 m^3 per day and a surface dilution factor of 200). The major user of the notified substance will be in Adelaide and the discharge from the textile plant's waste water treatment system is expected to be treated at the Bolivar sewerage treatment works (Average flow rate of

between 130 and 160 ML per day). The discharge of 2.34 kg of laccase TOS into this sewerage system would result in a concentration of 18 ppb in discharge from the Bolivar sewerage treatment works. This would undergo a further 10-fold dilution in receiving waters to give a PEC of 1.8 ppb. This concentration is four orders of magnitude lower than the lowest NOEC observed for fish, daphnia or algae. Thus, the discharge of laccase from textile plants is not expected to be hazardous to aquatic organisms. Dilution rates in other capital cities are likely to be similar or greater.

Analysis of the enzyme concentrate did not detect the host organism. Hence, the hazards posed by the potential for release to the open environment of genetically modified *Aspergillus oryzae* do not need to be assessed.

The environmental hazard from the proposed use the notified substance is rated as low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Although the toxicological tests preclude comment on the toxicological profile of the pure active enzyme, the tests are pertinent to the isolated enzyme preparation to which workers are exposed. That is, the active enzyme (3.5%) in association with organic impurites. Accordingly, the enzyme preparation is not likely to be toxic by acute oral exposure, nor by repeat exposures. It may cause slight irritation of the skin and the conjunctivae of the eye, but not sensitisation of the skin. The enzyme preparation may cause adverse effects if inhaled. Although no data was supplied by the notifier, dusts and aerosols of the enzyme preparation may induce pulmonary sensitisation as indicated on the MSDS supplied by the notifier. Finally, it is unlikely that genotoxic effects will result from exposure to the enzyme preparation.

As Laccase is supplied in a liquid form as an ingredient (less than 2%) of the product DeniLite^{TM}, the main risk to worker health lies with workers exposed to aerosols of Laccase possibly generated during transfer from jerry can to the dosing container. In addition the DeniLite^{TM} contains ethoxylated alcohols that may cause eye irritation in workers. The notifier states that it is normal procedure for workers in the textile industry to use gloves and eye protection. However, the Material Safety Data Sheet (MSDS) recommends respiratory protection as well. If workers are exposed to aerosols of the product containing the enzyme without respiratory protection, then the risk of adverse health effects may be significant. It is also imperative that spills are cleaned up, as failure to do so could result in the generation of hazardous enzyme dusts.

As DeniLite^{TM} is only used in industrial settings, the general public will not come into contact with the enzyme, except in the case of an accidental spill. Hence the risk of adverse health effects on the general public is negligible.

13. RECOMMENDATIONS

To minimise occupational exposure to Laccase enzyme and the ethoxylated fatty acid solvent, the following guidelines and precautions should be observed when handling the DeniLite^{TM} product:

- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 (16) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (17);
- Industrial clothing should conform to the specifications detailed in AS 2919 (18);
- Impermeable gloves or mittens should conform to AS 2161 (19);
- All occupational footwear should conform to AS/NZS 2210 (20);
- Respiratory devices conforming to AS/NZS 1716 (21) should be worn if exposed to dusts or aerosols of the enzyme
- Spillage of the notified chemical should be avoided, spillages should be cleaned up promptly with absorbents which should then be put into containers for disposal;
- Good personal hygiene should be practised to minimise the potential for ingestion;
- A copy of the MSDS should be easily accessible to employees.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the product DeniLite^{TM} containing the notified enzyme was provided in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (22).

This MSDS was provided by the applicant as part of the notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of the notified chemical shall be required if any of the circumstances stipulated under subsection 64(2) of the Act arise. In particular, secondary notification will be required if the concentration of the active enzyme in the total organic substances (TOS) rises above 5%. No other specific conditions are prescribed.

16. REFERENCES

1. Volkin D B and Klivanov A M 1989, *Minimising Protein Inactivation*, in Protein Function - A Practical Approach, IRL Press, Oxford, UK.

2. Gobas, F., Opperhuizen, A. & Hutzinger, O. 1986, 'Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation', *Environmental Toxicology and Chemistry*, vol. 5, pp. 637-646.

3. Lissau B G 1996, *Laccase from Polyporus pinsitus, SP807 - Acute Oral Toxicity (Limit Test) in Rats*, Project no., 1.35-90, Novo Nordisk,, Bagsvaerd Denmark.

4. Kieran P C 1990, *Laccase SP 807 Batch PPX 5326 Acute Inhalation Toxicity Study in Rats (Limit Test)*, Project no., 13355, Inveresk Research, Scotland

5. Bergman A 1996, *Laccase Batch 5660 Acute Dermal Irritation/Corrosion in Rabbits*, Project no., QR-9615214, Novo Nordisk, Bagsvaerd, Denmark.

6. Lissau B G 1996, *Laccase Batch PPX 5326 Primary Skin Irritation in Rabbits*, Project no., QR-9611665, Novo Nordisk, Bagsvaerd, Denmark.

7. Bergman A 1996, *Laccase Batch 5660 Acute eye Irritation/Corrosion in Rabbits*, Project no., QR-9615248, Novo Nordisk, Bagsvaerd, Denmark.

8. Lissau B G 1996, *Laccase Batch PPX 5326 Eye Irritation in Rabbits*, Project no., QR-9611655, Novo Nordisk, Bagsvaerd, Denmark.

9. Hamilton J A and Donald E 1997, *Laccase SP 807 PPX 5660 Buehler Sensitisation Test in Guinea Pigs*, Project no., 14661, Inveresk Research, Scotland

10. Organisation for Economic Co-operation and Development 1995-1996, *OECD Guidelines for the Testing of Chemicals on CD-Rom*, OECD, Paris.

11. Walker S A and Clubb S 1996, *Laccase SP 807 Batch PPX 5326 4-Week Oral Toxicity Study in Rats with Administration by Gavage*, Project no., 11551, Inveresk Research, Scotland

12. Pedersen P B 1996, *Laccase SP807 Batch No. PPX 5326: Testing for Mutagenic Activity with Salmonela typhimurium TA 1535, TA 100, TA 1537, TA 98 in a Liquid Culture Assay*, Project no., 1.35-90, Novo Nordisk, Bagsvaerd, Denmark.

13. Pedersen P B 1996, *Laccase SP807 Batch No. PPX 5326: Testing for Mutagenic Activity with Salmonela typhimurium TA 1535, TA 100, TA 1537, TA 98 an Escherichia coli WP2uvrA in a Plate Incorporation Assay*, Project no., 1.35-90, Novo Nordisk, Bagsvaerd, Denmark.

14. Innes D C 1996, *Laccase Sp807 Batch 5326 Chromosomal Aberrations Assay with Human Peripheral Lymphocyte Cultures in vitro*, Project no., 14024, Inverersk Research, Scotland

15. National Occupational Health and Safety Commission 1994, *Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(1994)]*, Australian Government Publishing Service, Canberra.

16. Standards Australia 1994, *Australian Standard 1336-1994, Eye protection in the Industrial Environment*, Standards Association of Australia, Sydney.

17. Standards Australia/Standards New Zealand 1992, *Australian/New Zealand Standard 1337-1992, Eye Protectors for Industrial Applications*, Standards Association of Australia/Standards Association of New Zealand, Sydney/Wellington.

18. Standards Australia 1987, *Australian Standard 2919-1987, Industrial Clothing*, Standards Association of Australia, Sydney.

19. Standards Australia 1978, *Australian Standard 2161-1978, Industrial Safety Gloves and Mittens (excluding electrical and medical gloves)*, Standards Association of Australia, Sydney.

20. Standards Australia/Standards New Zealand 1994, *Australian/New Zealand Standard 2210-1994, Occupational Protective Footwear*, Standards Association of Australia/Standards Association of New Zealand, Sydney/Wellington.

21. Standards Australia/Standards New Zealand 1994, *Australian/New Zealand Standard 1716-1994, Respiratory Protective Devices*, Standards Association of Australia/Standards Association of New Zealand, Sydney/Wellington.

22. National Occupational Health and Safety Commission 1994, *National Code of Practice for the Preparation of Material Safety Data Sheets [NOHSC:2011(1994)]*, Australian Government Publishing Service, Canberra.

Attachment 1

The Draize Scale for evaluation of skin reactions is as follows:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale for evaluation of eye reactions is as follows:

CORNEA

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
Diffuse beefy red	3 severe	Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
		Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe