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Orally Administered Enzyme Food Supplement Safety Overview

Enzyme Technical Association

January 9, 2012

I. Introduction

This paper has been prepared by the Enzyme Technical Association ("ETA") as an initial response to the Technical Report and new Abbreviated Labelling Standards ("AbLS") for Enzymes released on September 14, 2011 by the Natural Health Products Directorate ("NHPD"). The Technical Report and AbLS were prepared without virtually any input from industry and very little input from the scientific community.[1] Most significantly, the Technical Report and AbLS call into question the safety of enzymes used as Natural Health Products ("NHPs") for dietary supplementation/digestive aids based on a review prepared by a single naturopathic doctor, without taking into account significant and critically important sources of information, let alone basic protein science. We do not address the contents of the Technical Report and AbLS directly in this paper, other than to note our strong objection to the lack of transparency, complete absence of stakeholder participation in their preparation, and our general disagreement with methods used and conclusions reached in those documents.

This paper will provide: 1) a brief historical overview of the use of enzymes as supplements to the diet and digestive aids; 2) a brief overview of enzymes and modern safety assessment procedures; 3) currently available toxicity data references that should be reviewed by the NHPD; and 4) a discussion of a preliminary review of adverse events reported to Health Canada involving proteases.

As explained in greater detail below, the use of enzymes as supplements and digestive aids is largely an extension of their ubiquitous presence in food. Their acceptance and use over the last 70 years as supplements to the diet is premised in many respects on the fact that these enzymes are largely found as components of food and therefore raise little concern regarding safety. This broad recognition of safety and the somewhat imprecise definition of the term "digestive aid" have resulted in relatively few published studies of enzymes as "digestive aids" in the recent scientific and medical literature.

[1] ETA and its membership were approached in late 2009, and asked to provide Health Canada NHPD data regarding the unit relationship between gelatin digestion units ("GDU") and the United States Pharmacopeia's Food Chemicals Codex plant proteolytic activity ("FCC PU"), when applied to the measurement of bromelain. ETA prepared a paper (in the extremely short time frame requested) and formally responded to NHPD on February 5, 2010. ETA was never asked for safety data or other information about the use of enzymes by NHPD.

However, given the long history and common use in food, efficacy testing is not required to demonstrate safety of enzymes. Microbially (which includes fungal) sourced enzymes have been the subject of significant safety and toxicity testing for their use as direct additives and processing aids in the food (and feed) industries. The safety of enzyme supplements should be determined by the history of safety use and the accumulated safety and toxicity data that have been generated over the last 40 years for food-uses of the same enzymes, as well as the international recognition of food enzymes as being intrinsically safe proteins.

As noted at the outset, this paper is intended to be an initial response to NHPD, and is by no means an exhaustive review. As neither ETA nor its individual members were approached for assistance during the development of the Technical Report and the AbLS, the small window for a response to NHPD has not allowed for thorough collection and assessment of data and consultation with industry and academic experts.[2] ETA therefore respectfully requests that Health Canada provide a minimum of six (6) to twelve (12) months to prepare a review of available data to supplement this initial report and provide NHPD with the specific information it seeks.

ETA further requests the immediate withdrawal of the current version of the Technical Report due to significant shortcomings and the fact that its conclusions are not supported by applicable data. ETA would be pleased to support and assist NHPD with the development of a revised Technical Report that more accurately addresses the use and safety of enzymes as digestive aids. ETA sincerely hopes that NHPD will engage in an open, transparent process that includes stakeholders from academia, medicine, industry and consumer groups to develop guidelines that address the use of enzyme NHPs and ensure the safety of Canadian citizens.

II. History of Enzyme Dietary Supplementation and Digestive Aid Use

Foods modified by and containing (animal, plant and microbial) enzymes prior to ingestion have been consumed by man for millennia. Early examples of enzyme applications are cheese and bread-making, dry aging of meats, and a variety of fermentation processes including brewing, wine and vinegar production and lactic acid fermentations. Yeast has been used medically not only as a source of vitamins but also to combat constipation and to stimulate normal digestion by the action of yeast proteases and amylases.[3]

The enzyme industry as it exists today began in the late 19th century. By 1894, Dr. Jokichi Takamine had been granted U.S. Patent 525,823 "Process of making diastatic enzyme" which detailed the process and extraction of amylases from koji (*Aspergillus oryzae*). His patented product, Taka-diastase, was marketed by Parke, Davis & Company as a digestive aid throughout the world.

By 1932, Dr. Edward Howell formed a company in Illinois to provide supplemental enzymes to replace those destroyed in cooking, canning and food processing. Dr. Howell's 1947 survey

[2] As NHPD is aware, ETA submitted a draft practitioner survey for comment to NHPD on November 23, 2011, and is prepared to circulate that document to collect the type of practitioner information requested by NHDP in its September 14th BEEP. We have attached the draft survey for NHPD's convenience.

[3] 15, IRWIN W. SIZER, MEDICAL APPLICATIONS OF MICROBIAL ENZYMES; ADVANCES IN APPLIED MICROBIOLOGY, (D. Perlman ed.) (1972).

"Status of Food Enzymes in Digestion and Metabolism" cites use of papain as an aid to digestion and a benefit to "digestive disturbances of widely different kinds."[4] Fungal amylase is similarly cited as used in digestive tract therapy, as are lipases and pancreatic extracts.[5]

The use of enzymatic digestive aids is documented in medical reference texts going back at least six decades. For example, in the 1948 Physician's Desk Reference ("PDR"), 9 enzyme compounds were listed including products such as Winthrop Stearns "Stamyl," containing trypsin, amylopsin, lipase and hemicellulase.[6] By 1966, the PDR contained 37 "gastro-intestinal" use enzymes, with preparations that included proteases, plant derived amylases and cellulases.[7] However, many enzyme supplements were available through health food and other non-pharmaceutical providers that are not captured in PDR references.

In his 1972 review, Dr. Irwin Sizer recognized that enzymatic "digestive aids effective in the small intestine have been extensively used for a long period of time."[8] Dr. Sizer noted that digestive aid enzymes were "most often fungal in origin" and preparations from *Aspergillus oryzae* and *Aspergillus niger* were most commonly used due to their high content of amylase and protease.[9] Similarly, Dr. Sizer noted that cellulases were being used to aid digestion of foods containing indigestible cellulose fibers such as cucumbers, cabbage, and radishes. Cellulases from *Aspergillus oryzae* and *Tricoderma viride* are cited in publications dating to 1962.[10] Lipases from *Aspergillus oryzae* or *Candida lipolytica* were taken orally by individuals with fatty stools as early as 1958.[11]

More recently, McGrath and Walsh list amylase, cellulase, invertase, alpha-galactosidase, papain, pepsin, bromelain, superoxide dismutase, lactase and pancreatin as enzymes widely used as digestive aids.[12]

The use of enzymatic digestive aids has continued to flourish to the present. From our initial review, we have identified industry documentation of active marketing of the following enzymes for dietary supplementation use in the North American market for 20 years or longer:[13]

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[4] EDWARD HOWELL, THE STATUS OF FOOD ENZYMES IN DIGESTION AND METABOLISM (The National Enzyme Company 1946).
[5] *Id.*
[6] PHYSICIAN'S DESK REFERENCE (J. Jones et al. eds. Medical Economics Inc.) (1948).
[7] PHYSICIAN'S DESK REFERENCE (H. Bull et al. eds. Medical Economics Inc.) (12th ed. 1966).
[8] 15, IRWIN W. SIZER, MEDICAL APPLICATIONS OF MICROBIAL ENZYMES; ADVANCES IN APPLIED MICROBIOLOGY, (D. Perlman ed.) (1972).
[9] *Id*.
[10] *Id*.
[11] *Id*.
[12] GARY WALSH,DIRECTORY OF THERAPEUTIC ENZYMES 278-279 (Barry M. McGrath ed. 2006).
[13] Industry survey and data collection performed by ETA. Confidential documents may be summarized in greater detail at NHPD's request.

Table 1. Selected Enzymes Marketed as Digestive Aids in North America Prior to 1994

Alpha-galactosidase - *Aspergillus niger* Amylase - *Aspergillus oryzae* Amylase - *Bacillus subtilis, Bacillus amyloliquefaciens, Aspergillus niger* Amylase (β-amylase) (Malt diastase) – barley malt Cellulase - *Aspergillus niger, Trichoderma longibrachiatum (reesei)* Invertase - *Saccharomyces cerevisiae* Lactase - *Aspergillus oryzae* Lactase - *Kluyveromyces lactis* Lipase – *Aspergillus oryzae* Lipase - *Aspergillus niger, Rhizopus oryzae, R. japonicus* Lipase - *Arthrobacter ureafaciens, Candida cylindracea, Rhizomucor miehei, Rhizopus delemar*	Pancreatin - Porcine pancreas Pancreatin - bovine Pancrelipase - Bovine and porcine pancreas Protease, botanical - Bromelain (*Ananas comosus)* and Papain *(Carica papaya)* Protease, animal - porcine pepsin Protease, animal - bovine or porcine (trypsin), bovine or porcine (chymotrypsin), bovine (pepsin) Protease, microbial - *Aspergillus niger, Aspergillus oryzae, Bacillus subtilis* Protease, microbial - *Aspergillus oryzae, Aspergillus melleus, Bacillus licheniformis, Bacillus thermoproteolyticus, Rhizopus niveus* SuperOxide Dismutase - *Bacillus spp.*

Based on industry estimates, in 1994 the market (wholesale) for enzymes used as digestive aids was U.S. $35 million in the U.S. and Japan each; U.S. $47 million in France/Italy/UK/Germany; and roughly U.S. $55 million for the rest of the world.[14] According to the Nutritional Business Journal's 2011 supplement business report, digestive enzymes now rank as 20th of the top 100 nutritional supplements and make up 4% of the U.S. nutritional supplement market. The digestive enzyme category has grown consistently over the last 10 years from approximately U.S. $80 million in 2000 to U.S. $209 million in 2010.

There are clearly significant numbers of individuals using enzymes for digestive purposes the world over. These data should also be contrasted with the dearth of reported adverse events associated with such use of these enzymes (discussed below). While ETA recognizes that a lack

[14] Market estimate by ETA member in 1994.

of reported adverse events does not guarantee safety, the supporting data are particularly compelling: a 50-plus year (conservatively) history of very safe use.

A complete survey of naturopathic use of enzymes has not been completed as of yet. As noted above, a practitioner use survey has been prepared and submitted to NHPD for review. ETA is prepared to perform a large scale survey regarding practitioner (naturopathic and allopathic) uses of digestive enzymes. Additionally, our initial survey of the literature has found reference to naturopathic use of microbially derived lipase, amylase, protease, and lactase.[15] However, because of time constraints, ETA has been unable to consult with and obtain additional information from leading naturopathic practitioners to supplement this paper.

III. Benchmarking to the Safety Profile of Food Enzymes and Testing Methodology

As NHPD is aware, enzymes are proteins with highly specialized catalytic functions, produced by all living organisms. Enzymes are responsible for many essential biochemical reactions in microorganisms, plants, animals, and human beings. Like all other proteins, enzymes are composed of amino acids; however, they differ in function in that they have the unique ability to facilitate biochemical reactions without undergoing change themselves. This catalytic capability is what makes enzymes unique. Enzymes are protein molecules that act as highly efficient catalysts. Enzymes not only work efficiently and rapidly, they are also readily biodegradable.

Enzymes work based on the three-dimensional structure formed by the amino-acid chain that comprises the enzyme protein. This three-dimensional structure determines the active site of the enzyme, and allows binding to the substrate(s). It follows logically then, that enzymes of a certain activity class – for example amylases – all have similar active sites and molecular structure/shape in order to catalyze the same type of reaction, regardless of enzyme source – animal, plant, or microbial. This is in fact, the case. In general, there is a remarkable similarity between microbial enzymes and those of higher organisms.[16] Enzyme parameters, such as pH optimum, activation energy, temperature sensitivity, inhibition, substrate specificity and affinity, are quite comparable but not identical in all living organisms. Similarly, the amino acid sequence of a particular enzyme bears considerable resemblance in different organisms although the further apart two species are, the greater the number of amino acid substitutions that have occurred in the peptide chain.[17]

The long-term and well-defined presence of an enzyme in human food therefore carries with it *prima facie* evidence of safety. While ETA of course recognizes that differences in molecular structure, weight and other properties must be carefully considered when systemic treatments of specific diseases are assessed, the same level of concern is not justified for oral use of supplement enzymes that humans have been consuming for thousands of years as part of the diet.

Accordingly, while there may generally be more information available regarding the use of animal derived enzymes as digestive aids, the differences between animal sourced and

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[15] *See, e.g.*, Mario Roxas, *The Role of Enzyme Supplementation in Digestive Disorders*, 13 Alternative Med. Rev. 307-313.
[16] 15, IRWIN W. SIZER, MEDICAL APPLICATIONS OF MICROBIAL ENZYMES; ADVANCES IN APPLIED MICROBIOLOGY, (D. Perlman ed.) (1972).
[17] *Id*.

microbially sourced enzyme supplements is in many ways minor and may largely be addressed with appropriate safety and toxicity testing. As discussed above, use of enzymes as digestive aids is an extension of their presence in food (and in some cases the body). The modern food industry now predominantly relies on enzymes from microbial (fungal and bacterial) and plant sources – it is the rare enzyme that continues to be sourced from animals.

It is exactly the consistency of composition, structure, and activities that have allowed the food industry to move enzyme production to microbial sources. We note that the use of microbially sourced enzymes is not a new technology, but has been employed for over 40 years (discounting microbially sourced enzymes that have been used for hundreds of years in miso, soy, winemaking, etc.). Manufacturers have subjected their products to significant safety and toxicity testing that has been reviewed by Canadian, U.S., European, Australian/New Zealand and Japanese governmental bodies as well as the World Health Organization.

As NHPD is aware, the enzymes used as digestive aids are found in the general food supply. For example, the Food and Drug Regulations, Division 16, Table V (Food Additives That May Be Used as Food Enzymes) allow use without limitation of amylase, bromelain, hemicellulase, cellulase, lactase, lipase, papain, protease, and trypsin (to be addressed further below).

The long history of enzyme use (microbially, plant and animal sourced) in food has resulted in the recognition that enzymes can be categorized as "non-toxic" and "intrinsically safe" proteins.[18] The safety studies performed as part of numerous regulatory approvals for food enzymes around the world substantiate that enzymes are not mutagenic or clastogenic, nor are they reproductive or developmental toxins.

Modern Safety Assessment of Enzymes for Food-Use

Digestive aid enzymes are generally components of commonly consumed foods, and have been ingested by man for thousands of years. Accordingly, the methods of safety assessment used for food may be utilized as a benchmark for the safety assessment of orally taken enzyme digestive aids.

The leading peer-reviewed literature on the safety of microbial enzymes used in food was published in 2001 and authored by Pariza and Johnson.[19] The Pariza and Johnson review and methodology have been accepted as the standard for review of microbially derived enzymes by the scientific community at large, as well as the U.S. Food and Drug Administration ("FDA"), and has been submitted to Health Canada for review. According to Pariza and Johnson, a 100-fold Safety Margin compared to the no observable adverse effect level ("NOAEL") established in a 90-day oral toxicity study provides an acceptable level of safety for food use. Specifically, Pariza & Johnson conclude:

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[18] *See* Gerald Reed, ENZYMES IN FOOD PROCESSING 549–554 ,(AcademicPress, New York) (2nd ed. 1975); *see also* Zofia S.Olempska-Beer et al., *Food Processing Enzymes From Recombinant Microorganisms – A Review*, 45 Reg. Toxicology & Pharmacology 144–58; *see also* Noordervliet, PF, and DA Toet, 1987. Safety in enzyme technology, Biotechnology, vol. 7A. VCH Verlagsgesellschaft, Weinheim, Germany, pp. 711–741.

[19] Michael W. Pariza, & Eric A. Johnson, *Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century*, 33 Reg. Toxicology & Pharmacology 173-86.

> *"… a repeated-dose oral study (14–91 days) in one animal species, preferably the rat because of the historical data available on this species. The test article can be administered either in the feed or via gavage. The lowest dose used for this study should be at least 100 times the estimated mean human exposure (based on TOS). This test will detect toxicity that would be associated with the known microbial toxins that are active via the oral route. The NOAEL should provide at least a 100-fold margin of safety for human consumption, calculated using standard methods (Klaassen, 1996; Lehman and Fitzhugh, 1954; ILSI, 1997).*"[20]

Similarly, the Joint FAO/WHO Expert Committee on Food Additives ("JECFA"), in its General Specifications and Considerations for Enzyme Preparations Used in Food Processing (2006),[21] cites Pariza and Johnson for the evaluation of enzymes, as well as the Scientific Committee on Food ("SCF"). The SCF concludes that the following tests should be used in the evaluation for food enzymes:

> For enzyme preparations derived from microorganisms, the following tests are normally required:
>
> a) 90-day oral toxicity test in a rodent species;
> b) Two mutagenicity tests:
>
> 1. a test for gene-mutation in bacteria;
> 2. a test for chromosomal aberrations.[22]

The ETA supports the Pariza/Johnson and JECFA conclusions, and our members have used these safety assessments around the world for several decades. Historically these tests have been performed on any enzyme intended for use in foods. The enzymes utilized in the manufacturing of dietary (digestive) supplements are the same enzymes used as food additives. The strains and enzymes being examined by NHPD are all well defined and have been subject to numerous regulatory reviews for food use in Canada, the U.S., Europe, Australia/New Zealand and Japan.

Additionally, Pariza and Johnson set forth that the safety of food enzymes is largely tied to the production strain used to produce the enzyme.[23] Enzymes used as digestive aids are all produced from well defined microbial strains that are approved and actively used for food enzyme production.

We further note that in general, JECFA considers enzymes to be substances of very low toxicity which do not represent a hazard to health and, accordingly, a numerical Acceptable Daily Intake ("ADI") was not deemed necessary. This is expressed by JECFA in relation to food enzymes in the following way:

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[20] *Id*. at 181.
[21] General Specifications and Considerations for Enzyme Preparations Used in Food Processing (2006), *available at* http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm.
[22] Scientific Committee on Food, *Guidelines for the Presentation of Data on Food Enzymes* (Apr. 11, 1991); Report of the Scientific Committee on Food: Twenty-seventh series, Catalogue No. EUR 14181, 1992, pp. 13–22. *See* pg. 19.
[23] Pariza and Johnson, supra note 17. We have included as Appendix I additional references addressing the safety of enzymes and safe strain lineage of production organisms.

"ADI 'not specified' when used in the applications specified and in accordance with good manufacturing practice."[24]

In line with this approach, JECFA has never assigned an ADI to any of the seventy-four (74) food use enzymes it has evaluated.[25] As briefly noted above, for food additive use, Health Canada has not set maximum use levels for any of the enzymes listed in Table V. Similarly, in the U.S., the FDA has not set limits on any food use enzymes.

Finally, we must briefly address a statement by NHPD in its September 14th BEEP. Specifically, NHPD states: "It has been *established* that the long-term safety of most enzymes in oral form for digestive purposes is not supported *based on the concern that the chronic use of enzymes may decrease natural enzyme production*." September 14th BEEP at 3 (emphasis added).

As far as ETA can identify, this statement, which apparently provides the primary basis for NHPD's current regulatory actions, is based on the uncited, completely unsupported statement in the Technical Report that reads: "*[t]heoretically* in healthy adults, chronic use of digestive enzymes *may* down regulate endogenous enzyme production." Id. at 10 (emphasis added). There is no evidence of such down regulation that we are aware of, and citation to any such evidence is conspicuously absent from the Technical Report.

ETA is very concerned that NHPD has taken drastic regulatory action, involving substances with long records of safe use, based entirely on an unsupported "theory" put forth by a single naturopathic physician.

IV. Sources of Safety and Toxicity Data for Currently Marketed Food-Use Enzymes

We provide below an initial survey of sources for publically available safety and toxicity testing performed on a variety of food-use enzymes. As noted, ETA has not had sufficient time for a thorough analysis of the data and assessment of exposure in relationship to digestive aid uses, but would be pleased to work with NHPD in assessing the available information. It is unclear whether NHPD considered or reviewed any safety/toxicity data as part of its review of enzymes used for digestive purposes.

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[24] JECFA defines ADI "not specified" as used to refer to "a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the [JECFA], represent a hazard to health. For that reason, and for the reasons stated in individual evaluations, the establishment of an [ADI] expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e., it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance." *See* JECFA Glossary of Terms, *available at* *www.who.int/entity/foodsafety/chem/jecfa/glossary.pdf*.

[25] *See* Evaluations of the JECFA on Food Additives, http://apps.who.int/ipsc/database/evaluations/search.aspx?fc=35.

Table 2. Selection of Publically Available Food-Use Enzyme Safety and Toxicity Testing

Enzyme	Publically Available Data
Fungal & Bacterial amylase	FDA GRAS Notification 22 FDA GRAS Notification 24 FDA GRAS Notification 79 FDA GRAS Notification 126 21 C.F.R. 184.1148 JECFA Evaluations-FAS 22-JECFA 31/5 JECFA Evaluations -TRS 759-JECFA 31/17 JECFA Evaluations -FAS 22-JECFA 31/11 (1987) JECFA Evaluations -TRS 789-JECFA 35/15 JECFA Evaluations -FAS 28-JECFA 37/67 JECFA Evaluations -TRS 806-JECFA 37/10 Australia New Zealand Application A467
Fungal & Bacterial protease	FDA GRAS Notification 89 FDA GRAS Notification 90 FDA GRAS Notification 333 21 C.F.R. 184.1150 JECFA Evaluations -TRS 789-JECFA 31/15 JECFA Evaluations -FAS 22-JECFA 31/8 JECFA Evaluations -TRS 759-JECFA 31/17 JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/12 JECFA Evaluations -FAS 1/NMRS 50A—JECFA 15/9 Australia New Zealand Application A1057
Fungal lipase	FDA GRAS Notification 81 FDA GRAS Notification 111 FDA GRAS Notification 216 JECFA Evaluations -NMRS 54/TRS 557-JECFA 18/20 Australia New Zealand Application A1036 Australia New Zealand Application A569 Australia New Zealand Application A519 Australia New Zealand Application A517 Australia New Zealand Application A516 Australia New Zealand Application A402 Australia New Zealand Application A435
Bromelain	21 C.F.R. 1024 JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/11
Papain	21 C.F.R. 184.1585 JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/11
Cellulase	FDA GRAS Notification 292 JECFA Evaluations -FAS 30-JECFA 39/15 JECFA Evaluations -TRS 828-JECFA 39/10 Australia New Zealand Application A1011
Hemicellulase	JECFA Evaluations -FAS 22-JECFA 31/19 (1987)

Enzyme	Publically Available Data
	JECFA Evaluations -TRS 789-JECFA 31/15
Pepsin	21 C.F.R. 184.1595 JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/11
Beta glucanase	FDA GRAS Notification 149 FDA GRAS Notification 195 FAS 22-JECFA 31/15 (1987) JECFA Evaluations -TRS 789-JECFA 35/15
Lactase	FDA GRAS Notification 132 21 C.F.R. 184.1387

The above table (Table 2) contains active "links" that will take NHPD directly to regulatory submissions and evaluations for the listed food-use enzymes at the respective websites.[26]

Furthermore, we believe that NHPD should have readily accessible safety data on file in numerous Masterfile Submissions. It is our understanding that Masterfiles have been submitted for at least the following enzymes: lactase, xylanase, cellulose, fungal protease, amylase, α-Galactosidase, lipase, bromelain and papain. In addition to Masterfiles, Health Canada should have access to the safety and toxicity data for each enzyme and strain included in Division 16 Table V.

V. There Are Few Reported Side Effects and Adverse Events Associated with Digestive Enzymes

Enzymes are regarded as safe substances with few reported side effects.[27] We briefly note that like all proteinaceous substances, repeated inhalation of enzymes contained in aerosols can cause an allergic response. However, we are not aware of such responses in orally ingested enzymes nor are aerosolized digestive aid enzymes available to the public. Similarly, prolonged skin contact with proteolytic enzymes can cause skin irritation (and potentially eye irritation). However, this is only in cases where raw, powdered enzyme is handled and does not apply to encapsulated proteases to which consumers have access. Outside these limited cases, enzymes have a remarkably benign safety record.

ETA performed a preliminary analysis of "Canada Vigilance" reports of adverse reactions associated with "proteases" marketed in Canada between 1/1/1965 and 3/31/2011. Our initial analysis shows:

A total of 45 reports are listed. However, two cases each seem to be represented by three reports (000101016, 000101017 and 000101018, and 000113490, 000113491 and 000114792); thus the 45 reports describe a total of 41 individual cases. Typically and similar to the US FDA's AERS system database, the reports are sparse with respect to clinical detail and demographic characterization often is incomplete. Notwithstanding, important information is available.

[26] At NHPD's request, we would be pleased to provide "long form" html web addresses in addition to the active links contained in this document.
[27] Mario Roxas, supra note 13.

Among the 41 individual cases, in 26, an animal-derived pancreatic enzyme formulation/preparation (e.g., Pancrease or Creon) is identified as the "suspect agent" and one, reported in triplicate (000101016, 17 and 18) identifies intravenously administered "CA-17" as the suspect agent. Overall then, 14 cases putatively involving ingestion of non-animal derived protease or non-animal derived protease containing "digestive aids" are described. Age is listed for 12 of these 14 cases and the range is 20-68 years with a mean of 42 ± 16. Sex is listed for all 14 and 10 are female. The reporter's ADR severity assessment is available for all 14. Eight were considered "serious," i.e., involved death (0), were considered life-threatening (2, 000191425, 000365432), involved disability (0), hospitalization (4, 000169033, 000186677, 000212424 and 000304877), congenital anomaly (0) or "other medically important conditions" (2, 000187233, 000355939). Outcomes are listed for 10 of the 14, of whom nine recovered completely. One (000187233) is listed as not recovered, but no further details are available in the report (this case is discussed further below).

Of special interest among the cases considered to have suffered "serious" adverse events are:

Report #	Case Description
000186677	A 32 year old female who took "Plant Enzyme M7" for 19 days as well as "Citridal" (possibly Citrical®, a calcium citrate formulation, or citricidal, an extract of grapefruit seed) and was being treated with methotrexate for an undescribed medical problem. She was hospitalized for "thrombocytopenia" (not further detailed) and recovered completely. This patient's thrombocytopenia seems far more likely to have been causally related to methotrexate, or her underlying medical problem (rheumatoid arthritis) than to Plant Enzyme M7.
000187233	Formula 2 Multivitamin Mineral Complex, Formula 3 Vitamin Complex Tab, Herbalife – Dong Quai, Herbalife – Factor 1000, Herbalife – Florafiber, Herbalife – Formula 1, Herbalife – Formula 4 Canola Oil Capsule, Herbalife – Herbal Aloe Drink, Herbalife – Meal Replacement, Herbalife – Protein Performance Powder Nutri-9 A male of unrecorded age who developed dizziness, dyspnea, an irregular heart beat and a "sleep disorder," was hospitalized for an undescribed length of time and is said not to have recovered (by the time of the report). No details of this patient's diagnostic evaluation or treatment (if any) are provided. Ascription of this patient's signs and symptoms to any one of the 11 NHPs he was taking when he became ill is simply not possible given the paucity of details reported.
000191425	This patient was a 29 year old female who had been taking "Enriching Greens®" and "Revital X®" daily for 214 days as well as azathioprine, methylprednisolone sodium succinate parenterally, oxybutynin, prednisone, Rebif® subcutaneously (Rebif is interferon beta-1 and is approved to treat multiple sclerosis), Tylenol® and Vitamin B12 and had an underlying diagnosis of "autoimmune disorder" as well as, presumably, multiple sclerosis. She developed liver test abnormalities, all of undescribed magnitude and duration (increased alanine transaminase, aspartate transaminase, alkaline phosphatase, "blood bilirubin" and an elevated

Report #	Case Description
	prothrombin time). Although the magnitudes of the increases in her transaminases and serum bilirubin are not described, these abnormalities, in concert with her elevated prothrombin time, make it likely that the reporter's assessment of the patient's illness as potentially life threatening was correct. "Toxic hepatitis" with hepatic necrosis was diagnosed. Further details regarding diagnosis and treatment (if any) are not provided. She apparently recovered completely presumably after being hospitalized for an undescribed period of time. Ascription of this patient's serious and potentially life threatening liver injury to either of the NHPs she had been taking without adverse incident for 214 days, given her underlying illness, an autoimmune disorder, presumably multiple sclerosis, and use of several drugs with well-known potential for potentially lethal liver injury (e.g., Tylenol [acetaminophen], Rebif, azathioprine and an oral contraceptive) cannot be made with any certainty.
000304877	This 58 year old male had been taking "Dual Action Cleanse Colon Clear Formula" and "Dual Action Cleanse Total Body Purifier" for two years without incident when he developed upper abdominal pain and tenderness and nausea associated with an elevated serum lipase value. He was diagnosed with acute pancreatitis, hospitalized for an undescribed period of time and recovered completely. No details of his diagnostic evaluation are provided. A causal relationship between one or both of the NHPs he was taking and his episode of acute pancreatitis cannot be ruled out completely, but seems very unlikely.

Overall, the Canada Vigilance protease-associated data for 1/1/1965 to 3/31/2011, a span of more than 46 years, show a total of 14 cases with no deaths and full recovery for nine of the 10 cases for which outcomes are available.[28] Ascription of the four most concerning adverse events to NHP is tenuous and poorly supported by information available in the database. An attempt should be made to obtain further information for all of these cases and especially for the four reviewed individually above.

The reporting rate of 14/46 years, or 0.30/year (3/10 years), is very low even if substantial underreporting is assumed. These data alone do not constitute a basis for concluding that NHP proteases marketed as digestive aids are unsafe. The Canada Vigilance databases for the other NHP enzymes marketed as digestive aids should certainly be analyzed.

[28] Similar searches have identified a small number of Canada Vigilance reports for the following enzymes: alpha-galactosidase (5); amylase (45); bromelain (38); cellulase (4); hemicellulase (3); invertase (5); lactase (27); lipase (41); maltase/glucoamylase (1); protease (45); and SOD (2). Additional time is required for further analysis.

Clearly, the adverse event databases should be thoroughly reviewed and analyzed with respect to adverse events reported in association with NHP non-animal derived enzymes marketed as digestive aids.[29]

Additional sources of publically available safety data include:

1. FDA's Adverse Event Reporting System (AERS) including the Spontaneous Reporting System (SRS) (pre-1997), http://www.fda.gov/Drugs/InformationOnDrugs/ucm135151.htm
2. World Health Organization (WHO) Individual Case Safety Reports (ICSR), http://www.who.int/medicines/areas/quality_safety/safety_efficacy/pharmvigi/en/index.html; also accessible through the Uppsala Monitoring Centre Vigibase System, http://www.umc-products.com/DynPage.aspx?id=73564&mn1=1107&mn2=1132&mn3=6048
3. Toxic Exposure Surveillance System (TESS), http://www.1-800-222-1222.info/stats/tess.asp
4. Drug Abuse Warning Network (DAWN), http://www.icpsr.umich.edu/icpsrweb/SAMHDA/studies/31264
5. Food and Agriculture Organization of the UN and World Health Organization Joint Expert Committee on Food Additives (FAO/WHO JECFA), http://apps.who.int/ipsc/database/evaluations/search.aspx
6. Food Standards Australia New Zealand (FSANZ), http://www.foodstandards.gov.au/consumerinformation/additives/

[29] The Canada Vigilance assessment was performed by Thomas Q. Garvey III, M.D. at the request of ETA.

VI. Conclusions and Recommendations

Enzymes have been ingested by man for thousands of years and have been used to supplement the diet and aid in digestion for at least 70 years. This widespread use has resulted in little evidence of safety concern, which we believe is reflected in the adverse event reporting records as well as the scientific literature.

ETA and its members consider the safety and well being of the public our top priority and will support NHPD in its efforts to assess the safety and uses of enzyme digestive aid NHPs. However, NHPD has taken regulatory action against products that have been safely marketed for many years without performing a scientifically valid assessment.

As we have set forth in this paper, dietary enzyme safety should be measured using evidence of safe use and appropriate safety and toxicity testing. We have provided NHPD with an outline to aid with the safety assessment of enzymes and sources of available testing data.

ETA respectfully requests that NHPD stay its current regulatory action and engage industry, academia and other interested parties in an open and systematic review. Should genuine issues of concern regarding safety arise; appropriate action can be taken in an informed manner.

Sincerely,

Scott Ravech
Co-Chair, ETA Dietary Supplements Committee

Danielle Harrison
Co-Chair, ETA Dietary Supplements Committee

John Carroll
Chair, ETA

Anthony Pavel
Secretary and General Counsel, ETA

Appendix I. Additional Citations Regarding Enzyme and Strain Safety

Barbesgaard, P., H. P. Heldt-Hansen and B. Diderichsen. "On the safety of *Aspergillus oryzae* - a review," *Applied Microbiology and Biotechnology* 36 (1992): 569-572.

Berkowitz, D. and J. Maryanski. "Implications of biotechnology on international food standards and codes of practice," Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 18th Session, Geneva (July 3-12, 1989).

Conference organized by Associacas Nacional de Biossequranca. "Industrial Enzymes for Food Production: Past, Present and Future Perspectives," Brasilia, May 6, 2001.

De Boer, A. S. and D. Borge. "On the safety of *Bacillus subtilis* and *Bacillus amyloliquefaciens* - a review," *Applied Microbiology and Biotechnology* 36 (1991): 1-4.

De Boer, A. S., F. Priest and B. Diderichsen. "On the safety of *Bacillus licheniformis* - a review," *Applied Microbiology and Biotechnology* 40 (1994): 595-598.

FAO/WHO. *Biotechnology and food safety*, *Report of a Joint FAO/WHO Consultation*. FAO Food and Nutrition Paper 61. Rome, Italy (1996).

FAO/WHO. *Safety assessment of foods derived from genetically modified microorganisms, Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology*. Geneva, Switzerland (September 24-28, 2001).

FAO/WHO. *General specifications and considerations for enzyme preparations used in food processing.* Food and Nutrition Paper. FAO JECFA Monographs 3. Accessible at: http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm 2006.

IFBC (International Food Biotechnology Council). Chapter 4: "Safety evaluation of foods and food ingredients derived from microorganisms." In "Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification." *Regulatory Toxicology and Pharmacology* 12 (1990): S1-S196.

International Food Biotechnology Council. "Safety evaluation of foods and food ingredients derived from microorganisms." In "Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification." *Regulatory Toxicology and Pharmacology* 12 (1990): S114-S128.

Jonas, D.A., E. Antignac, J.M. Antoine, H.G. Classen, A. Huggett, I. Knudsen, J. Mahler, T. Ockhuizen, M. Smith, M. Teuber, R. Walker, and P. de Vogel. "The safety assessment of novel foods, guidelines prepared by ILSI Europe Novel Food Task Force," *Food and Chemical Toxicology* 34 (1996): 931-940.

Nevalainen, H., P. Sucminen and K. Taimisto. "On the safety of *Trichoderma reesei* - a review," *Journal of Biotechnology* 37 (1994): 193-200.

OECD (Organisation for Economic Cooperation and Development). "Safety evaluation of foods derived by modern biotechnology."

Olempska-Beer, Z.S., R.I. Merker, M.D. Ditto, and M.J. DiNovi. "Food-processing enzymes from recombinant microorganisms - a review," *Regulatory Toxicology and Pharmacology* 45 (2006): 144-158.

Pariza, M.W. and E.M. Foster. "Determining the safety of enzymes used in food processing," *Journal of Food Protection* 46 (1983):453-468.

Pariza, M.W. and E.A. Johnson. "Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century," *Regulatory Toxicology and Pharmacology* 33 (2001): 173-186.

Pariza, M.W. and M. Cook. "Determining the safety of enzymes used in animal feed," *Regulatory Toxicology and Pharmacology* 56 (2010): 332–342.

Schuster, E., N. Dunn-Coleman, J.C. Frisvad and P.W.M. van Dijck. "On the safety of *Aspergillus niger* - a review," *Applied Microbiology and Biotechnology* 59 (2002): 426-435.

Enzyme Technical Association

DRAFT

Practitioner Enzyme Supplement Usage Survey

Product Name: _____

Distributor: _____

Manufacturer: _____

Recommended Dosage: _____

Frequency of Dose: _____

Duration of Use: _____

Practitioner's Name (Voluntary): _____

Type of Practice: _____

Location of Practice: _____

City, State/Province, Country

Below please list the ingredients and the amount in each capsule/tablet/or other dosage form. Alternately, you may attach a copy of the product label or other product literature detailing the contents of the product.

PRODUCT INGREDIENTS	ACTIVITY	MGs

PRODUCT INGREDIENTS	ACTIVITY	MGs

Product Type: ☐ Digestive Aid ☐ Systemic Support ☐ Other, please specify _____

SUBMIT FORM

DRAFT

How long have you used this product in your practice? ☐ < 1 YR ☐ 1-2 YRS ☐ 3-5 YRS ☐ 5-10 YRS ☐ >10 YRS

Approximately how many patients/clients have used this product? ☐ <10 ☐ 10-50 ☐ 50-100 ☐ >100

Please describe to whom you recommend this product? ☐ EVERYONE ☐ ONLY HEALTY INDIVIDUALS ☐ ONLY INDIVIDUALS WITH DIGESTIVE ISSUES ☐ OTHER: _____

What age group(s) do you recommend this product for? ☐ ALL ☐ CHILDREN ☐ TEENS ☐ ADULTS ☐ ELDERLY

Have benefits been observed or reported with use of this product? ☐ YES ☐ *If YES, please describe:*

Have any adverse effects been observed or reported?: ☐ YES ☐ NO *If YES, please describe symptoms, duration, frequency of occurrence, etc:*

Have you had patients discontinue use of the product due to adverse effects? ☐ YES ☐ NO

How often do patients discontinue use due to adverse effects? ☐ NEVER ☐ RARELY ☐ OFTEN ☐ OCCASIONALLY

Comments:

Have patients complained of problems after **completing** the recommended dosing regimen? ☐ YES ☐ NO *If YES, please describe:*

Do you have any additional comments about your clinical experience with this product?