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Opinion of the Scientific Committee on Food on

ß-cyclodextrin produced using cycloglycosyltransferase obtained from *Paenibacillus macerans*

(Adopted by the SCF on 22/6/2000)

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Terms of Reference

To evaluate the safety-in-use of ß-cyclodextrin manufactured by the action of the enzyme cycloglycosyltransferase obtained from *Paenibacillus macerans* when used as a food additive.

Background

ß-cyclodextrin (BCD) is a food additive with applications as carrier and stabiliser of food flavours, food colours, and some vitamins. It is a cyclic heptamer composed of seven glucose units joined "head-to-tail" by α-1,4 bonds. It is employed in food as a flavour protecting agent at levels not exceeding 1 g/kg food. The producer organism was formerly known as *Bacillus macerans* but is now listed as *Paenibacillus macerans* (5).

In this submission the petitioner proposes manufacture of BCD by a method using the enzyme cycloglycosyltransferase (CGTase) n°: 2,4,1,19 derived from a mutant strain of *Paenibacillus macerans* (1).

The safety-in-use of BCD manufactured by the action of a CGTase obtained from *Bacillus circulans* on partially hydrolysed starch has already been evaluated by the SCF in 1996 when BCD was allocated an ADI of 0-5 mg/kg b.w. (2). The safety-in-use of BCD manufactured by the action of a CGTase obtained from *Bacillus macerans* has been evaluated already by JECFA in 1993 (3) and 1995 (4).

Evaluation

Only limited information on the manufacturing process for producing the enzyme CGTase from *Paenibacillus macerans* were supplied by the petitioner. However, it is known that the producer organism *Paenibacillus macerans* IFO 3490 had been selectively mutated by conventional treatment with UV light and certain mutagens so as to be no longer capable of forming spores and also to express CGTase at high levels. The petitioner has investigated its non-pathogenicity and non-toxigenicity only by searching the published literature between 1967-1999 for any relevant reports on adverse reactions. However, taking into account the purity of the CGTase, the purity of the BCD crystals and that there are several purification steps included in the production processes, the Committee considers this to constitute a dilution factor of several orders of magnitude for any *Bacillus* toxins possibly elaborated by the producer organism. The Committee therefore considers it unnecessary to subject the final crystalline BCD to additional analytical examination required to evaluate the safety of use of *Bacillus* species in animal nutrition as set out in the SCAN opinion (6).

CGTase is manufactured by a fermentation procedure involving aerobic submerged culturing of a lyophilised seed culture of *Paenobacillus macerans* IFO 3490. Measurements of pH, enzyme activity and analysis of fermentation-produced gases are used as the parameters for controlling the fermentation process. The resulting enzyme solution is purified by removing the bacterial cells by centrifugation and subsequent filtration through a ceramic filter. The solution is then concentrated by ultrafiltration, kept for 1 hour at 37°C, filtered again, and finally stabilised and diluted to a standard enzyme activity by the addition of 5% NaCl and 10% ethanol. The final enzyme preparation is analysed for compliance with the JECFA specification for CGTase before marketing.

The safety of the CGTase, prepared by the new manufacturing process, was examined in a 13-week gavage study in rats which did not show any treatment-related adverse findings.

BCD is produced by the action of the CGTase on a carbohydrate substrate. It is then precipitated from the starch hydrolysate reaction mixture as a toluene complex and separated from the reaction mixture by filtration or centrifugation. The toluene is removed from the precipitated complex by steam distillation, the residual crude cyclodextrin solution being subsequently purified and concentrated. BCD is eventually recovered after a crystallisation step followed by centrifugation and drying as a crystalline preparation. Any DNA or protein carried over by the CGTase would be found only in the aqueous phase separated from the precipitated BCD-toluene complex. Any traces of DNA and proteins on the BCD crystals would be denatured by the subsequent heat treatment to remove the solvent, the carbon decolourisation and the drying of the final BCD crystals, thus making them biologically inactive.

Comparison of the HPLC chromatograms of the mother liquor of BCD produced from *Bacillus circulans* and the mother liquor from BCD produced from *Paenibacillus macerans* shows the same patterns in both cases. The final product has also been shown to comply with the established JECFA specification for BCD.

There are no considerations relating to intake and nutritional aspects specifically needed for BCD manufactured by fermentation with CGTase from *Paenibacillus macerans* which have not already been taken into account in the safety evaluation by the SCF for the BCD made by fermentation using the *Bacillus circulans* CGTase as set out in the SCF Opinion of 1996 (2).

Conclusion

The Committee considers that BCD produced by the use of CGTase derived from *Paenibacillus macerans* is comparable with the BCD produced by CGTase derived from *Bacillus circulans* and complies with the existing JECFA specification for BCD. The Committee considers that the use as a food additive of BCD manufactured by this new method is acceptable.

The BCD produced by the use of CGTase derived from *Paenibacillus macerans* should be included in the ADI of 0-5 mg/kg bw set earlier by the SCF for BCD manufactured by the action of a CGTase obtained from *Bacillus circulans.*

References

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