10 October 2001
05/02

DRAFT ASSESSMENT REPORT

(FULL ASSESSMENT - SECTION 15 OF THE ANZFA ACT)

APPLICATION A435

LIPASE FROM GENETICALLY MODIFIED *ASPERGILLUS ORYZAE*

EXECUTIVE SUMMARY

- The Australia New Zealand Food Authority (ANZFA) received an Application (A435) on 6 February 2001, from Novo Nordisk for the approval of a new source of the enzyme triacylglycerol lipase (EC 3.1.1.3), for use as a processing aid in the food industry. The Applicant seeks to include provision for lipase sourced from a strain of *Aspergillus oryzae (A. oryzae)*, which carries the gene coding for a lipase isolated from *Fusarium oxysporum*.

- Four submissions were received in response to the public consultation- two supported the proposal, one disagreed and the other had no comments. The main issues raised in the submissions were (i) the labelling of processing aids obtained from genetically modified organisms (GMOs), (ii) lack of technological justification and (iii) the similarity to previous applications.

- The scientific evaluations concluded that the use of lipase produced in *A. oryzae* carrying the donor gene from *Fusarium oxysporum*, is technologically justified and poses no additional risk to public health and safety. None of section 10 objectives in the *Australia New Zealand Food Authority Act 1991* are compromised by the proposed change to the *Food Standards Code*. It is recommended that the draft variation should come into effect on the date of gazettal.

- The Regulatory Impact Statement concluded that the benefits outweighed the cost in relation to the proposal to amend Standard 1.3.3 - Processing Aids to permit lipase from the new source organism *A. oryzae* carrying the donor gene from *Fusarium oxysporum*.

BACKGROUND

ANZFA received an Application (A435) on 06 February 2001, from Novo Nordisk for the approval of a new source of the enzyme, triacylglycerol lipase (EC 3.1.1.3), for use as a processing aid in the food industry. The applicant sought to include a provision for lipase sourced from a strain of *A. oryzae*, which carries the gene coding for a lipase isolated from *Fusarium oxysporum.*

The enzyme lipase is currently permitted for use as a processing aid, when sourced from a genetically manipulated strain of *A. oryzae* containing the gene for lipase isolated from *Humicola lanuginosa*, and is listed in Standard A16, Table IV- Enzymes, Group III- Microbial Origin (Volume 1) and Standard 1.3.3 - Processing Aids, Table to clause 17 – Permitted Enzymes of Microbial Origin (Volume 2) of the *Food Standards Code*. The applicant seeks to include another genetically modified strain of *A. oryzae*, carrying the gene coding for lipase isolated from *Fusarium oxysporum* to this list.

Standards A16 (Volume 1) and Standard 1.3.3 (Volume 2) of the *Food Standards Code* make provision for the appropriate use of approved processing aids in food manufacture. A processing aid is a substance used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food.

OBJECTIVE

To promote innovation in the food industry by approving the use of another source of lipase while protecting public health and safety.

RELEVANT PROVISIONS

Food Standards Code

Standard A16, Table IV- Enzymes, Group III- Microbial Origin (Volume 1) and Standard 1.3.3 - Processing Aids, Table to clause 17 – Permitted Enzymes of Microbial Origin (Volume 2) of the *Food Standards Code* do not include triacylglycerol lipase produced by *Aspergillus oryzae*, carrying the gene coding for lipase isolated from *Fusarium oxysporum*.

New Zealand Food Regulations

253 (2d) Food conditioners, permits the use of lipase without any reference to host organisms.

Codex

There is no Codex Standard for lipase produced by *Aspergillus oryzae*, carrying the gene coding for lipase isolated from *Fusarium oxysporum*.

REGULATORY OPTIONS

Option 1. Not approve the use of triacylglycerol lipase produced by *Aspergillus oryzae* carrying the lipase gene from *Fusarium oxysporum*.

Option 2. Approve the use of triacylglycerol lipase produced by *Aspergillus oryzae* carrying the lipase gene from *Fusarium oxysporum*.

It is considered that this change to the *Food Standards Code* is a liberalising measure under the Sanitary/Phytosanitary Agreement. Matters raised in this Full Assessment therefore **will** be notified to the WTO.

PUBLIC CONSULTATION

The Preliminary Assessment Report for A435 was released for public comment between 8 May 2001 and 20 June 2001. Four submissions were received in response to the public consultation. Two submitters supported the proposal to amend the *Food Standards Code* to widen the existing permission for lipase. One submitter disagreed with the application and proposed that the *status quo* be maintained. The fourth submitter had no comments on the proposed application. A table elaborating the comments from public submissions is included as an attachment to this report (Attachment 2).

ASSESSMENT

TOXICOLOGICAL EVALUATION

Application A435 to approve the use of lipase from a genetically modified microorganism involves the use of two organisms - *Aspergillus oryzae* (the source organism) and *Fusarium oxysporum* (the donor organism). *Aspergillus oryzae* is currently listed in Standard 1.3.3 as a microorganism permitted for use in the production of certain enzymes, and has a history of safe use.

There are no nutritional issues associated with the use of lipase produced using recombinant DNA technology. The enzyme is used as a processing aid only, and is not expected to be present in the final food as a result of its proposed food uses. If a residue did occur in the food it would be in the form of inactivated enzyme, and in any case would be metabolised like any other protein.

The safety of the source organism is an important consideration in the safety assessment for recombinant lipase. *A. oryzae* is not considered to be pathogenic, is widely distributed in nature and is commonly found in foods. Enzymes from *A. oryzae* are extensively used in food processing, and have been for many years. The organism from which the lipase gene is derived (*Fusarium oxysporum*) is a plant pathogen and has been associated with human infections. Specific strains from this species has been reported to produce various secondary metabolites, among others, fusaric acid, monoliformine and cearalenone. However, only a limited and well-characterized DNA fragment from the donor strain is used in the construction of the genetically modified strain. Further, the production strain is not detectable in the final enzyme product and the toxicology data also confirmed the safety of this product. The DNA used for transforming the *A. oryzae* host strain does not contain antibiotic resistant genes.

The genetic modification process involves the transfer of the lipase gene from *F. oxysporum* to *A. oryzae*. The recombinant organism was found to be stable during production fermentations. Southern blotting was used to investigate the stability of the integration of the lipase gene after large-scale fermentation, and found that the inserted DNA was stably integrated into the host genome.

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants arising from the host organism. The production organism in this case is non-toxic and non-pathogenic and, as long as good manufacturing practice is followed, the enzyme produced should be safe.

Lipase from the source organism, *A. oryzae* carrying the gene from *F. oxysporum* complies with the recommended purity specifications for food-grade enzymes issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[1] and the Food Chemicals Codex[2].

Three toxicological studies were submitted in support of this application. These consist of a 13-week oral toxicity study in rats, a bacterial mutagenicity assay (Ames Test) and a human lymphocyte cytogenetic assay. The tests were conducted in accordance with the Organisation for Economic Co-operation and Development Guidelines for Testing of Chemicals no. 408 (adopted 1998), no. 471 (1997) and no. 473 (July 1997) respectively. The test material was produced in the same manner as the commercial preparations. Enzyme activity was found to be 4,000 LU/g (defined as the activity of one gram of pure enzyme protein), and the total organic substance (TOS) content 3%.

This assessment of the genetically modified lipase produced by *A. oryzae* carrying the lipase gene produced by *Fusarium Oxysporum* found that:

- the source organism has a long history of safe use;
- the lipase gene is stably integrated into the host genome;
- the enzyme preparation complies with the JECFA specifications;
- the enzyme preparation causes no mutagenic or cytogenic effects in *in vitro* studies; and
- the NOEL from sub-chronic rat feeding studies is 0.830g TOS/kg/day.

Because the host organism is safe and because the genetic modifications are well characterised and specific utilising well-known plasmids for the vector constructs, and the introduced genetic material does not encode and express any toxic substances, it is concluded that the use of this genetically modified lipase as a processing aid in food would pose no significant risk to human health.

The full toxicological evaluation is available as an attachment to this full assessment (Attachment 3).

FOOD TECHNOLOGY ASSESSMENT

The use of the enzyme triacylglycerol lipase as a processing aid for the oils and fats industry for oil degumming and in the food industry to improve emulsifying properties is technologically justified and is not expected to result in its presence in food. A detailed Food Technology report is attached (Attachment 4).

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[1] FAO (1992) General Specifications for Enzyme Preparations. Compendium of Food Additives Specifications, Vol. 1, Annex 1.

[2] FCC (1996) Food Chemicals Codex 4th Edition, 1996. Gopinath, C., Prentice, D. & Lewis, D (1987) In: Atlas of Experimental Toxicological Pathology, M.T.P. Press Ltd., 13, 11-21.

ISSUES ARISING FROM PUBLIC SUBMISSIONS

Four submissions were received in response to the public consultation. Two submitters supported the proposal to amend the *Food Standards Code* to widen the existing permission for lipase. One submitter disagreed with the application and proposed that the *status quo* be maintained while another had no comments on the proposed application.

The main issues raised by one submitter were 1) the labelling of processing aids obtained from genetically modified organisms (GMOs), 2) lack of technological justification as other GE and non-GE lipases are available and 3) this application is similar to previous applications and therefore ought not to be accepted.

1. Labelling

Issue

The **National Council of Women of Australia** stated that the use of unlabelled processing aids derived from genetically modified organisms amounts to deceiving the public.

Background

Processing aids are not currently required to appear in ingredient lists under general labelling provisions in the FSC and the NZFR. There are numerous GM processing aids used by the food industry. Processing aids are generally present to fulfil a technological purpose relating to treatment or processing, but do not perform a technological function in the final food.

Evaluation and Conclusion

The labelling of foods produced using gene technology, was decided on at the Australia New Zealand Food Standards Council (ANZFSC) meeting on 28 July 2000. The ANZFSC decided to exempt processing aids and food additives except where novel DNA and/or protein is present in the final food. While the gene coding for the lipase enzyme from the donor strain is novel which is however not present in the final food, the enzyme itself is not considered novel.

2. Technological Justification

Issue

The **National Council of Women of Australia** considered that there was no technological justification for the use of this product as other GE- and non-GE lipases are available.

Evaluation and Conclusion

The enzyme used by this method leads to improved yields in the de-gumming process, the purity of the vegetable oil and the emulsifying properties of lecithin and egg yolk as well as improved storage stability of the oil. The food technology evaluation concluded that the use of genetically modified triacylglycerol lipase as a processing aid in food is technologically justified. The enzyme is not expected to carry over into the final food when used as a de-gumming agent. When used in the emulsification process, the enzyme improves the emulsifying properties of lecithin and egg yolk and is heat inactivated after the reaction.

No reaction products, which could not be considered normal constituents of the diet, are formed during the production or storage of the enzyme treated food.

3. Similarity of applications

Issue

The National Council of Women of Australia commented that this application is similar to earlier GE lipase applications and hence should not be approved.

Evaluation and Conclusions

ANZFA considers that the additional source of lipase is safe, and will not lead to deceptive practices. This enzyme is used for applications different from currently permitted enzymes.

REGULATORY IMPACT ANALYSIS

The objective of regulatory impact analysis is to examine the impact of the permission to use lipase from a new source organism, as a processing aid in Standard 1.3.3.

As the use of lipase from genetically modified source organism *A. oryzae* requires pre–market approval it is not appropriate to consider non–regulatory options to address this application. Processing aids used in Australia and New Zealand are required to be listed in Standard 1.3.3. New entries in the schedule to Standard 1.3.3 are required to undergo an evaluation to ensure there is no public health and safety concerns.

IDENTIFICATION OF AFFECTED PARTIES

Parties affected by the options listed above include:

- State, Territory and New Zealand Health Departments;
- manufacturers and producers of food products that use lipase as a processing aid; and
- consumers.

OPTION 1

The status quo would be maintained and no specific permission would be given in the Code for the use of lipase from genetically modified *A. oryzae* carrying the *Fusarium oxysporum* gene.

BENEFITS

Government No perceived benefits.

Consumers No perceived benefits.

Industry No perceived benefits.

COSTS

Government No perceived cost at present. However, in the future, if other countries approve lipase from the new genetically modified source organism, lack of approval in Australia or New Zealand may be construed as a non-tariff barrier to trade.

Industry Industry would be denied the availability of an alternative source of lipase.

Consumers No costs.

OPTION 2

The Code would be amended to specifically permit the use of lipase from *A. oryzae* carrying the *Fusarium oxysporum* lipase gene and inserted by plasmid pMStr20.

BENEFITS

Government Approval of lipase from a new genetically modified source organism would promote international trade and reduce technical barriers to trade, while continuing to protect public health and safety.

Industry Promotes fair trade in food. This option will allow manufacturers to use alternative source of lipase.

Consumers No benefit.

COSTS

Government No cost.

Industry No cost.

Consumers No cost.

Evaluation

OPTION 1

Parties disadvantaged by not permitting this particular processing aid, are the manufacturers of lipase and producers who may use it in the manufacture of their final food products.

OPTION 2

This is the preferred option. Approval would allow an alternative safe source of lipase with no cost to government, industry or consumers.

WORLD TRADE ORGANISATION (WTO) NOTIFICATION

Australia and New Zealand are members of the WTO and are bound as parties to WTO agreements. In Australia, an agreement developed by the Council of Australian Governments (COAG) requires States and Territories to be bound as parties to those WTO agreements to which the Commonwealth is a signatory. Under the agreement between the Governments of Australia and New Zealand on Uniform Food Standards, ANZFA is required to ensure that food standards are consistent with the obligations of both countries as members of the WTO.

In certain circumstances Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment. Notification is required in the case of any new or changed standards that may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists).

It is considered that this change to the Food Standards Code is a liberalising measure under the Sanitary/Phytosanitary Agreement. Matters raised in this Full Assessment therefore **will** be notified to the WTO.

CONCLUSIONS

The draft assessment report concludes that approval of the use of lipase from a new source organism is technologically justified and poses no significant risk to public health and safety.

Approval for use will provide Australian manufacturers with a processing aid which is claimed to be more cost-effective and technologically efficient to manufacture and use.

The draft variation should come into force on gazettal.

FOOD STANDARDS SETTING IN AUSTRALIA AND NEW ZEALAND

The Governments of Australia and New Zealand entered an Agreement in December 1995 establishing a system for the development of joint food standards. On 24 November 2000, Health Ministers in the Australia New Zealand Food Standards Council (ANZFSC) agreed to adopt the new *Australian New Zealand Food Standards Code*. The new Code was gazetted on 20 December 2000 in both Australia and New Zealand as an alternate to existing food regulations until December 2002 when it will become the sole food code for both countries. It aims to reduce the prescription of existing food regulations in both countries and lead to greater industry innovation, competition and trade.

Until the joint *Australia New Zealand Food Standards Code* is finalised the following arrangements for the two countries apply:

- **Food imported into New Zealand other than from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code,* as gazetted in New Zealand, or the New Zealand *Food Regulations 1984,* but not a combination thereof. However, in all cases maximum residue limits for agricultural and veterinary chemicals must comply solely with those limits specified in the New Zealand *(Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.*

- **Food imported into Australia other than from New Zealand** must comply solely with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code,* but not a combination of the two.

- **Food imported into New Zealand from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* as gazetted in New Zealand, but not a combination thereof. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

- **Food imported into Australia from New Zealand** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code,* but not a combination of the two. However, under the provisions of the Trans-Tasman Mutual Recognition Arrangement, food may **also** be imported into Australia from New Zealand provided it complies with the New Zealand *Food Regulations 1984.*

- **Food manufactured in Australia and sold in Australia** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* but not a combination of the two. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

In addition to the above, all food sold in New Zealand must comply with the New Zealand *Fair Trading Act 1986* and all food sold in Australia must comply with the Australian *Trade Practices Act 1974,* and the respective Australian State and Territory *Fair Trading Acts.*

Any person or organisation may apply to ANZFA to have the *Food Standards Code* amended. In addition, ANZFA may develop proposals to amend the Australian *Food Standards Code* or to develop joint Australia New Zealand food standards. ANZFA can provide advice on the requirements for applications to amend the *Food Standards Code*.

INVITATION FOR PUBLIC SUBMISSIONS

ANZFA has completed a Draft Assessment (Full Assessment under s.15 of the ANZFA Act) of the Application, prepared draft variations to the *Food Standards Code* and will now conduct an Final Assessment (Inquiry under s.17 of the ANZFA Act) to consider the draft variations and its regulatory impact.

Written submissions containing technical or other relevant information which will assist ANZFA in undertaking a final assessment on matters relevant to the application, including consideration of its regulatory impact, are invited from interested individuals and organisations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

Submissions providing more general comment and opinion are also invited. ANZFA's policy on the management of submissions is available from the Standards Liaison Officer upon request.

The processes of ANZFA are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of ANZFA and made available for inspection. If you wish any confidential information contained in a submission to remain confidential to ANZFA, you should clearly identify the sensitive information and provide justification for treating it in confidence.

The *Australia New Zealand Food Authority Act 1991* requires ANZFA to treat in confidence trade secrets relating to food and any other information relating to food, the commercial value of which would be or could reasonably be expected to be, destroyed or diminished by disclosure.

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A435** at one of the following addresses:

Australia New Zealand Food Authority
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222 Fax (02) 6271 2278

Australia New Zealand Food Authority
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942 Fax (04) 473 9855

Submissions should be received by ANZFA by: **21 November 2001.**

General queries on this matter and other ANZFA business can be directed to the Standards Liaison Officer at the above address or by Email on <slo@anzfa.gov.au>. Requests for more general information on the Authority can be directed to the Information Officer at the above address or by Email <info@anzfa.gov.au>.

ATTACHMENTS

1 Draft Variation to the *Food Standards Code*.
2 Summary of Public Submissions.
3 Toxicological Report.
4 Food Technology Report

ATTACHMENT 1

DRAFT VARIATIONS TO *FOOD STANDARDS CODE*

To commence: On gazettal

[1] ***Standard A16*** *of Volume 1 is varied by omitting* Footnote 9 *to* Table IV - Enzymes, Group III - Microbial Origin, *substituting* -

[9] Lipase may be produced from a genetically manipulated strain of *Aspergillus oryzae* containing the gene for lipase isolated from (i) *Humicola lanuginosa* and inserted by plasmids pBoel1960 and p3SR2 or (ii) *Fusarium oxysporum*.

[2] ***Standard 1.3.3*** *of Volume 2 is varied by deleting the entry for* Lipase, triacylglycerol EC [3.1.1.3] *and corresponding sources from the Table to clause 17, substituting* -

Lipase, triacylglycerol EC [3.1.1.3]	*Aspergillus niger* *Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Fusarium oxysporum* *Aspergillus oryzae*, containing the gene for Lipase, triacylglycerol isolated from *Humicola lanuginosa* *Rhizopus arrhizus* *Rhizomucor miehei* *Rhizophus niveus* *Rhizophus oryzae*

ATTACHMENT 2

SUMMARY OF PUBLIC SUBMISSIONS RECEIVED
A435 – LIPASE AS A PROCESSING AID

No.	Organisation	Position	Comments
1	**National Council of Women of Australia**	Supports Option 1	Considers that without labelling the genetically engineered processing aid will deceive public. Considers that this application is not technologically justifiable as other GE and non-GE lipases are available. Use of this lipase should not be approved because of its similarity to other applications.
2	**Public Health Services, Queensland Health**	Supports Option 2	Endorses ANZFA's approach regarding conditions of use such as a requirement to comply with specifications for identity and purity.
3	**Food Technology Association, Victoria Inc.**	Supports option 2.	Supports provided there are no health and safety issues. Requests that they be maintained on the circulation list for any further changes in this matter.
4	**Public Health Directorate, Ministry of Health, NZ**	No comments	Has no comments regarding this application

ATTACHMENT 3

SAFETY ASSESSMENT REPORT

A435 – LIPASE FROM GENETICALLY MODIFIED *ASPERGILLUS ORYZAE*

INTRODUCTION

Application A435 to approve the use of lipase from a genetically modified microorganism involves the use of two organisms - *A. oryzae* (the source organism) and *F. oxysporum* (the donor organism).

The enzyme is used as a processing aid only, and is not expected to be present in the final food. Any residue would be in the form of inactivated enzyme, which would be metabolised like any other protein.

The source (production) organism - A. oryzae

The safety of the source organism is an important consideration in the safety assessment for recombinant lipase. *A. oryzae* is not considered to be pathogenic, is widely distributed in nature and is commonly found in foods (Barbesgaard et al, 1992). Enzymes from *A. oryzae* are extensively used in food processing, and have been for many years (Rogers, 1977).

The donor organism – F. oxysporum

The organism from which the lipase gene is derived (*Fusarium oxysporum*) is a plant pathogen and has been associated with human infections. Specific strains from this species have been reported to produce various secondary metabolites, among others, fusaric acid, monoliformine and zearalenone (Marasas *et al*., 1984). However, only a limited and well-characterized DNA fragment from the donor strain is used in the construction of the genetically modified strain. None of the secondary metabolites were detectable in the final enzyme product.

Nature of the genetic modification

The genetic modification process involved the transfer of the lipase gene from *F. oxysporum* to *A. oryzae*. The recombinant organism was found to be stable during production fermentations. Southern blotting was used to investigate the stability of the integration of the lipase gene after large-scale fermentation, and found that the inserted DNA using plasmid pMStr20 was stably integrated into the host genome.

Purity of enzyme preparation and proposed specifications

Historically, enzymes used in food processing have been found to be non-toxic, and the main toxicological consideration is in relation to possible contaminants. The production organism in this case is non-toxic and non-pathogenic. The detailed specifications to which the preparation was found to conform are shown in Table 1.

Table 1. Complete specification of lipase preparation

Criteria	Specification
Heavy Metals	not more than 30 ppm
Lead	not more than 5 ppm
Arsenic	not more than 3 ppm
Total viable count	not more than $5x10^4$
Total coliforms/g	not more than 30
Enteropathogenic E. coli/25g	negative by test
Salmonella/25g	negative by test
Antimicrobial activity	negative by test
Mycotoxins	negative by test
Production organism	negative by test

Lipase from the source organism, *A. oryzae* carrying the gene from *F. oxysporum* complies with the recommended purity specifications for food-grade enzymes issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC, 1996).

TOXICOLOGY STUDIES

Three toxicological studies were submitted in support of this application. These were a 13-week oral toxicity study in rats, a bacterial mutagenicity assay (Ames Test) and a human lymphocyte cytogenetic assay. The tests were conducted in accordance with current OECD Guidelines and they were conducted in accordance with the principles of Good Laboratory Practice (OECD, 1997, 1998). The test material was produced in the same manner as the commercial preparations. The enzyme has activity towards triglyceride and phospholipid substrates and is measured in LEU (T), an assay based on the hydrolysis of lecithin under constant pH and temperature. The activity was measured to be 22,700 LEU(T)/g with an amount of 8.3% Total Organic Solids.

Toxicity study by oral (gavage) administration to Sprague-Dawley rats for 13 weeks. Scantox Lab. No. 35125, Novo Norsdik Study No. 20006004: Lipase, batch PPW 6703. *January 16, 2001*.

Methods

Three groups of Sprague-Dawley Mol:SPRD rats (10/sex/group) were dosed lipase by gavage at 0.083, 0.249 and 0.830 g TOS/Kg/day using a constant dose volume of 10ml/kg bw/day for 13 weeks as per OECD Guidelines (No. 408, 1998). A similar constituted group received the vehicle (tap water) and served to generate contemporaneous control data.

Rats were observed twice daily for evidence of systemic toxicity or ill health and detailed clinical observations were conducted once weekly. Body weight and food consumption was recorded weekly. An eye examination of all animals was conducted before the study period and on all control and high dose animals before termination of the study.

Haematological, coagulation, and blood chemistry parameters were also measured, and urinalysis carried out, in week 13 of the study. After 13 weeks all animals were killed and subjected to a detailed necropsy, including organ weight analysis and histopathology.

Results

There were two premature deaths in the rats receiving lipase and one from the control group. For the remaining animals there were no clinical signs shown during the 13-week study period, although abscesses were observed in the one animal each from the control and high dose groups.

Food consumption was reduced in the high dose male groups only, for the first 3 to 6 weeks (max -7%). Thereafter the amount consumed was similar to that of the control group, and over the 13 week period there were no significant differences between control animals and those receiving lipase. However there was a drop in the food consumption towards the end among all groups (from a maximum of 140g in week 4 to 115g in week 13 for females and from a maximum of about 205g in weeks 4-6 to about 170g in week 13). The reduction in food uptake was very similar between control and test groups. The amount of food scattered was comparable between all groups, suggesting that all diets were palatable, even at the highest lipase dose, and the reduction in food consumption did not significantly affect weight gain.

There were no apparent differences between treated and control animals in the open field testing (ambulation, rearing, grooming and faecal boli), ophthalmoscopy and stimuli-induced clinical observations that could be related to treatment with test article.

Minor haematological differences in the levels of eonosine, alanine amino-transferase and aspartate amino-transferase activities were noted between control and test groups.

Minor differences in the urine volume and detectable nitrite levels were also noted between control and test groups:

No significant histopathological changes were observed apart from moderate diffuse subacute pericarditis in one low-dose male and mammary gland adenomatous fibrodenoma in one high-dose female and pappilomatus adenocarcinoma in one control female.

Discussion and conclusions

Sub-chronic administration of lipase at the doses mentioned above was associated with effects upon food intake, heart and renal function. Effects on the lack of appetite seen in high dose animals in the initial period may represent an adaptive response to the gavage administration.

Cardiac and other related changes (myocarditis and high plasma aspartate amino-transferase activities) are normal in aging rats. As such, they are of little toxicological significance.

In conclusion daily treatment with test substance at concentrations of up to 0.830 g TOS/kg/ day for 13 weeks resulted in no treatment related effects. The NOEL for lipase is therefore 0.830g TOS/kg/day, which is the highest dose used in this study.

Test for Mutagenic Activity with *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *Escherichia coli* WP2uvrA
Study No. 20008020: Lipase, batch PPW 6703, by P.B. Pedersen, Toxicology Enzyme Business (Novo Nordisk A/S), Denmark. *June 28, 2000*.

Lipase (the same preparation as for the subchronic study) was examined for mutagenic activity in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA in accordance with OECD Guidelines (No. 471, July 1997). A liquid culture assay was applied and bacteria exposed to six doses of the test substance in a phosphate buffered broth for three hours with 5mg/ml as the highest concentration. After incubation the test substance was removed by centrifugation, plated, and the number of both revertants to prototrophy and viable cells estimated.

The part of the study comprising *E. coli* was conducted using the direct plate incorporation assay. Six doses of the test substance were applied with 5 mg/plate as the highest dose level followed by successive bi-sections between doses. The test was carried out both in the presence and absence of metabolic activation (in the form of a liver preparation, S-9, and co-factors required for mixed function oxidase activity). The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens (2-Aminoanthracene, 9-Aminoacridine, N-methyl-N-nitro-N-guanidine, N-ethyl-N-nitro-N-guanidine, benzo(a)pyrene and 2-Nitrofluorene).
No dose-related or reproducible increases in revertants to prototrophy were obtained with any of the bacterial strains exposed to lipase either in the presence or absence of metabolic activation. A repeat experiment confirmed these results. It was concluded that the test material lipase PPW 6703 did not exhibit any mutagenic activity under the conditions of the test.

Chromosome aberration assay in cultured human lymphocytes.
Novo Nordisk Study No. 996050: Lipase, batch PPW 6703, by R. Marshall, Toxicology Enzyme Business (Novo Nordisk A/S), Denmark. *December 06, 2000*.

The potential of lipase(Batch PPW 6703) to damage the chromosomal structure was tested in an *in vitro* cytogenetics assay, using duplicate human lymphocyte cultures from a single female donor, in accordance with OECD Guidelines (No. 473, July 1997). Tests were carried out in the presence and absence of S-9 metabolic activation, over a broad range of doses. The highest dose for chromosome analysis from cultures sampled at 20 hours should be one at which at least 50% mitotic inhibition has occurred or should be the highest dose tested. In Experiment 1, where the treatment in the absence and presence of S-9 was for 3 hours followed by 17 hours recovery period prior to harvest, the highest concentration chosen for analysis, 5000 µg/ml, induced approximately 12% and 42% mitotic inhibition in the absence and in the presence of S-9 respectively. In Experiment 2, treatment in the absence and presence of S-9 was continuous for 20 hours. Treatment in the presence of S-9 was only for 3 hours followed by a 17-hour recovery period. Concentration chosen in this experiment, 5000 µg/ml and 1638 µg/ml, induced approximately 0% and 53% mitotic inhibition in the absence and presence of S-9 respectively.

Treatment did not produce biologically or statistically significant increases in the frequency of aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S-9 metabolic activation.

Positive controls (4-Nitroquinoline-1-oxide and Cyclophosphamide) gave the expected increases in the frequency of aberrant metaphases, indicating the efficacy of the metabolic activation mix and the sensitivity of the test procedure.

CONCLUSION

Lipase produced from *A. oryzae* has already been shown to be safe for use as processing aids for food. This assessment of the lipase produced by *A. oryzae* carrying the lipase gene from *F. oxysporum* found that:

- The source organism has a long history of safe use;
- The lipase gene is stably integrated into the host genome;
- The enzyme preparation complies with JECFA specifications;
- The enzyme preparation produced no evidence of genotoxic potential in *in vitro* assays;
- The NOEL from the sub-chronic rat feeding study is 0.830g TOS/kg bw/day.

From the information available, it is concluded that the use of the lipase from this source as a processing aid in food would pose no public health and safety risk.

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ATTACHMENT 4

FOOD TECHNOLOGY REPORT

Introduction

The Australia New Zealand Food Authority received an application from Novozyme A/S on 6th February 2001, seeking approval to include triacylglycerol lipase with *Aspergillus oryzae* as host strain and *Fusarium oxysporum* as donor strain in the Table to clause 17, Standard 1.3.3 – Processing Aids. The enzyme referred in this application is used as a processing aid in the oils and fats industry for vegetable oil de-gumming, obtaining lysophospholipid emulsifiers with altered emulsifying properties and improving the emulsifying properties of egg yolk.

Lipases

Lipases hydrolyse the three ester bonds of triacylglycerol (a lipid) to release free fatty acids from glycerol. Triacylglycerol lipase can be used in the oils and fats industry for de-gumming purposes. De-gumming is the term used for removing phospholipids during oil purification to ensure satisfactory taste and quality and to improve storage stability of the food oil or fat being produced.

Triacylglycerol lipase also hydrolyses one of the ester bonds of diacylphospholipids to release one free fatty acid and 2-acyl-1-lysophospholipid. This mode of action can be employed to improve the emulsifying action of lecithin and egg yolk. Lecithin is a phospholipid naturally occurring in egg yolk and soybean.

The applicant supplied a letter from Weston Technologies supporting the application for approval of the enzyme. Weston Technologies stated that using the enzyme allows them more flexibility in their recipes enabling them to reduce or possibly stop the use of emulsifiers in their bread production.

Triacylglycerol lipase

Possible alternative names are lipase, triacylglycerol acylhydrolase and phospholipase.

The marketing name is Lecitase Novo.

It has an Enzyme Commission identification number of EC 3.1.1.3 and a CAS number of 9001-62-1.

The host source is *Aspergillus oryzae* with the donor gene for lipase isolated from the donor organism *Fusarium oxysporum*.

The molecular weight of the enzyme is ~ 28 kDaltons (kDa).

The enzyme is sold as a pale brown water-soluble liquid.

The enzyme is produced using submerged fed-batch pure culture fermentation techniques common in the enzyme manufacturing industry. The production uses GMP (Good Manufacturing Practices), uses ingredients that are accepted for general use in foods and under controlled conditions that produce a food safe fermentation. Specific details of the process are commercial-in-confidence.

All equipment and processes are designed and operated to prevent microbial contamination. The aseptic fermentation vessels are cleaned and sterilised using water and steam. Microbiological testing is performed throughout the process to ensure there are no contaminating organisms.

Once the fermentation has completed the enzyme preparation is separated from the broth, partially purified, concentrated and stabilised. Solids, which include cell mass, are removed using diatomaceous earth filtration or centrifugation. Ultra filtration is used to remove low molecular weight impurities as well as evaporation to increase the activity/dry matter ratio. A final diatomaceous earth filtration is performed to remove any traces of production strain and microbial contaminants. The enzyme concentrate is stabilised by addition of salt and sugar.

Enzymes are proteins that catalyse chemical reactions and are used in very small amounts. During use of the enzyme as a de-gumming agent it remains predominately in the aqueous phase being poorly miscible in the oil. Residues are also removed during the further oil purification steps. The enzyme is deactivated with heat treatment after it is used to improve emulsification. In this usage the enzyme is not expected to be present in the final food. In the other proposed uses of this enzyme, it improves the emulsifying properties of lecithin and egg yolk.

The Applicant states that no reaction products, which could not be considered normal constituents of the diet, are formed during the production or storage of the enzyme treated food.

Lipase from *Aspergillus oryzae* is covered by the specification in Food Chemical Codex (FCC) 4th Ed., 1996. There is no Codex Standard for lipase produced from *Aspergillus oryzae* carrying gene from *Fusarium oxysporum*.

The product complies with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex, 4th Ed., 1996.

Conclusion

The use of the enzyme triacylglycerol lipase as a processing aid for the oils and fats industry for oil de-gumming and in the food industry to improve emulsifying properties is technologically justified.

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