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APPENDIX C: Dietary exposure

Contents:

1 Application areas

CB108 Lactase will be used in dairy processing. In the production of dairy products, the enzyme will convert lactose into GOS and glucose. CB108 Lactase will be able to generate GOS *in situ* in raw milk or whey even with low lactose content (roughly 5% lactose content in milk), as benefit to provide 1) a low lactose/lactose free dairy product with reduced total sugars and caloric content in the final dairy product and 2) enable dairy products to contain galacto-oligosaccharides prebiotic material. Example of final dairy products produced with enzymated milk or whey would be milk, milk drinks, yogurts, fermented milk drinks, cheese, …etc.

CB108 Lactase will also be used in the production of purified GOS for the infant formula industry. GOS is primarily used in infant formula to mimic the effect of the human milk oligosaccharides (HMOs) on babies. GOS are present in both human milk and bovine milk, but in low concentration especially in human milk.

Consequently, according to the food group classification system used in Standard 1.3.1- Food Additives Schedule 15 (15-5), CB108 Lactase will be used in:

- 1 Dairy products (excluding butter and fats)
- 13.1 infant formula products

2 Level of use

In dairy processing, CB108 lactase can be used in milk, or whey to convert lactose to glucose and GOS. The proposed application rate of CB108 Lactase is 151-601 mg TOS /kg raw material (milk, whey).

In GOS production, proposed application rate of CB108 Lactase is 317-932 mg TOS/kg raw material (lactose).

3 Level of residues in food

3.1 Estimated Food Intake

Commercial food enzyme preparations are generally used following the *Quantum Satis* (QS) principle, i.e. at a level not higher than the necessary dosage to achieve the desired enzymatic reaction – according to Good Manufacturing Practice. The amount of enzyme activity added to the raw material by the individual food manufacturer has to be determined case by case, based on the desired effect and process conditions. Therefore, the enzyme manufacturer can only issue a recommended enzyme dosage range. Such a dosage range is the starting point for the individual food producer to fine-tune his process and determine the amount of enzyme that will provide the desired effect and nothing more. Consequently, from a technological point of view, there are no 'normal or maximal use levels' and CB108 Lactase is used according to the QS principle. A food producer who would add much higher doses than the needed ones would experience untenable costs as well as negative technological consequences.

The dosage of a food enzyme depends on the activity of the enzyme protein (in this case CB108 Lactase) present in the final food enzyme preparation (i.e. the formulated food enzyme). However, the activity Units as such do not give an indication of the amount of food enzyme actually added. Microbial food enzymes contain – apart from the enzyme protein in question – also some substances derived from the producing microorganism and the fermentation medium. The presence of all organic materials is expressed as Total Organic Solids[1] (TOS, FAO/WHO, 2006). From a safety point of view, the dosage on basis of TOS is more relevant. Consequently, the use levels are expressed in TOS in the Table below. If wanted, these TOS values can easily be converted to activity Units on basis of the Units/TOS ratio. Whereas the dosage of a food enzyme depends on the enzyme activity present in the final food enzyme preparation, the dosage on basis of TOS is more relevant from a safety point of view. Therefore, the use levels are expressed in TOS.

The Table below shows the range of recommended use levels for each application where the food enzyme may be used.

Application	**Raw material (RM)**	**Recommended use levels (mg TOS/kg RM)**	**Maximal recommended use levels (mg TOS/kg RM)**
Dairy processing	Milk, Whey	151-601	601
GOS production	Lactose	317-932	932

CB108 Lactase from *Bacillus subtilis* may be used in the manufacture of a wide variety of foods, food ingredients and beverages. Due to this wide variety of applications, the most appropriate way to estimate the human consumption in the case of food enzymes is using the so-called Budget Method (Hansen, 1966; Douglass *et al.*, 1997). This method enables to calculate a Theoretical Maximum Daily Intake (TMDI) based on conservative assumptions regarding physiological requirements for energy from food and the energy density of food rather than on food consumption survey data.

The Budget Method was originally developed for determining food additive use limits and is known to result in conservative estimations of the daily intake.

The Budget Method is based on the following assumed consumption of important foodstuffs and beverages (for less important foodstuffs, e.g. snacks, lower consumption levels are assumed):

[1] In the case of food enzymes, which are – per legal definition – not formulated, TOS is the same as Dry Matter minus ash. The amount of ash (e.g. mineral salts used in the fermentation) does generally not exceed a few percent.

Average consumption over the course of a lifetime/kg body weight/day	Total solid food (kg)	Total non-milk beverages (l)	Processed food (50% of total solid food) (kg)	Soft drinks (25% of total beverages) (l)
	0.025	0.1	**Essential foods 0.0125** **Non-essential foods 0.00125**	**Soft drinks 0.025** **Sport nutrition 0.0025**

3.2 Estimated intake of CB108 Lactase

The recommended use levels of the enzyme CB108 Lactase are given, based on the raw materials used in the various food processes. For the calculation of the TMDI, the maximum use levels are chosen. Furthermore, the calculation takes into account how much food or beverage is obtained per kg raw material and it is assumed that all the TOS will end up in the final product.

Application		Raw material (RM)	Maximal recommended use level (mg TOS/kg RM)	Example Final food (FF)	Ratio RM/FF	Maximal level in FF (mg TOS/kg food)
Beverages	Dairy processing	Whey	601	Whey drinks, Sports drinks	1	**601**
Solid food	Dairy processing	Milk	601	Yogurt, Yogurt drinks, Milk drinks, plain milk	1	**601**

The Total TMDI can be calculated on basis of the **maximal** values found in food and beverage. With the current dossier dairy processing represents the maximum exposure for solid food and beverages, respectively.

In dairy processing the CB108 Lactase is use for production of protein enriched milk products and whey drinks. These foods and drinks are considered non-essential as they represent a maximum of 1-10% of the daily energy intake. For calculation of TMDI for solid foods and beverages the ‘non-essential foods’ and ‘sport nutrition’ consumption data has therefore been used.

Consequently, the Total TMDI will be:

TMDI in food (mg TOS/kg body weight/day)	TMDI in beverage (mg TOS/kg body weight/day)	Total TMDI (mg TOS/kg body weight/day)
601x0.00125=0.75	601x0.0025=1.50	**2.25**

It should be stressed that this Total TMDI is based on conservative assumptions and represents a highly exaggerated value because of the following reasons:

- It is assumed that ALL producers of the above mentioned foodstuffs and beverages use the specific enzyme Galactosidase from *Bacillus subtilis*.
- It is assumed that ALL producers apply the HIGHEST use level per application;
- For the calculation of the TMDI's in foodstuffs as well as in beverages, only THOSE foodstuffs and beverages were selected containing the highest theoretical amount of TOS. Thus, foodstuffs and beverages containing lower theoretical amounts were not taken into account;
- It is assumed that the amount of TOS does not decrease as a result of the food production process;
- It is assumed that the final food containing the calculated theoretical amount of TOS is consumed DAILY over the course of a lifetime;
- Assumptions regarding food and beverage intake of the general population are overestimates of the actual average levels (Douglass *et al.*, 1997).

Although the guidance of the EFSA CEF panel on the submission of a dossier for food enzymes (EFSA, 2009a) proposes the Budget Method for calculation of the daily intake, it also mentions that where the food enzyme is used for the production of foods specifically designed for infants (0-12 months) or young children (12-36 months), *ad hoc* conservative exposure estimates must be provided.

The dietary exposure for infant and young children was therefore calculated by means of the Estimated Daily Intake (EDI). The EDI can be calculated based on the maximal dose levels and consumption data from reliable surveys.

The average GOS yield is approx. 45% from lactose. The yield of 45% corresponds to a RM/FF ratio of 2.22 kg lactose per kg GOS. This gives a use level of 2,069 mg TOS/kg GOS.

According to EU approval on the use of GOS in infant formulae, there should not be more than 7.2g GOS/kg infant formulae.

The calorie density of infant formulas range from 0.60-0.70 kcal/g and the energy intake of infant (1 month) is 115 kcal/kg bw/day (SCF, 2003). Using the lowest calorie density (0.60 kcal/g) as a worst case scenario, the intake of infant formula will be maximum 192 g infant formula/kg bw/day (1 month old).

The above data, finally leading to an estimated GOS intake of 1.38 g GOS/kg bw/day.

Based on the maximal level of Galactosidase used in GOS production (see table above), the EDI can be calculated as follows:

EDI (infants 1 to < 2 months old) (according to EU guideline) (mg TOS/kg body weight/day)
2,069 x (1.38/1,000) = 2.85

Similarly, according to FSANZ code 2.9.1, the permitted maximum amount of GOS consumption is "if only galacto-oligosaccharides are added—290 mg/100 kJ of galacto-oligosaccharides", which is equal to 290 mg/23 Kcal. As the energy intake of infant (1 month) is 115 kcal/kg bw/day (SCF, 2003), the intake of GOS will be 1.450 g/kg body weight/day (1 month old). Accordingly, the EDI can be calculated as follows:

EDI (infants 1 to < 2 months old) (according to FSANZ guideline) (mg TOS/kg body weight/day)
2,069 x (1.45/1,000) = 3.00

The EDI calculation for infants results in a dietary intake which is of similar order of magnitude than the intake calculated by the Budget Method. This indicates that the overestimation of intake according to the calculation of the Budget Method is high enough to cover the consumption of infant formulas.

4 Safety assessment

CB108 Lactase is an enzyme produced from *B. subtilis* which was genetically modified to express the lactase gene from *B. bifidum*.

DuPont IB has determined by scientific procedures that production organism *B. subtilis* is safe as a production organism as it pertains to the DuPont IB *B. subtilis* Safe Strain Lineage (see Appendix B2, B3). For the determination of the safety of CB108 Lactase, we use the results of toxicology studies conducted on BIF917 lactase preparations derived from *B. subtilis*.

Summarizing the results obtained from the several toxicity studies the following conclusions can be drawn:

- No mutagenic or clastogenic activity under the given test conditions was observed;
- The sub-chronic oral toxicity study showed a No Observed Adverse Effect Level (NOAEL) of at least 1,000 mg TOS/kg body weight/day.

Identification of the NOAEL

In the 90-day oral (gavage) study in rats for BIF917 lactase from *B. subtilis*, a NOAEL was established at 1000 mg total protein/kg bw/day equivalent to 1416.4 mg TOS kg bw/day. The study was designed based on OECD guideline No. 408 and conducted in compliance with both the FDA Good Laboratory Practice Regulations and the OECD Good Laboratory Practice.

NOAEL: 1000 mg TP/kg bw/day = 1416.4 mg TOS/kg bw/day

Determination of the Margin of Safety

The Margin of Safety (MoS) for human consumption can be calculated by dividing the NOAEL by the Total Theoretical Maximal Daily Intake (TMDI). As was shown in the section above, the Total TMDI of the food enzyme is 2.25 mg TOS/kg body weight/day. Consequently, the MoS is:

MoS = 1,416.4 mg TOS/kg bw/day / 2.25 mg TOS/kg bw/day

= 630

5 Conclusion

The safety of CB108 Lactase from *B. subtilis* as a food processing aid in dairy processing and GOS production is assessed with the Safe Strain Lineage concept and toxicology studies conducted on earlier strains of this lineage. Similar to all enzymes produced by the same DuPont IB *B. subtilis* lineage, CB108 Lactase produced by *B. subtilis* is not expected to be a mutagen, a clastogen, or an aneugen.

Based on a worst-case scenario that a person is consuming dairy products treated with CB108 Lactase (i.e., cumulative risk), this NOAEL still offers at least a 630 fold margin of safety. Based on a margin of safety of 630, the proposed uses of CB108 Lactase in dairy processing and GOS production are not a human health concern and are supported by existing toxicology data.

6 References

Denise Hoban, H-30869 (Milky Whey): Subchronic Toxicity 90-Day Gavage Study in Rats, Report No. DuPont-20510-1026, August 8, 2014.

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