FULL ASSESSMENT
AND REGULATORY IMPACT ASSESSMENT

SUBJECT: A371 – PHYTASE AS A PROCESSING AID

EXECUTIVE SUMMARY

- The Australia New Zealand Food Authority (ANZFA) received an application (A371) on 1 March 1999, from Novo Nordisk for the approval of the enzyme, 6-phytase (IUB 3.1.3.26), for use as a processing aid for starch, when produced in *Aspergillus oryzae* from a phytase gene isolated from *Peniophora lycii*. The commercial name for the enzyme product is Novozym 938.

- Nine submissions were received in response to the section 14 gazette notice. Three submitters supported the application. The Office of Regulation Review submitted comments pertaining to Regulatory Impact assessment. One submitter did not express any preference. Four submitters did not support the use of an enzyme derived from a genetically modified source organism, and on this basis did not support the application.

- The main issues raised by submissions were the labelling of processing aids obtained from genetically modified organisms (GMOs); and the importance of safety assessment for the new organism and the enzyme product.

- The scientific evaluations have concluded that the use of phytase produced in *Aspergillus oryzae,* from a phytase gene isolated from *Peniophora lycii,* is technologically justified and poses no additional risk to public health and safety. No significant concerns were raised in the public comment regarding the actual use or approval of the processing aid. None of ANZFA's section 10 objectives are compromised by the proposed change to Standard A16. It is recommended that the draft variation should come into effect on the date of gazettal.

- The Regulatory Impact Statement concluded that the amendment to Standard A16 of the *Food Standards Code* to permit phytase from the new source organism *Aspergillus oryzae* carrying the donor gene from *Peniophora lycii,* is necessary, cost effective and of benefit to both producers and consumers.

BACKGROUND

ANZFA received an application (A371) on 1 March 1999, from Novo Nordisk for the approval of the enzyme, 6-phytase (IUB 3.1.3.26), for use as a processing aid for starch, when produced in *Aspergillus oryzae* from a phytase gene isolated from *Peniophora lycii*. The commercial name for the enzyme product is Novozym 938.

The enzyme phytase is currently permitted for use as a processing aid, when sourced from the organism *Aspergillus niger* in Standard A16 in the Australian *Food Standards Code*. The applicant seeks to vary the list of approved source organisms in Standard A16 - Processing Aids, for the enzyme phytase. The variation would constitute an extension of recognised source organisms to include a genetically modified strain of *Aspergillus oryzae*, carrying the gene coding for phytase isolated from *Peniophora lycii*.

Standard A16 makes 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.

No comparable standard for processing aids exists in the *New Zealand Food Regulations 1735.* Processing aids are either regulated as food additives or are not specifically regulated. Under the Review of the *Food Standards Code*, a joint processing aids standard for Australia and New Zealand has been proposed and the proposal (P188) has recently been released for public comment.

OBJECTIVE

To promote innovation in the food industry while protecting public health and safety.

RELEVANT PROVISIONS

Australian Food Standards Code

Standard A16 - Processing Aids

New Zealand Food Regulations

There is no comparable standard for processing aids in the NZFR. Processing aids are generally not treated in a uniform manner. A limited number of substances are identified in the NZ Food Regulations as processing aids, and these are exempt from the general labelling provisions.

Codex Alimentarius Commission

Codex have developed an 'Inventory of Processing Aids', which is not intended to be a complete or "positive" list or permitted processing aids.

REGULATORY OPTIONS

Option 1

The status quo would be maintained and no specific permission would be given in the *Food Standards Code* for the use of phytase from the source organism *Aspergillus oryzae*.

Option 2

The *Food Standards Code* would be amended to specifically permit the use of phytase from the source organism *Aspergillus oryzae*.

The proposed variation to the *Food Standards Code* constitutes a minor technical change and is not envisaged to effect trade for either technical or sanitary or phytosanitary reasons. Therefore a notification to the World Trade Organization is not required.

PUBLIC CONSULTATION

The preliminary assessment report for A371 was released for public comment between 26 June 1999 and August 4 1999. Nine submissions were received in response to the public notification. Three submitters supported the application to extend the list of approved source organisms in Standard A16. The Office of Regulation Review submitted comments pertaining to Regulatory Impact assessment. One submitter, The Victorian Food Safety Council did not express any preference, but simply noted that ANZFA would be undertaking a Full Assessment of the issue. Four submitters did not support the inclusion of a genetically modified source organism, and therefore did not support the application. A table elaborating the comments from public submissions is included as an attachment to this report (Attachment 3).

ASSESSMENT

TOXICOLOGICAL EVALUATION

Phytase from recombinant *Aspergillus oryzae*

Aspergillus oryzae has a history of safe use in the food industry and is widely used for the production of food grade enzymes. The joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded in 1987 that this organism is a traditionally accepted constituent of food.
Phytase from the organism *A.niger* has already been evaluated and approved for use as a food grade enzyme, in the Australian *Food Standards Code*.

Nutritionally, there are no positive or negative effects associated with the use of phytase. The active enzyme will not be present in the final food, because any residue is found in the form of inactivated enzyme that is metabolised as protein.

6-phytase (IUB 3.1.3.26) produced from the source organism, *A. oryzae* carrying a donor gene from *Peniophora lycii*, complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC), 4th ed., 1996. It also conforms to the General Specifications for Enzyme Preparations as proposed by the JECFA in Compendium of Food Additives Specifications, Vol. 1, FAO (1992).

Three toxicological studies were submitted in support of this application. These consist of a bacterial mutagenicity assay, *in vitro* chromosomal damage test and a 13-week oral toxicity study in the rat.

Phytase produced from the genetically modified source organism *A.oryzae* carrying a donor gene from *Peniophora lycii*, did not exhibit any toxicological effects that would be associated with its use as a processing aid.

The full toxicological evaluation is available as an attachment to this full assessment (Attachment 4).

FOOD SCIENCE AND TECHNOLOGY REPORT

6-Phytase will be used in exactly the same way as the phytase already permitted in the Australian *Food Standards Code* therefore a food technology report is not necessary.

ISSUES RAISED IN PUBLIC SUBMISSIONS

Labelling of Processing Aids

Seven submitters raised concerns that processing aids from GMOs are not required to be labelled under current regulation.

Evaluation

Recently, the labelling of processing aids was addressed in the Review of Ingredients Lists (Proposal P143), which was completed in February 1999. Processing aids were proposed to be generally exempt from the requirements to be declared in ingredient lists, unless they contain substances that require a mandatory declaration of their presence in food (proposed Standard 1.2.1 Mandatory Information, and 1.2.4 Labelling of Ingredients). Proposal P161 proposes the mandatory declaration of a list of foods and food additives that may cause severe adverse reactions. If the processing aid is one of these foods or a derivative of one of these foods then it will be required to be declared in the label.
The approach taken by the general review of processing aids would apply to the products within this application, therefore all comments regarding the labelling of processing aids whether from GMOs or not, have been referred to that review project.

The labelling of foods produced using gene technology, including whether there is a need for processing aids derived from GMOs to be labelled, is currently a matter under consideration by the Australia New Zealand Food Standards Council (ANZFSC) and will not be expressly dealt with under this application.

Processing aids from GMOs

The main issues raised by submissions were about the specific use of genetic modification to obtain the new source organism. There are concerns regarding the safety of such technology and the resulting products. Submitters were concerned that the phytase enzyme itself is genetically modified.

Background

Currently permission exists in the *Food Standards Code* for phytase to be sourced from the fungus *Aspergillus niger*. In this application, it is obtained from a related micro-organism *Aspergillus oryzae* (the source) which has been genetically modified, using recombinant DNA techniques, to carry a gene from another fungus *Peniphora lycii* (the donor). *A. oryzae* is a traditionally accepted constituent of food.

Evaluation

While the processing aid is the product of the genetic modification of a micro-organism, it is not itself modified. The resulting enzyme phytase is the same enzyme that would be obtained from the already approved *A niger*. However, the method of using recombinant technology to modify a source organism, allows for more efficient production of phytase, and therefore a cheaper final product.

The enzyme product (phytase) is collected during and after fermentation by the micro-organisms. There would be no micro-organisms remaining in the collected product, when added into a food manufacturing process. Any enzymes remaining in the food in which they are used as a processing aid are no longer biologically active as enzymes are used at very low concentrations and are usually inactivated, or even removed before the finished food is sold. Remaining inactivated enzymes would be metabolised as protein.

Toxicological evaluation

Three submitters urged that a toxicological evaluation on the new combination be undertaken to establish if any public health and safety threats exist from either the enzyme or the micro-organism.

Evaluation

Toxicological evaluations form part of the usual ANZFA assessment procedure for any new food additive, processing aid or similar type of product. The results of the toxicological evaluation undertaken as part of this assessment indicate that there are no concerns relating to either the toxicity or pathogenicity of *Aspergillus oryzae* carrying the *Peniophora lycii* gene. The results of the evaluation are in the scientific evaluation section of this paper, below.

REGULATORY IMPACT ANALYSIS

The objective of regulatory impact analysis is to examine labelling and other issues arising from permission to use phytase, from a new source organism, as a processing aid in Standard A16. A cost/benefit approach is undertaken to meet ANZFA's objectives as described in section 10 of the *Australia New Zealand Food Authority Act 1991*.

As the use of phytase from the new source organism *Aspergillus oryzae* requires pre–market approval it is not appropriate to consider non–regulatory options for the Regulation Impact Statement. Currently processing aids used in Australia are listed in Standard A16. New entries in the schedule to Standard A16 are required to undergo an evaluation to ensure there are no health and safety concerns with permitting their use. The standard is intended to reflect current use and prohibit inappropriate use of processing aids.

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 phytase as a processing aid; and
- consumers.

OPTION 1

The status quo would be maintained and no specific permission would be given in the *Food Standards Code* for the use of phytase from the source organism *Aspergillus oryzae*.

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 phytase from the new source organism, lack of approval in Australia may be construed as a non-tariff barrier to trade.

Industry Industry may be denied the availability of this processing aid, which may affect their ability to save on production costs in this area.

Consumers Consumers may be denied cheaper food products that would be a result of use reduced costs to food industry.

OPTION 2

The *Food Standards Code* would be amended to specifically permit the use of phytase from the source organism *Aspergillus oryzae*.

BENEFITS

Government	Approval of phytase from a new source organism may in the future promote international trade and reduction of 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 a cheaper more efficiently obtained processing aid in food production.
Consumers	Consumers may have greater access to cheaper products.

COSTS

Government	Cost of amending the *Food Standards Code*.
Industry	Possible loss in sales from consumer reaction to food which has been produced using a processing aid derived from a genetically modified organism.
Consumers	Consumers who object to the use of processing aids derived from genetically modified organisms in food may have reduced food choices. This is a commercial matter manufacturers will need to address. The issue of labelling of such products is under consideration by ANZFSC.

Evaluation

Option 1

Parties disadvantaged by the current state of regulation, which would not permit this particular processing aid are the manufacturers of phytase and producers who may use it in the manufacture of their final food products. This option would essentially deny Australian industry and consumers assess to a cheaper product.

Option 2

This is the preferred option. The assessment indicates that this application raises no new issues which would preclude phytase from a new source organism being included in Standard A16 – Processing Aids.

The amendment to Standard A16 of the *Food Standards Code* to permit phytase from the new source organism *Aspergillus oryzae* carrying the donor gene from *Peniophora lycii,* is necessary, cost effective and of benefit to both producers and consumers

ASSESSMENT AGAINST ANZFA OBJECTIVES

Protection of public health and safety

Toxicological evaluation of phytase from the new source organism *Aspergillus oryzae* indicates that there are no public health and safety concerns identified with its use, that are associated with either the enzyme, or the source or donor organisms. This is addressed in full by the Toxicology Report (in Attachment 4) and in the issues raised in public submissions. The enzyme phytase is already approved as a food-grade processing aid.

The provision of adequate information relating to food to enable consumers to make informed choices and to prevent fraud and deception.

Currently, there is no general requirement within the Australian *Food Standards Code* for the declaration of processing aids in ingredient lists. This is because their presence, if any, in the food is incidental to the final product. The labelling of processing aids is being addressed under Proposal P143 – Review of Ingredient Lists. Processing aids are proposed to be generally exempt from requirements to declare their presence in ingredient lists unless they contain substances that require a mandatory declaration of their presence in food, eg if they may cause severe adverse reactions.
The labelling of food produced using gene technology, including food produced using processing aids derived from GMOs, is an issue under consideration by ANZFSC.

Promotion of fair trading in food.

Approval for the use of phytase from *Aspergillus oryzae* in the manufacture of food will be a provision available for all manufacturers and should not impact on fair trading in food.

Promotion of trade and commerce in the food industry.

If approved, this application would aid promotion of trade and commerce in the food industry, through the availability of a more efficient and cost-effective methods of production to manufacturers of processing aids. This saving would arguably be passed on to consumers.

Promotion of consistency between domestic and international food standards.

There are no international standards that are relevant to the scope of this application.

OTHER RELEVANT MATTERS

Currently ANZFA is undertaking a review of Standard A16 and Standard A11 as part of the overall development of a Joint *Food Standards Code* for Australia and New Zealand. The proposed variation to A16 if accepted would finally appear in the joint provisions for the regulation of processing aids.

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).

A variation in the Code to extend the listed recognised source organisms of the processing aid phytase constitutes a minor technical change. This change will not effect trade issues for either technical or sanitary or phytosanitary reasons. Therefore a notification to the WTO on grounds relating to the Technical Barrier to Trade Agreement or Sanitary or Phytosanitary Agreement is not required.

CONCLUSIONS

The full assessment report concludes that approval of the use of phytase from a new source organism is technologically justified and poses no 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.

General processing issues have been referred to the Review of Processing Aids (P188). The issue of labelling of processing aids derived from genetically modified organisms is currently under consideration by ANZFSC.

The draft variation should come into force on gazettal.

ATTACHMENTS:

1 Draft Variation to the Food Standards Code.

2 Explanatory Notes.

3 Summary of Public Comment.

4 Toxicological Report – Complete.

DRAFT VARIATION TO THE AUSTRALIAN FOOD STANDARDS CODE

To commence: On gazettal

Standard A11 of the *Food Standards Code* is varied by inserting in columns 1 and 2 respectively of the Table in the Schedule, after the entry for "Phylloquinone" –

6-Phytase	FCC p107 (enzyme preparations)

Standard A16 of the *Food Standards Code* is varied by inserting in column 2 of the Table IV, Group III of the Schedule, in relation to the entry in Column 1 for "Phytase" -

Aspergillus oryzae

Attachment 2

EXPLANATORY NOTES

Document available separately

Attachment 3

PUBLIC COMMENT RECEIVED
A371 – PHYTASE AS A PROCESSING AID

No.	Organisation	Position	Comments
1	National Council of Women of Australia	In **support** of Option 1	Consider the exclusion from labelling for processing aids to be contrary to consumers having an informed choice. As this product is derived from gene technology it should not be allowed until consumers have information freely available. Applications for foods from GT should be rejected until the foods are tested approved and labelled accordingly. There are other processing aids available.
2	Donella Peters	In **support** of Option 1	As genetic engineering is a new and very untested technology, and we don't know what effects we may see from it some years down the track, this should not be allowed. There is too much potential for it to prove detrimental to out health and food producers should not be using us as guinea pigs.
3	Elaine Attwood	In **support** of Option 1	Same as for NCW. • Labelling for increased consumer awareness is important. • With the current disquiet surrounding all aspects of gene technology related to food no new permissions for any GT product should be granted. • Consumers should be able to make informed choices and cannot whilst PA's are exempt from labelling. • Plenty of other PA's available.

4	Arnold Ward	**Support** the Option 1	As the processing aids are already in use and are based on the natural organism, what possible reason can there be for introducing a genetically modified version? Whenever there is a genetic modification of a natural organism there is a always the potential for something to go wrong. • Provides excerpts from the literature and media. • Gives the example of L-tryptophan and FDA findings. • Discusses the faults of the substantial equivalence concept. • Requests copies of the tests performed by ANZFA that indicates that products made using the processing aids are absolutely safe. If not why not?
5	Office of Regulation Review (ORR)	**Do not state a position**	Provide comment on developing the Regulatory Impact Statement. • Suggest that if the products are genetically modified that this is made more explicit. • The RIS should indicate that Govts have intervened in the market for processing aids for reasons of Public Health and Safety, and hence manufacturers must seek amendment to seek new market access.
6	InforMed Systems Ltd.	**Support the application** with conditions. (see comments)	• Provided it can be shown that adequate documentation is provided about the safety of this product in the human diet. • Agrees that no scientific justification exists for labelling, cautions that such a requirement would be advisable in the present climate.

7	FTA Victoria	**Support the application** with conditions. (see comments)	Accepts the application provided that: • the toxicological safety assessment is satisfactory • consideration for the labelling of genetically modifiec processing aids. • further consideration will need to be given to other genetically modified enzymes.
8	Western Australia Health	**Support the application**	ANZFA should ascertain if there are any public health and safety concerns associated with the use of *Peniophora lycii* (donor organism) via toxicological evaluation
9	Victorian Food Safety Council Standards Sub-Committee	**Do not state a position**	Issues: • hopefully safety will be addressed during the Full Assessment. • Seek inclusion of detail of the source of the enzymes ir Standards A16 indicating that they may be derived from recombinant strains and an indication of how genes are inserted (this comment also referred to the review team for Standard A16) • Note that ANZFA are undertaking a full assessment.

TOXICOLOGICAL ASSESSMENT

Phytase – processing aid for starch

1. Introduction

Aspergillus oryzae has a history of safe use in the food industry and is widely used for the production of food grade enzymes. The joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded in 1987 that this organism is a traditionally accepted constituent of food.

Nutritionally, there are no positive or negative effects associated with the use of phytase. The active enzyme will not be present in the final food, because any residue is found in the form of inactivated enzyme that is metabolised as protein.

2. Purity of enzyme preparation and proposed specifications

6-phytase (IUB 3.1.3.26) produced from the source organism, *A. oryzae*, complies with the purity criteria recommended for enzyme preparations in Food Chemicals Codex (FCC), 4th ed., 1996, and also conforms to the General Specifications for Enzyme Preparations as proposed by the JECFA in Compendium of Food Additives Specifications, Vol. 1, FAO (1992).

3. Evaluation of the submitted studies

Three toxicological studies were submitted in support of this application. These consist of a bacterial mutagenicity assay, *in vitro* chromosomal damage test and a 13-week oral toxicity study in the rat.

3.1 Phytase (Batch Number PPQ5938): Testing for mutagenic activity with strains of *Salmonella typhimurium* and *Escherichia coli* in the direct plate incorporation assay. Novo Nordisk Study No. 978139. Author: P. B. Pederson, Denmark, 22 April 1998.

Phytase (Batch Number PPQ 5938) was examined for mutagenic activity in histidine auxotrophs of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and tryptophan-dependent *Escherichia coli* (WP2uvrA). Bacteria were exposed in a plate incorporation assay to six doses of the test substance in two complete and independent experiments, in the presence or absence of metabolic activation (S9 mixture). The experiments complied with OECD Guidelines for testing chemicals, 'Bacterial Reverse Mutation Test'. Proposal for Replacement of Guidelines 471 and 472, (1996).

The test material was a fluid enzyme preparation containing an abundance of various nutrients, including low concentrations of amino acids like histidine and tryptophan.

Positive controls possessed sensitivity for crystal violet (rfa-character) and for Mytomycin C (uvrB), and were resistant to ampicillin (pKM101), tested in the presence and absence of metabolic activation. All positive control chemicals induced significant increases in revertant colony numbers.

The maximum concentration of test material used was 50 mg/ml. Prepared plates were incubated for about 64 hours after which the number of revertant colonies were counted.

No dose-related or reproducible increases in revertants to prototrophy were obtained with any of the bacterial strains exposed to phytase (Batch Number PPQ 5938) at concentrations ranging from 156 to 5000 μg per plate, either in the presence or absence of metabolic activation.

Conclusion: The test material phytase PPQ 5938 did not exhibit any mutagenic activity under the conditions of the test.

Phytase: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Novo Nordisk Study No. 665/206-D5140. Author: M. Burman, Covance Laboratories Limited, England, 7 July 1998.

The potential of phytase SP 938, batch PPQ 5938, to damage the chromosomal structure was tested in human lymphocyte culture *in vitro*.

48-hour cell cultures established from whole human blood were exposed to the test substance in the presence and absence of metabolic activation as follows:

- 3 hour treatment plus 17 hour recovery;
- 3 hour treatment plus 17 hour recovery with metabolic activation (rat liver-derived Aroclor 1254 induced S9 mixture);
- 3 hour treatment plus 17 hour recovery with metabolic activation (rat liver-derived phenobarbitone and β-naphthoflavone induced S9 mixture); and
- 20 hour treatment plus 0 hour recovery.

The doses of phytase selected for cytogenetic analysis were determined by a cell toxicity pretest. The concentrations used in the test were 2450, 3500 and 5000 μg/ml.

One and one half hours before the harvesting, colchicine was added to a final concentration of approximately 1 μg to arrest dividing cells in metaphase. Purified water was added to cultures and designated as negative controls.
The positive control chemicals, 4-nitroquinoline 1-oxide (final concentration 2.5 μg/ml) and cyclophosphamide (final concentrations 25 and 45 μg/ml) were dissolved in sterile anhydrous analytical grade dimethyl sulphoxide.

100 metaphases were scored for chromosome aberrations from each culture and the controls. The procedure and experimental design complied with the OECD Test Guideline 473 (revised draft document, 1996) and the ICH Tripartite Harmonised Guideline on Genotoxicity: Specific Aspects of Regulatory Tests (1996).

Treatment with phytase SP 938, PPQ 5938, did not produce biologically or statistically significant increases in the frequency of metaphase with aberrant chromosomes at any concentration tested when compared to control values, either in the presence or absence of S-9 metabolic activation. No significant increase in polyploid, endoreduplicated or hyperdiploid cells was noted. Positive controls 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: Phytase SP 938, PPQ 5938 showed no clastogenic potential under the test conditions.

Phytase batch PPQ 5938: 13 week toxicity study in rats with administration by gavage. Novo Nordisk Study No. NN 976027. Authors: T. Martin and P. Rogerson, Inveresk Research, Scotland, 1 September 1998.

Sprague-Dawley rats (10/sex/group) were dosed with phytase SP 938, PPQ 5938 daily by gavage at doses of 0, 1, 3 or 10 mg/kg/day (equivalent to 0, 0.11, 0.32 or 1.07 total organic solid /kg/day) for 13 weeks.

The enzyme activity of the test batch was stated to be 69.800 FYT/g and had a total organic solid (TOS) content of 10.2%, a dry matter content of 89.5% and an ash content of 0.3%. The vehicle was sterile water. Stability of the test substance during the 13 week study was demonstrated.

This study complied with the OECD Guidelines for testing of Chemicals, Guideline 408, sub-chronic oral toxicity – rodent: 90-day study, adopted May 1981, and the EC Guidelines for classification, packaging and labelling of dangerous substances: 88/302/EEC, Part B, sub-chronic oral toxicity test: 90 day repeated oral dose using rodent species, adopted May 1988.

Rats were observed twice daily for clinical signs of toxicity and were palpated once weekly. A weekly record of body weights, food and water consumption was maintained. An eye examination of all animals was conducted before the study period and on all control and high dose animals during week 13 of the study. Haematological, coagulation and blood chemistry parameters were done in week 13 of the study. All animals were subjected to a detailed necropsy, including organ weight analysis and histopathology.

There were two premature deaths, both high dose males. One died during the blood sampling for laboratory investigations during week 13 and the other was killed prematurely due to eye incurred damage during blood sampling. The deaths were not attributable to treatment with phytase.

The observed clinical changes (hair loss and staining) did not occur in a dose-dependent manner, and not considered attributable to the administration of the test material.

Body weights and food and water consumption were comparable between all study groups.

There were no treatment-related ocular changes at week 13 and no notable intergroup haematological differences in either sex at the end of the study.

Clinical chemistry results showed that, in the low-dose males, blood urea was slightly increased compared to the control. However, this was considered coincidental due to the absence of a similar effect at higher dose levels. There were no notable intergroup clinical chemistry differences in females. Urinalysis results also revealed no notable intergroup differences in either sex.

In males, thyroid weight was slightly decreased in the low an intermediate dose groups ($P<0.001$ and $P<0.01$, respectively) when compared to the control. This result was not considered attributable to the administration of phytase because of the lack of an effect in the high dose group. In females, ovarian weight was slightly decreased in all treatment groups. However, due to the lack of a dose-related response, this effect was not considered to be caused by the administration of phytase. There were no other significant intergroup changes in organ weights in either males or females.

Histological analysis showed a number of mild lesions in the heart, kidney, liver and lungs, none of which were considered to be related to the administration of phytase. The findings were randomly distributed among all dose groups (including controls), and correspond to spontaneously arising, incidental mild inflammatory and degenerative lesions.

Conclusion: Administration of phytase SP 938, PPQ 5938 at dosages up to

10 mg/kg/day for 13 weeks to rats was not associated with any significant toxicity. The NOEL for his study was 10 mg/kg/day.