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Research Section

Safety evaluation of a lactase enzyme preparation derived from *Kluyveromyces lactis*

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Abstract—Neutralact®, the DSM brand name of a lactase enzyme preparation, obtained from a homologous rDNA strain of *Kluyveromyces lactis*, was subjected to a series of toxicological tests to document the safety for use as a processing aid in the dairy industry. The enzyme preparation was examined for subacute oral toxicity and mutagenic potential. As a result of these tests, no evidence of oral toxicity, mutagenicity or clastogenicity was found. Administration of the lactase enzyme preparation at doses of 500, 3000 and 10,000 mg/kg body weight/day for 28 days did not induce noticeable signs of toxicity. The no-observed-adverse-effect level (NOAEL) of the enzyme preparation in the acute toxicity study was 10,000 mg/kg body weight/day (equivalent to 114,000 NL units/kg body weight/day). It can be concluded that no safety concerns were identified in the studies conducted with this lactase enzyme preparation derived from *Kluyveromyces lactis* under controlled fermentation conditions.

Keywords: toxicology; lactase; *Kluyveromyces lactis*; safety.

Abbreviations: NLunits = neutral lactase units; TOS = total organic solids; OECD = Organisation for Economic Cooperation and Development; NOAEL = no-observed-adverse-effect level; EDI = estimated daily intake.

INTRODUCTION

A part of the world population suffers from lactose intolerance (Kretchmer, 1972). These people cannot benefit from the nutritional quality of milk and milk-derived products without having serious intestinal problems. Pretreatment of the milk with the enzyme lactase (β-galactosidase) converts lactose into galactose and glucose. These mono-sugars can be consumed without any problem even by humans with lactose intolerance.

The US Food and Drug Administration (FDA) affirmed lactase preparations, produced by *Kluyveromyces lactis* (formerly known as *Saccharomyces lactis*) as generally recognised as safe (GRAS) in 1984 (FDA, 1984). Maxilact®, the DSM brandname of a lactase preparation produced by the classical strain of *K. lactis*, has already been on the market for decades and has a history of safe use (AMFEP, 1997). Its safety evaluation was based on an LD_{50}, a 14-day subacute and a 90-day subchronic toxicity study in rat. Based on the results of these studies, it should be noted that the level of enzyme which is administered to the experimental animals at the highest dose is the same as would be ingested by a 25-kg child drinking 8000 litres of milk per day, a 50-kg adult drinking 16,000 litres of milk per day or a 75-kg adult drinking 24,000 litres of milk per day, all at the standard usage levels of one packet of Lactaid (a commercial lactase product containing 5000 ONPGU) per litre of milk. (Newberne, 1976).

The safety of microbial food enzymes can be established on basis of a history of safe use of the production organism, supplemented with scientific studies of the enzyme preparation (Battershill, 1993; SCF, 1992). The safety of the production organism *Kluyveromyces lactis* was reviewed extensively by Bonekamp and Oosterom in 1994. They concluded that *K. lactis* is generally recognised as a safe organism for use in lactase and chymosin production.

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Moreover, *K. lactis* is an excellent host for safe production of harmless products by recombinant strains. The safety of chymosin, produced by *K. lactis*, was examined comprehensively in the early 1990s. The production of calf chymosin by the heterologous rDNA *K. lactis* was evaluated by the Joint WHO/FAO Expert Committee of Food Additives (JECFA, 1991) and the Scientific Committee for Food (SCF, 1995). The US FDA affirmed chymosin derived from *K. lactis* as GRAS in 1992 (*Federal Register*, 1992).

Neutralact®, the DSM brandname of the enzyme preparation, containing lactase (β-galactosidase), is produced by a homologous rDNA strain of *K. lactis*, containing extra copies of the lactase gene. The strain does not contain any antibiotic markers or auxotrophic markers nor any other foreign DNA sequences. Based on the comparison of the biochemical characteristics of the classical and the rDNA derived product, the manufacturing processes used and the safety of the rDNA strain, there is no indication that introduction of extra copies of the lactase gene leads to any change in the product safety. Applying this information into the decision tree for evaluating relative safety of food ingredients derived from Genetically modified microorganism (IFBC, 1991; Pariza and Foster, 1983) the 'product is accepted as safe'.

Although from the above data it can be concluded that the chance that the homologous rDNA lactase production strain adds additional risks to the final product is negligible, an extensive test programme has been performed to confirm the conclusion on the safety of lactase derived from this *K. lactis* strain. This paper describes studies conducted to examine the lactase enzyme preparation for subacute oral toxicity as well as mutagenic potential in detail.

MATERIALS AND METHODS

The batch of lactase enzyme preparation, used for toxicity testing (referred to as "the tox-batch") was produced by the procedure used for the commercial large-scale manufacturing of lactase. The production process—performed according to the requirements of ISO9002—includes the fermentation process, recovery (downstream processing) and formulation of the product. As a result of the purification process, the final, non-standardised UF-concentrate (tox-batch) was obtained, which was characterised by chemical and microbial analysis (Table 1). The stability of the tox-batch during the period of investigations was confirmed by analysis of the enzyme activity. The initial enzyme activity of the tox-batch is approximately 11400 NL units/g with a TOS (total organic solids) value of 15.4%. (As 1 g of enzyme preparation corresponds to 11400 NL units, 1 NL unit corresponds to 1000*0.154/11400 = 0.014 mg TOS.)

Table 1. Analytical results of the lactase tox-batch

Parameter	Unit	Tox-batch
Appearance		Liquid
Colour		Brown
Identity (SDS–PAGE)		Conforms
Foreign matter		absent by test
Enzymatic assay		
Lactase	NLU[a]/g	11400
Dry matter	%	16.4
Ashes	%	1.0
Total organic solids (TOS[b])	%	15.4
Antifoam	%	< 0.01
Residual minerals		
Heavy metals (as Pb)	mg/kg	< 30
Pb	mg/kg	< 5
Cd	mg/kg	< 0.5
As	mg/kg	< 3
Hg	mg/kg	< 0.5
Contamination		
Mycotoxins	–	absent by test
Total plate count	CFU[c]/g	< 10E2
Moulds	CFU/g	< 10
Yeasts	CFU/g	< 10
Salmonella	CFU/25g	neg
Coliforms	CFU/g	< 30
Enteropath *E. coli*	CFU/25g	neg
Antimicrobial activity	–	absent by test
Stability:water 21°C 24 hr	%	> 90
stability:water 4°C 24 hr	%	> 90

[a]One neutral lactase unit (NLU) is defined as the amount of enzyme which liberates 1.30 μmol *o*-nitrophenol (ONP) per min from the 'pseudo-substrate' *o*-nitrophenol-β-D-galactopyranoside under the conditions of the assay.

[b]Total organic solids (TOS) is defined as 100%-(A + W + D)% where A is the ash content, W is the water content and D is the diluent content. TOS was 15.4% in the tox batch.

[c]CFU = colony forming units.

In order to confirm the safety of the enzyme preparation, the following studies were carried out at Notox, Safety and Environmental Toxicology BV, s'-Hertogenbosch, The Netherlands, during the period January to April 1998: Subacute 28-day oral toxicity in rat; Bacterial gene mutation, Ames test; *In vitro* chromosomal aberration test, human lymphocytes. All studies were carried out in accordance with current guidelines of the Organisation for Economic Cooperation and Development (OECD, 1984), and in compliance with the principles of good laboratory practice (GLP), according to OECD principles of GLP, May 1981.

Oral toxicity studies

Subacute 28-day oral toxicity in rats

The tox-batch was examined in a 28-day study with four groups of five male and five female young specified pathogen free (SPF)-bred Wistar rats, which received the tox-batch undiluted, daily, by oral gavage. Based on previous studies the doses of 0 (control), 500, 3000 and 10,000 mg/kg body weight/day were selected. Food and water were available *ad lib*.

Routine clinical observations, body weight and food consumption, were measured throughout the

study periods, whereas functional observation tests were performed throughout the last week of the study. During wk 4, prior to scheduled post mortem examination, blood was collected from each animal for clinical laboratory investigations. At the end of wk 4, all animals were necropsied and macroscopic observations were recorded. The weights of organs as listed in Table 2 were measured at autopsy and organ:body weight ratios were calculated. Tissues (Table 2) collected from all animals of the control and highest dose group, as well as all gross lesions of all animals (all dose groups) were processed and slides were examined (De Hoog, 1998).

Mutagenicity

Ames test

The tox-batch was examined for its mutagenic potency in four histidine-requiring *Salmonella typhimurium* mutant strains TA 98, TA 100, TA 1535 and TA 1537 and one tryptophan-requiring *Escherichia coli* mutant strain WP2uvrA, in two independent experiments (Ames *et al*., 1975; Maron and Ames, 1983; OECD 1984). Tester bacteria were exposed to five concentrations ranging from 0.1 to 5 mg/ml in phosphate buffered nutrient broth both in the absence and presence of a rat liver-derived metabolic activation system (S-9 mix). Negative and positive controls were run simultaneously with the test (Verspeek-Rip, 1998).

In vitro chromosomal aberration test with human lymphocytes

The tox-batch was examined for its effect on the organisation and structure of chromosomes of cultured human peripheral lymphocytes following the methods of OECD (1984). The test was conducted with and without the inclusion of a rat liver-derived metabolic activation system (S-9 mix).

In the absence of S-9 mix, the tox-batch was tested at concentrations of 1000, 3330 and 5000 μg/ml for a 24-hr treatment time with a 24-hr fixation time and at 5000 μg/ml for a 48-hr treatment time with a 48-hr fixation time in the first experiment. In the second experiment the tox-batch was tested at concentrations of 1000, 3330 and 5000 μg/ml for a 24-hr treatment time with a 24-hr fixation time.

In the presence of 1.8% (v/v) S-9 fraction, the tox-batch was tested at concentrations of 1000, 3330 and 5000 μg/ml for a 3-hr treatment time with a 24-hr fixation time and at 5000 μg/ml for a 3-hr treatment time with a 48-hr fixation time in the first experiment. In the second experiment the tox-batch was tested at the same three concentrations for a 3-hr treatment time with a 24-hr fixation time (Table 6).

All dose levels together with the negative (vehicle) and positive controls (mitomycin C and cyclophosphamide) were selected for the analysis of chromosomal aberrations. At least 200 metaphase chromosome spreads were analysed from all selected cultures (Bertens, 1998).

RESULTS

Subacute 28-day oral toxicity study in rats

Stability of the tox-batch in vehicle was demonstrated by analysis of the lactase enzyme activity (Table 1). No mortality occurred during the study, and there were no clinical signs of toxicity or behavioural changes over the 28-day observation period that were considered to be related to treatment. In Table 3, the data of body weight, food consumption, relative food consumption, and enzyme dose are given. No differences among overall body weight gain, food consumption, relative food consumption considered to be an effect of treatment, were noticed. Moreover, functional observations made during wk 4 did not reveal any treatment-related effect (results not shown). Haematology parameters of treated rats were considered not to have been affected by the treatment. Minor statistically significant differences arising between controls and females receiving 3000 mg/kg body weight/day, were considered to have arisen by chance and not to represent a change of biological significance as no dose–response relationship was detected and all values remained within the range of background data for rats of this age and strain (Table 4).

There were no treatment-related differences noted between biochemistry parameters of control and treated rats. Values in treated animals achieving a level of statistical significance when compared to controls were considered to have arisen as a result of slightly high (aspartate aminotransferase) or low (creatinine) control values or by chance. In the absence of a treatment-related distribution or corroborative findings in the opposite sex, the values were considered to be of no toxicological significance. Moreover, all values remained within the normal range of historical data for rats of this age and strain. Macroscopic observations at necropsy did not reveal any alterations that were considered to have arisen as a result of treatment. In one female treated at 10,000 mg/kg body weight/day, an enlarged spleen and liver were noted (results not shown). Based on the absence of any clinical signs and of corroborative findings in the other animals, these findings were considered to be within the range of biological variation for rats of this age and strain, and do not represent a chance of toxicological significance. Watery fluid in the uterus, found in two females, is related to a stage in the oestrous cycle and is a normal finding. A cyst in the uterus or ovaries was found in two females and was considered to be within the range of biological variation for rats of this age and strain.

Organ weights and organ:body weight ratios of treated animals were considered to be similar to those of control animals. In one female treated at 10,000 mg/kg body weight/day, an increased spleen and liver weight was noted, correlating with the macroscopic findings in this animal (results not shown). Adrenal and kidney weights were also

Table 2. Organs and tissues weighed, preserved and microscopically examined in the 28-day oral toxicity study with lactase tox-batch

Organ/tissue	28-day study		
	Weighed	Fixed	Light microscopy
All gross lesions		x	x
Adrenal glands	x	x	x
Aorta		x	x
Brain	x	x	x
Caecum		x	x
Colon		x	x
Cervix		x	[a]
Clitoral gland		x	[a]
Duodenum		x	x
Epididymides	x	x	x
Eyes with optic nerve, Harderian gland		x	[a]
Femur including joint		x	[a]
Heart	x	x	x
Ileum		x	x
Jejunum		x	x
Kidneys	x	x	x
Larynx		x	[a]
Lacrimal glands, exorbital		x	[a]
Liver	x	x	x
Lung, infused with formalin		x	x
Lymph nodes—mandibular, mesenteric		x	x
Nasopharynx		x	[a]
Mammary gland area (female)		x	[a]
Oesophagus		x	x
Ovaries		x	x
Pancreas		x	x
Peyer's patches		x	x
Pituitary gland		x	x
Preputial gland		x	[a]
Prostate gland		x	x
Rectum		x	x
Salivary glands—sublingual, mandibular		x	[a]
Sciatic nerve		x	x
Seminal vesicles		x	[a]
Skeletal muscle		x	[a]
Skin		x	[a]
Spinal cord—cervical, midthoracic, lumbar		x	x
Spleen	x	x	x
Sternum with bone marrow		x	x
Stomach		x	x
Testes	x	x	x
Thymus	x	x	x
Thyroid with parathyroids		x	x
Tongue		x	[a]
Trachea		x	x
Urinary bladder		x	x
Uterus		x	x
Vagina		x	[a]

[a]Not examined since there were no signs of toxicity of target organ involvement.

Table 3. 28-Day oral toxicity study with lactase tox-batch: results of BW, FC, RFC

Group/sex	Enzyme dose (mg/kg bw/day)	Food consumption (g/animal/day) wk 1/2; 2/3; 3/4; 4	Mean body weight (g/animal) wk 1/2; 2/3; 3/4; 4	Relative food consumption (g/kg bw/day) wk 1/2; 2/3; 3/4; 4
1M[a]	0	27; 28; 28; 28	254; 291; 320; 342	105; 95; 86; 82
2M	500	26; 28; 27; 28	257; 301; 325; 345	101; 94; 84; 82
3M	3000	27; 28; 28; 29	262; 306; 337; 359	101; 92; 84; 82
4M	10,000	27; 27; 28; 29	259; 296; 327; 353	102; 92; 86; 82
1F	0	18; 19; 20; 20	185; 198; 210; 224	96; 98; 96; 91
2F	500	19; 19; 20; 21	191; 208; 221; 234	98; 93; 91; 91
3F	3000	18; 19; 19; 20	183; 200; 214; 226	98; 93; 89; 89
4F	10,000	18; 19; 18; 19	185; 205; 218; 226	98; 91; 84; 84

[a]n = 10 animals per group.

slightly increased. As no corroborative findings were noted in the other animals, these findings were considered not to represent a change of toxicological significance. The slight increase in adrenal:body weight ratio in females receiving 10,000 mg/kg body weight/day was considered to be mainly a result of increased adrenal weights in this female and considered of no toxicological significance. Moreover,

Table 4. Subacute 28-day oral toxicity study of lactase tox-batch in rats: relevant haematological and biochemical results[d]

Blood parameters	Treatment levels							
Dose levels (mg/kg/day tox-batch)	Group 1 M control	Group 2 M 500 mg/kg	Group 3 M 3000 mg/kg	Group 4 M 10,000 mg/kg	Group 1 F control	Group 2 F 5000 mg/kg	Group 3 F 300 mg/kg	Group 4 F 10,000 mg/k
RBC	7.79[c]	7.38	7.24	7.20	6.65	6.30	5.98[a]	6.30
10***12	0.26	0.56	0.43	0.18	0.14	0.36	0.11	0.70
Hb	9.8	9.6	9.5	9.5	8.9	8.6	8.4	8.5
mmol/litre	0.2	0.4	0.4	0.3	0.3	0.5	0.1	0.9
HCT	0.422	0.418	0.413	0.413	0.365	0.351	0.338	0.354
L/litre	0.013	0.020	0.021	0.012	0.013	0.020	0.007	0.030
ALAT	0.70	0.61	0.57	0.69	0.59	0.61	0.57	0.72
Ukat/litre	0.06	0.04	0.08	0.11	0.08	0.03	0.13	0.47
ASAT	2.92	2.43[a]	2.17[b]	2.90	2.67	2.20	1.97	2.75
Ukat/litre	0.19	0.24	0.31	0.40	0.42	0.31	0.29	0.95
CHOL	1.61	2.06	2.12[a]	2.04	2.38	2.28	2.32	2.17
mmol/litre	0.37	0.24	0.29	0.30	0.25	0.23	0.28	0.42
CREA	29	31	32	31	34	41[b]	37[a]	36
Umol/litre	2	5	4	3	2	1	2	2
UREA	5.5	6.2	6.2	5.9	6.5	8.5[b]	7.2	7.7
mmol/litre	0.8	1.5	0.7	0.5	0.5	0.6	1.1	1.0
Phos	2.66	2.65	2.80	2.70	2.45	2.43	2.12[a]	2.36
mmol/litre	0.13	0.21	0.16	0.12	0.24	0.10	0.04	0.18
Sodium	139.1	139.0	138.9	138.2[a]	138.4	137.6	137.8	137.7
mmol/litre	0.6	0.5	0.5	0.6	1.0	0.9	0.7	1.3

[a,b]Dunnett's test based on pooled significance at 5% or 1% level.
[c]Values are mean ± stand. dev. of n = five rats. Parameters haematology: red blood cells (RBC), haemoglobin (HB), hematocrit (HCT); Parameters biochemistry: alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), total cholesterol (CHOL), creatinine (CREA), urea and inorganic phosphorus (Phos).
[d]Data not shown from blood parameters: mean cell haemoglobin concentration (MCHC); mean cell volume (MCV); mean cell haemoglobin (MCH); total leukocyte count; platelet count; red cell distribution; prothrombin time; partial thromboplastin time; differential leukocyte count; total bilirubin; glucose; total protein, albumin; alkaline phosphatase and electrolytes.

Table 5. Bacterial mutagenicity assays with lactase tox-batch: results

Tox-batch treatment	Addition (μg per plate)	Mean revertant colonies/plate with strain									
		TA 98		TA 100		TA 1535		TA 1537		WP2uvrA	
		−S9	+S9[a]	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
First assay											
Tox-batch	5000	49[b]	49	61	66	17	15	6	4	17	17
Tox-batch	3330	43	58	78	59	11	11	5	5	16	13
Tox-batch	1000	52	52	48	66	17	20	2	7	15	12
Tox-batch	333	49	58	59	62	12	16	4	5	15	16
Tox-batch	100	44	57	58	61	18	16	11	7	15	16
Purified water	0	43	51	66	84	16	14	8	4	16	16
Positive control	[c]	614	1021	440	565	268	260	498	480	186	138
Second assay											
Tox-batch	5000	19	20	103	101	9	9	3	4	26	21
Tox-batch	3330	15	17	111	114	8	8	3	3	26	27
Tox-batch	1000	15	21	100	126	12	4	5	4	26	30
Tox-batch	333	12	27	91	110	7	9	4	2	22	26
Tox-batch	100	19	21	92	127	8	7	4	5	26	25
Purified water	0	16	27	100	105	9	6	5	4	24	24
Positive control	[c]	655	1151	719	1209	272	179	291	371	301	276

[a]The S9 mix was checked for sterility and found sterile.
[b]Each experiment was carried out using triplicate plates.
[c]Positive controls:sodium azide: 1 μg with strain TA1535-S9; 9-aminoacridine: 60 μg with strain TA1537-S9; daunomycine: 4 μg with strain TA98-S9; methylmethanesulphonate: 650 μg with strain TA100-S9; 4-nitroquinoline *N*-oxide: 10 μg with strain WP2uvrA-S9; 2-ami-

for adrenals, absolute organ weights are more relevant for evaluation as the weight, generally, does not vary with body weight. Other significant changes between organ weights of treated and control animals were considered not to be a sign of toxicity, since no dose–response relationship could be detected. There were no microscopic findings noted that were considered to be treatment related. All microscopic findings were within the range of background pathology encountered in Wistar rats of this age and strain and occurred at similar incidences and severity in both control and treated rats.

Table 6. *In vitro* human lymphocyte assay with lactase tox-batch: results

Test details	Treatment (μg/ml)	Number of metaphases per 1000 cells (% of control)[a]	Cells with abberrations (including gaps)	Cells with aberrations (excluding gaps)
Experiment 1				
24-hr treatment, 24-hr fixation time −S9	0	100	2	1
	1000	98	2	2
	3330	99	2	1
	5000	94	2	2
	Mitomycin C (0.2)	30	55***	54***
48-hr treatment, 48-hr fixation time −S9	0	100	2	1
	5000	85	4	1
	Mitomycin (0.2)	95	55***	52***
3-hr treatment, 24-hr fixation time +S9	0	100	3	3
	1000	89	3	2
	3330	73	2	1
	5000	96	0	0
	Cyclophosphamide (15)	47	97***	95***
3-hr treatment, 48-hr fixation time +S9	0	100	3	3
	5000	86	2	1
Experiment 2				
24-hr treatment, 24-hr fixation time −S9	0	100	2	2
	1000	98	4	3
	3330	99	3	3
	5000	97	5	4
	Mitomycin C	59	52***	52***
3-hr treatment, 24-hr fixation time +S9	0	100	1	1
	1000	102	6	6
	3330	89	3	3
	5000	88	6	6
	Cyclophosphamide	48	56***	55***

***Very highly significant; $P < 0.001$ of increase in frequency of aberrant metaphases in treated cultures, compared to negative control values.
[a]Duplicate cultures.

It was concluded that administration of the tox-batch at doses of 500, 3000 and 10,000 mg/kg body weight/day did not induce noticeable signs of toxicity.

The NOAEL of the tox-batch in the subacute study was therefore 10,000 mg/kg body weight/day (De Hoog, 1998).

Mutagenicity—Ames test

No increases in the number of revertants were obtained in any of the five bacterial strains at the concentrations tested, either in the presence or absence of S-9 mix. The positive control substances, sodiumazide, 2-aminoanthracene, 9-aminoacridine, daunomycine, methylmethanesulfonate and 4-quinoline-*N*-oxide, gave the expected strong increase in the number of revertants (Table 5). It is concluded that the tox-batch did not show mutagenic activity under the conditions of the test (Verspeek-Rip, 1998).

In vitro chromosomal aberration test with human lymphocytes

No biologically or statistically significant increases in the frequency of metaphases with aberrant chromosomes, compared to solvent control values, were seen in cultures treated with the tox-batch, including or excluding gap-type aberrations, at all sampling times ($P > 0.05$), both in the absence and presence of S-9 mix. The results for the concentrations that were scored in this study are summarised in Table 6. The known clastogens mitomycin C (direct-acting clastogen) and cyclophosphamide (requires metabolic activation to achieve optimum activity) induced statistically significant increases in the frequency of metaphases with aberrant chromosomes, compared to the solvent control values, at all sampling times ($P < 0.001$ in all cases), thus demonstrating the sensitivity of the procedure and the metabolic activity of the S-9 mix employed (Table 6).

It is concluded that the tox-batch did not show any evidence of clastogenic activity under the conditions of the test (Bertens, 1998).

DISCUSSION AND CONCLUSION

The results of the 28-day oral toxicity study presented in this article can be used to calculate the safety margin of the consumption of the lactase enzyme preparation from *Kluyveromyces lactis*.

Whereas the dosing of an enzyme preparation in its application is always based on the enzyme activity present in the preparation, the calculation of the daily intake of a preparation, as well as the safety margin, should be based on weight. The best way to express the weight is on the basis of the total organic solids (TOS). This TOS parameter is used to distinguish the proportion of the enzyme preparation from

the source material from that contributed by diluents and other additives and ingredients.

For the calculation of the safety margin, it is necessary to know: (a) the NOAEL of the 28-day oral toxicity study expressed as TOS; (b) the concentration of the enzyme-TOS in the final food products; and (c) the human consumption of the food products concerned.

In the 28-day oral toxicity study, no adverse effects were observed at the highest dose given, that is, 10,000 mg/kg body weight/day. As the percentage TOS in the enzyme preparation was 15.4%, the highest dose corresponds with 1540 mg TOS/kg body weight/day.

The maximum recommended dose of the enzyme preparation in milk is 3000 NLU/litre. As 1 NL unit corresponds to 0.014 mg TOS the maximum recommended doses in 1 litre milk thus correspond to 42 mg TOS. The average US consumer uses an equivalent of 250 kg milk annually in the form of milk, skim milk powder and cheese (*World Dairy Facts*, 1999; USDA, 1996), which is about 0.7 litres per person per day. The consumption of a person with an average weight of 60 kg thus results in a maximum estimated daily intake (EDI) of 42*(250/365 L)/60 = 0.48 mg enzyme-TOS/kg body weight/day. The safety margin would thus be: NOAEL/EDI = 1540/0.48 = 3208.

The data presented in this article indicate that there are no reasons for safety concerns when using the lactase from *Kluyveromyces lactis* in the preparation of dairy products.

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