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# Evaluation of certain food additives

Ninety-fifth report of the Joint FAO/WHO Expert Committee on Food Additives


Food and Agriculture Organization of the United Nations

World Health Organization

World Health Organization

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# Dedication

It was with great sadness that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) noted the passing of Dr Jim Smith, Past President of the Canadian Institute of Food Science and Technology.

Jim was a longstanding Member of the Committee from 2003. His contribution to food safety risk assessment, and in particular to the work of JECFA, is gratefully recognized. Under his technical leadership, JECFA pioneered the evaluation of food additives, processing aids and flavours, which laid the foundation for this type of assessment at national and international level. Jim's contribution to the work of JECFA over the years was unique, and was the foundation of solid, objective and consistent assessments.

His always-positive attitude and big smile helped the Committee navigate through many difficult agendas. His warm personality, bright mind and great sense of humour will always be remembered. Jim will be sorely missed by his peers and friends in the scientific community.

In recognition of his services, the Committee dedicates this report to the memory of Dr Jim Smith.

# List of participants

## Ninety-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Virtual meeting, 6–17 and 22 June 2022

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# List of acronyms and abbreviations

| | |
|---|---|
| AAU | α-amylase unit |
| ADI | acceptable daily intake |
| ADME | absorption, distribution, metabolism and elimination |
| ATCC | American Type Culture Collection |
| BAMU | β-amylase unit |
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CCFA | Codex Committee on Food Additives |
| COVID-19 | coronavirus disease |
| EHC | Environmental Health Criteria |
| EFSA | European Food Safety Authority |
| FAO | Food and Agriculture Organization of the United Nations |
| GMP | Good Manufacturing Practices |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LU | lipase unit |
| MOE | margin of exposure |
| MSDI | maximized survey-derived intake |
| NOAEL | no-observed-adverse-effect level |
| PLA2 | phospholipase A2 |
| SGF | simulated gastric fluid |
| SIF | simulated intestinal fluid |
| SPET | single portion exposure technique |
| TMDI | theoretical maximum daily intake |
| TOS | total organic solids |
| WHO | World Health Organization |

# 1. Introduction

The Ninety-fifth meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) was convened by video conference on 6–17 and 22 June 2022. The meeting was opened on behalf of FAO by Dr Divine Njie (Deputy Director, Food Systems and Food Safety Division) and on behalf of the Director-General of WHO by Dr Moez Sanaa (Head of Unit, Standards and Scientific Advice Unit, Department of Nutrition and Food Safety).

In his opening remarks, Dr Njie welcomed all meeting participants and stressed that, despite the challenges of the ongoing coronavirus disease (COVID-19) pandemic and the ambitious meeting agenda (Annex 1), the work of JECFA had progressed and continued to provide sound scientific advice to the Codex Alimentarius Commission and the Member States, largely thanks to the efforts and work of the JECFA experts. He reminded the participants of their responsibility to impart the most unbiased and best scientific advice possible, and that they had been invited to serve solely in their capacity as scientific experts to provide sound scientific advice and not as representatives of their employer or country. He closed by reiterating his sincere gratitude to all participants for providing their time and expertise to this JECFA meeting.

Dr Moez Sanaa welcomed all meeting participants on behalf of WHO and thanked all experts for their commitment and dedication to the work of JECFA. He underlined the importance of their work in relation to the work of the Codex Alimentarius Commission in developing international food safety standards.

## 1.1 Procedural matters

Because of the travel restrictions as a result of the COVID-19 pandemic in many countries, it was not possible to convene a physical meeting; instead, it was decided to hold the meeting online by video-conferencing. In view of the time differences in the countries of origin of the invited experts, the video-conference was restricted to a 4-hour time slot (12:00–16:00 Central European Summer Time) each day.

All participating experts reaffirmed that online meetings did not permit the necessary in-depth, robust scientific discussions that have been a characteristic of past JECFA physical meetings, and were therefore not a suitable substitute. In particular, the experts felt that the online format did not foster the atmosphere of trust, inclusiveness and openness that has marked all JECFA physical meetings, making participation for new experts especially challenging.

The experts considered that the success of the Ninety-fifth meeting was mainly a result of the cohesion between them, which stemmed from the trust built on the relationships they had formed during previous in-person meetings. The experts also decried the significant difficulty of holding any informal meetings outside the scheduled meeting times because of the widely differing time zones. They noted that such informal interactions during the physical meetings were instrumental in solving problems and discussing issues in depth, bilaterally or in small groups, and added that such informal settings often gave rise to equitable solutions to difficult problems.

The experts also emphasized that an invitation to a physical JECFA meeting at the FAO or WHO headquarters gives rise to a more significant recognition by the expert's employer of the weight, reach, responsibility and workload required for full participation in a JECFA meeting. The same degree of acknowledgement was not granted by employers for this online meeting, as the experts remained available locally. This lack of recognition of the workload and significance of participation in a JECFA meeting led to an increase in other demands on the experts, resulting in greater distractions and more frequent scheduling conflicts.

Cumulatively, the experts concluded that maintaining the online-only format would be counterproductive for participation in future JECFA meetings.

In recognition of the difficulties experienced and the tremendous efforts made, the Joint FAO/WHO Secretariat expressed its deep gratitude to all the experts for their commitment and flexibility, especially as the scheduled meeting times were exceedingly inconvenient for many.

The meeting report was adopted on 22 June 2022.

## 1.2 Declarations of interests

The Secretariat informed the Committee that all experts participating in the Ninety-fifth JECFA meeting had completed a declaration-of-interest form. The declarations were assessed as to the extent to which any interest could be reasonably expected to influence the experts' judgement. The declared interests were considered unlikely to impair the individual's objectivity or have any significant influence on the impartiality, neutrality and integrity of the work. Neither FAO nor WHO received any public comments in response to the online posting of the names and brief biographies of the individuals considered for participation in the expert meeting. The interests of all participants were disclosed at the beginning of the meeting to all meeting attendees.

## 1.3 Adoption of the agenda

After discussion among the experts, several changes were made to the agenda to (i) remove three enzymes (acid prolyl endopeptidase from *Aspergillus niger* expressed in *Aspergillus niger*; asparaginase from *Aspergillus niger* expressed in *Aspergillus niger*; and glucose oxidase from *Penicillium chrysogenum* expressed in *Aspergillus niger*) for which no data were submitted; (ii) ensure the enzyme names were formatted consistently (see Section 2.1); (iii) distinguish between different enzymes with the same name under this new naming convention by including a unique JECFA enzyme identifier (see Section 2.1); and (iv) take into account the updated names of certain donor organisms (the donor organisms of the enzymes discussed in agenda items 7.1.2, 7.1.4 and 7.1.7 were updated from *Bacillus stearothermophilus* to *Geobacillus stearothermophilus*, from *Talaromyces emersonii* to *Rasamsonia emersonii*, and from *Thermomyces lanuginosus* to *Thermomyces lanuginosus* and *Fusarium oxysporum*, respectively). These changes were reflected in both the report and relevant monographs. The meeting agenda (Annex 1) was adopted with no further modifications.

## 1.4 Meeting summary

Please see Annex 2 for a summary of food additives and flavourings discussed, as well as specifications agreed or revised.

# 2. General considerations

## 2.1 Guidance on the naming and identification of JECFA enzyme preparations

### 2.1.1 Naming enzyme preparations

The Committee reviewed the list of enzyme preparations for evaluation at the present meeting and noted that there were two different formats for the title. Reflecting on the past evaluations and considering ease of use, the Committee decided that the name given to the enzyme preparation should correspond to the name of the enzyme activity or activities that most accurately characterizes the preparation, the donor(s) of the genetic material and the production microorganism. The Committee therefore decided that enzyme preparations on the agenda of the present and future meetings would conform to the following format wherever possible: [principal enzyme activity (activities)] from [name of donor organism(s)] expressed in [systematic name of production organism], for example, "α-amylase from *Bacillus licheniformis* expressed in *Bacillus subtilis*".

### 2.1.2 Identification of JECFA enzyme preparations

In considering the agenda for the present meeting (Annex 1), the Committee noted that by following the naming convention above, two of the enzyme preparations would have the same name, that is, agenda items 7.1.1 (α-amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*) and 7.1.2 (α-amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*).

In line with Recommendation 5 from Section 2.1.2 of the Report of its Eighty-ninth meeting, 2020 (Annex 3, reference 246), "The Committee supported establishment of a separate JECFA numbering system for identifying enzyme preparations for which JECFA had completed safety evaluations (similar to that used for flavourings)", the Committee decided that an identification system would be used for all enzyme preparations. The identifier would consist of two parts: the JECFA meeting number followed by the number of agenda point of the substance, that is, JECFAXX-Y (e.g. JECFA95-1). The examples noted above therefore become 7.1.1 α-amylase (JECFA95-1) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis,* and 7.1.2 α-amylase (JECFA95-2) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*.

## 2.2 Data submission on Class 1, Type iii enzyme preparations

Under the current JECFA enzymes guidelines described in Environmental Health Criteria 240 *(1)*, toxicological data and dietary exposure information are not required for Class 1, Type iii preparations. However, for many of the enzyme preparations that the Committee considers, toxicological data and dietary exposure assessment data are available. The Committee therefore wishes to emphasize that, when such data exist, they should be submitted to the Committee for evaluation.

## 2.3 Consideration of information labelled confidential

The Committee discussed the requests of sponsors for confidentiality for some information, and determined that any information that can be found in the public domain will be included in JECFA publications as necessary.

## 2.4 Data required to support the evaluation of enzyme preparations

The Committee expressed its frustration that many of the current data submissions were inconsistent with key aspects of the guidelines published by the Committee. Although the Committee has spent significant time attempting to use the available data to finalize the evaluation, the data gaps have been too large to do so. This has not only led to a huge demand on deliberation time of the Committee, but also demonstrated that the Committee's time would be better spent in the evaluation of submissions prepared in accordance with the guidelines.

The Committee noted when preparing the Specifications monographs for individual enzyme preparations that a considerable amount of supporting information was not made available, even when requested on more than one occasion. In particular, the Committee recognized that the establishment of a validated assay to support the identification and quantification of an enzyme was of paramount importance. The Committee was further reminded that such information was a requirement of the submission checklist (items 21 and 22) and was supported by the decisions of the Eighty-ninth meeting of the Committee (Recommendation 1c from the section 2.1.2 of the Report of its Eighty-ninth meeting, 2020; Annex 3, reference 246): "The Committee approved the proposed checklist of data requirements for the risk assessment of enzyme preparations in submissions for review by JECFA…".

In addition, the details of the assays supplied included the use of an enzyme reference or calibrant, rather than a direct link to an original enzyme assay from which a meaningful unit definition could be derived. The consequence

of the absence of such data has resulted in the Committee designating many of the enzyme specifications "tentative" and toxicological evaluations "temporary" at the present meeting.

It should also be noted that, for one enzyme preparation (phospholipase A2 [PLA2; JECFA95-8] from porcine pancreas expressed in *Aspergillus niger*), the Committee became aware that highly relevant toxicological studies, now known to have been submitted to at least one regulatory body in 2005, were not submitted to JECFA. The sponsor had asked the Committee to consider toxicological studies on a related enzyme preparation in lieu of studies on the requested enzyme preparation itself, a situation that the Committee considered unacceptable.

The Committee asks the JECFA Secretariat to urge sponsors and Codex Members to ensure that all required information is available for evaluation prior to requesting inclusion in the CCFA JECFA Priority List.

## Reference

1. Chapter 9: Principles related to specific groups of substances. In: Environmental health criteria 240. Principles and methods for the risk assessment of chemicals in food. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; International Programme on Chemical Safety (IPCS); 2009 (updated 2020) (https://inchem.org/documents/ehc/ehc/ehc240_chapter9.pdf, accessed 22 June 2022).

# 3. Specific food additives (other than flavouring agents)

## 3.1 Safety evaluations

### 3.1.1 α-Amylase (JECFA95-1) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*

#### Explanation

At the request of the Codex Committee on Food Additives (CCFA) at its Forty-eighth Session *(1)*, the Committee evaluated the safety of α-amylase (Enzyme Commission No. 3.2.1.1; Chemical Abstracts Service [CAS] No. 9000-90-2) from *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) expressed in *Bacillus licheniformis*. The Committee had not previously considered this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-1 to distinguish it from similarly named enzyme preparations. The Committee had previously evaluated several other α-amylases, including from *G. stearothermophilus*, and α-amylase from *G. stearothermophilus* expressed in *Bacillus subtilis* (Annex 3, reference 94), for which an acceptable daily intake (ADI) "not specified"[1] was established.

The term "α-amylase" refers to the enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product containing the enzyme of interest, which is used in the toxicity studies; and the term "enzyme preparation" refers to the product formulated for commercial use.

The Committee has previously assessed the safety of enzyme preparations derived from *B. licheniformis* (e.g. pullulanase, Annex 3, reference 205; serine protease, Annex 3, reference 205), and established an ADI "not specified" for these enzyme preparations. On this basis, the present Committee considered that α-amylase (JECFA95-1) from *G. stearothermophilus* expressed in *B. licheniformis* met the criteria of a Class 1, Type iii enzyme, as described in Environmental Health Criteria (EHC) 240 *(2)*. A Class 1, Type iii enzyme preparation is produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted data.

The enzyme catalyses the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units. The enzyme preparation is intended to be used as a processing aid in starch/carbohydrate processing, for example, potable alcohol production.

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

The Committee conducted a literature search in Google Scholar with the linked search terms "alpha-amylase" AND "*Geobacillus stearothermophilus*" and "alpha-amylase" AND "*Bacillus licheniformis*". This search identified 298 references. None of the identified publications provided additional toxicity data relevant to this evaluation.

### Genetic background

The production organism *B. licheniformis* is a non-pathogenic and non-toxigenic bacterium. It is ubiquitous in nature, having been isolated from environments as diverse as freshwater, saltwater, soil, plants, animals and air *(3)*. *B. licheniformis* has a history in the production of enzymes intended for use in food processing.

The production strain JSF07-170-3 was obtained from *B. licheniformis* Bra 7 strain by inactivation of the genes encoding α-amylase, chloramphenicol resistance and a sporulation factor. The α-amylase gene from *G. stearothermophilus* ASP-154 strain was modified and transferred into *B. licheniformis* Bra 7. The donor strain was deposited in the American Type Culture Collection (ATCC) as *B. stearothermophilus* ATCC 39709. The stability of the introduced gene sequence was confirmed by the production strain performance over at least 60 generations.

### Chemical and technical considerations

This α-amylase enzyme is produced by pure culture fermentation of the *B. licheniformis* production strain. Manufacture of the enzyme preparation includes fermentation, recovery and formulation. After fermentation, the broth containing the α-amylase enzyme is separated from the biomass via a series of filtration steps, then concentrated. The resulting enzyme concentrate is formulated and standardized into a liquid preparation. The entire process is performed in accordance with current Good Manufacturing Practices (GMP) and with food-grade raw materials. The enzyme concentrate is tested to ensure that it is free from the production organism and any antibiotic activity.

The primary sequence of α-amylase produced by *B. licheniformis* consists of 486 amino acids; its molecular weight calculated from the determined amino acid sequence is 55 kDa. The α-amylase produced by *B. licheniformis* is not expected to have any significant subsidiary or secondary activity.

One α-amylase unit (AAU) is defined as the amount of enzyme required to hydrolyse 10 mg of starch per minute under specified conditions (pH 6.0, $T$ = 60 °C). However, the activity of α-amylase is determined spectrophotometrically by measuring the release of *p*-nitrophenol from *p*-nitrophenyl maltoheptoside at 410 nm (pH 5.6, $T$ = 25 °C, 5 min), compared with a proprietary enzyme standard with activity expressed in AAU. The mean activity from three batches of the liquid enzyme concentrate was 32 377 AAU/g.

α-Amylase catalyses the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units. The enzyme preparation is intended for use as a processing aid in starch/carbohydrate processing, brewing/cereal beverage processing and potable alcohol production at maximum levels of 31.6 mg total organic solids (TOS)/kg raw material. The TOS includes the enzyme of interest and residues of organic materials (e.g. proteins, peptides and carbohydrates) from the production organism during manufacture.

The α-amylase enzyme is inactivated by heat or removed during processing. It is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

Potential immunological cross-reactivity with known allergens was assessed by comparing the amino acid sequence of α-amylase with those of known allergens *(4,5)*. The analyses included a search for amino acid matches between α-amylase and other proteins with more than 35% identity over the full length of the α-amylase and over a sliding window of 80 amino acids, as well as full identity over eight contiguous amino acids.

The results indicated that the amino acid sequence of the α-amylase aligned with Asp o 21, an α-amylase originating from the fungus *Aspergillus oryzae*. This is a respiratory allergen associated with occupational rhinitis and asthma in bakers. When compared with the α-amylase amino acid sequence, the enzymes showed a 37.5% identity over a sliding window of 80 amino acids, with 25.1% identity over the full length of the protein. No identity over eight contiguous amino acids was observed. The α-amylase did not align with any known food allergen.

The α-amylase was assessed for its resistance to proteolysis using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) assays in vitro *(6)*. In the SGF assay, SDS-PAGE and Western blot analyses showed that the α-amylase was readily converted to short-chain peptides within 30 seconds of exposure to SGF.

The Committee concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity.

### Toxicological studies

An acute oral toxicity study was conducted in female rats with the enzyme concentrate as the test material *(7)*, and no toxicity was observed at 1776 mg TOS/kg body weight (bw), the only dose tested.

A 13-week oral toxicity study was conducted in rats with the enzyme concentrate as the test material *(8)*. The enzyme concentrate was administered via gavage at doses up to 66.81 mg TOS/kg bw per day. In the absence of any

adverse effects, a no-observed-adverse-effect level (NOAEL) of 67 mg TOS/kg bw per day was identified (rounded by the Committee from 66.81 mg TOS/kg bw per day), the highest dose tested.

The enzyme concentrate was not genotoxic in a bacterial reverse mutation assay *(9)* and in an in vitro mammalian chromosome aberration assay *(10)*. The Committee had no concerns about potential genotoxicity of this α-amylase enzyme concentrate.

A comparison of the amino acid sequence of the enzyme with those of known protein toxins *(5,11)* revealed no biologically relevant homology. The Committee concluded that this enzyme was unlikely to be a toxin.

### Assessment of dietary exposure

The Committee evaluated two estimates of dietary exposure to TOS from this α-amylase enzyme preparation, one submitted by the sponsor and the other estimated by the Committee. Both estimates were derived with the budget method, a screening method based on maximum physiological levels of consumption of solid foods and non-milk beverages, and on maximum use levels of the enzyme preparation. Maximum use levels of 0.44 mg TOS/kg for solid foods and 5.37 mg TOS/L for non-milk beverages were used. The highest concentration from all proposed uses for non-milk beverages was not used in the budget method calculation in this assessment. It was assumed that the highest TOS of 83.1 mg/kg for the production of potable alcohol would not be present in the final distilled product as a result of the production process. In addition, the Committee noted that the entire consumption of non-milk beverages would not be distilled alcoholic beverages, which supports this assumption. In both dietary exposure estimates it was assumed that 50% of solid foods and 25% of non-milk beverages would contain the enzyme preparation. Different assumptions were made about the amount of solid foods consumed. The resulting theoretical maximum daily intakes (TMDIs) from solid food and non-milk beverages were 0.14 mg TOS/kg bw per day by the sponsor and 0.2 mg TOS/kg bw per day by the Committee. The Committee concluded that the dietary exposure estimate of 0.2 mg TOS/kg bw per day was appropriate for use in the evaluation. For the dietary exposure assessment, it was assumed that 100% of the TOS from the enzyme preparation remains in the final food. The Committee noted that the enzyme is either removed or inactivated during the processing of food ingredients and will have no function in the final food.

### Evaluation

The Committee concluded that dietary exposure to this α-amylase enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee

identified a NOAEL of 67 mg TOS/kg bw per day, the highest dose tested, in a 13-week oral toxicity study in rats. When this NOAEL is compared with the dietary exposure estimate of 0.2 mg TOS/kg bw per day, a margin of exposure (MOE) of more than 330 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified"[2] for α-amylase (JECFA95-1) from *G. stearothermophilus* expressed in *B. licheniformis*, when used in the applications specified, at the levels of use specified and in accordance with current GMP. This ADI "not specified" was made temporary because of the tentative nature of the specifications.

A toxicological monograph with a dietary exposure assessment was prepared.

A new tentative specifications monograph and a chemical and technical assessment were prepared.

### Recommendations

The Committee requested the following information, by the end of 2023, to complete the safety assessment:

- validated method of analysis to determine α-amylase activity, including the validation report;
- unit definition for α-amylase activity based on the method of assay; and
- analytical data using the validated method for at least five different batches of commercially available products.

## References

1. Report of the Forty-eighth Session of the Codex Committee on Food Additives. Xi'an, China, 14–18 March 2016. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2016 (REP16/FA).

2. Chapter 9: Principles related to specific groups of substances. In: Environmental health criteria 240. Principles and methods for the risk assessment of chemicals in food. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; International Programme on Chemical Safety (IPCS); 2009 (updated 2020) (https://inchem.org/documents/ehc/ehc/ehc240_chapter9.pdf, accessed 22 June 2022).

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[2] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

4. AllergenOnline [online database]. AllergenOnline version 20; 2022 (http://allergenonline.org, accessed 10 February 2020).

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### 3.1.2 α-Amylase (JECFA95-2) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*

#### Explanation

At the request of the CCFA at its Forty-seventh session *(1)*, the Committee evaluated the safety of α-amylase (Enzyme Commission No. 3.2.1.1; CAS No. 9000-90-2) from *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) expressed in *Bacillus licheniformis*. The Committee has not previously evaluated this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-2 to distinguish it from similarly named enzyme preparations. The Committee had previously evaluated several other α-amylases, including from *G. stearothermophilus*, and α-amylase from *G. stearothermophilus* expressed in *Bacillus subtilis* (Annex 3, reference 94), for which an ADI "not specified"[1] was established.

The term "α-amylase" refers to the α-amylase enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

containing the enzyme of interest, which is used in the toxicity studies; and the term "enzyme preparation" refers to the product formulated for commercial use.

The Committee has previously assessed the safety of enzyme preparations derived from *B. licheniformis* (e.g. pullulanase, Annex 3, reference 205; serine protease, Annex 3, reference 211) and established an ADI "not specified" for these enzyme preparations. On this basis, the present Committee considered that α-amylase (JECFA95-2) from *G. stearothermophilus* expressed in *B. licheniformis* met the criteria of a Class 1, Type iii enzyme, as described in EHC 240 *(2)*. A Class 1, Type iii enzyme preparation is produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted data.

The enzyme catalyses the endohydrolysis of (1→4)-α-D-glucosidic linkages in amylose and amylopectin. The α-amylase enzyme preparation is intended for use as a processing aid in starch/carbohydrate processing, brewing/cereal beverage processing, potable alcohol production and removal of starch in sugar processing.

The Committee conducted literature searches in PubMed and EBSCO. Searches were conducted with the linked search terms "α-amylase" AND "stearothermophilus" AND ("safety" OR "toxic" OR "allergy"). Searches were repeated using "alpha-amylase". Similar searches were conducted in both databases replacing "stearothermophilus" with "licheniformis". Finally, searches were conducted using the names of both organisms, together with "safety" OR "toxic" OR "allergy". One publication of peripheral relevance, which was considered at the Thirty-seventh JECFA meeting (Annex 3, reference 95), was identified as a result of all literature searches.

### Genetic background

The production organism, *B. licheniformis*, is a non-pathogenic and non-toxigenic bacterium. It is ubiquitous in nature, having been isolated from environments as diverse as freshwater, saltwater, soil, plants, animals and air *(3)*. *B. licheniformis* has a history in the production of enzymes intended for use in food processing *(4)*.

The production strain was obtained from a *B. licheniformis* parental strain through a combination of chemical mutagenesis and genetic modifications. The parental strain is deposited in Deutsche Sammlung von Mikrooorganismen und Zellkulturen (DSMZ), Germany. The production strain was obtained by deleting genes encoding several proteases and sporulation, and modifying a gene to reduce the expression of an unwanted background protein. Further manipulations were performed using plasmids containing the required regulatory elements

to allow for integration and expression of a modified α-amylase gene from *G. stearothermophilus*. The stability of the production strain and the absence of any genes of concern were confirmed by Southern blot hybridization.

### Chemical and technical considerations

This α-amylase enzyme is produced by controlled submerged fed-batch fermentation of a pure culture of the *B. licheniformis* production strain. Manufacture of the α-amylase enzyme preparation consists of fermentation, recovery and formulation. After fermentation, the broth containing the α-amylase enzyme is separated from the biomass via a series of filtration steps. The broth is purified and concentrated to increase the enzyme content, and the concentrate is formulated into a liquid enzyme preparation. The entire process is performed in accordance with current GMP and with food-grade raw materials. The final enzyme preparation contains no major food allergens from the fermentation medium, and is free from the production organism and any antibiotic activity.

The primary sequence of the α-amylase enzyme produced by *B. licheniformis* consists of 513 amino acids with a calculated molecular weight of 59 kDa. The α-amylase produced by *B. licheniformis* is not expected to have any secondary or subsidiary activity.

A definition for the unit of activity was not provided. The sponsor determined the activity of α-amylase spectrophotometrically by measuring the hydrolysis of a 4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-α-D-maltoheptaoside (ethylidene-G7PNP) substrate by the enzyme at 405 nm compared with a proprietary enzyme standard. The activity was expressed in units relative to an enzyme of a declared strength. The mean activity of α-amylase from five batches of enzyme concentrate was 1262 units/g.

α-Amylase catalyses the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units. The enzyme preparation is intended for use as a processing aid in starch/carbohydrate processing, brewing/cereal beverage processing, potable alcohol production and removal of starch in sugar processing at a maximum level of 9.48 mg TOS/kg in the starch-based raw material. The TOS includes the enzyme of interest and residues of organic materials (e.g. proteins, peptides and carbohydrates) derived from the production organism during the manufacturing process.

The α-amylase enzyme is inactivated by heat or removed during processing, and is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

Potential immunological cross-reactivity with known allergens was assessed by the sponsor in 2012 according to recommended criteria *(5)*. The amino acid sequence of α-amylase was compared with those of known allergens in two databases *(6,7)*. Three homology analyses were performed: (i) more than 35% identity over the full length of the alignment; (ii) more than 35% identity using a sliding window of 80 amino acids and a suitable gap penalty; and (iii) as for (ii) but with scaling enabled, in order to identify windows shorter than 80 amino acids but with high identity. Using the 80 amino acid window search, with and without scaling, several matches with more than 35% identity were found, all of which were the allergen Asp o 21, an α-amylase originating from the fungus *Aspergillus oryzae*. This is a respiratory allergen associated with occupational rhinitis and asthma in bakers. However, when compared over the full length of the sequence, the Asp o 21 α-amylase and the α-amylase from *B. stearothermophilus* expressed in *B. licheniformis* are only 21.2% identical.

The Committee repeated the comparison of the amino acid sequence of the enzyme with known allergens based on the recommended bioinformatics criteria *(5,8)* and as outlined in EHC 240 *(2)*. The amino acid sequence of the enzyme was compared with those of known allergens in the current version of the FARRP AllergenOnline database *(6)*. A search for matches with more than 35% identity in a sliding window of 80 amino acids, a search for exact matches over contiguous stretches of eight amino acids and a full-length FASTA sequence search did not identify homology to any allergens not previously identified by the sponsor in 2012.

No information concerning the digestibility of this α-amylase in SGF or SIF was available. The Committee noted that a similar α-amylase (JECFA95-1) from *G. stearothermophilus* expressed in *B. licheniformis* was readily hydrolysed in SGF.

The Committee concluded that dietary exposure to the α-amylase is not anticipated to pose a risk for allergenicity.

### Toxicological studies

A 13-week study of oral toxicity in rats of an α-amylase enzyme concentrate from a closely related production strain of *B. licheniformis (9)* was available and was considered relevant on the basis of safe strain lineage. The enzyme concentrate was administered by gavage at dosages up to 660 mg TOS/kg bw per day. No treatment-related adverse effects were observed. The Committee identified a NOAEL of 660 mg TOS/kg bw per day, the highest dose tested.

The enzyme concentrate was not genotoxic in either a bacterial reverse mutation assay *(10)* or an in vitro micronucleus induction assay in human

peripheral blood lymphocytes *(11)*. The Committee had no concerns about potential genotoxicity of the enzyme concentrate.

The Committee compared the amino acid sequence of the enzyme with those of known protein toxins *(12)*. There was no biologically relevant homology (~ 16%). The Committee therefore concluded that the enzyme is unlikely to be a toxin.

### Assessment of dietary exposure

The Committee evaluated two estimates of dietary exposure to the TOS from this α-amylase enzyme preparation, one submitted by the sponsor and the other estimated by the Committee. Both estimates were derived with the budget method, a screening method based on maximum physiological levels of consumption of solid foods and non-milk beverages, and on maximum use levels of the enzyme preparation. A maximum use level of 9.48 mg TOS/kg starch-based raw material for both solid foods and non-milk beverages was used. It was assumed that 50% of solid foods and 25% of non-milk beverages in the food supply are processed and would contain the enzyme preparation, and that processed solid foods contain 25% starch (or starch-derived) dry matter and processed beverages contain 10% starch hydrolysates. Different assumptions were made about the amount of solid foods consumed. The resulting TMDI from solid food and non-milk beverages was 0.0533 mg TOS/kg bw per day by the sponsor and 0.08 mg TOS/kg bw per day by the Committee. The Committee concluded that the dietary exposure estimate of 0.08 mg TOS/kg bw per day was appropriate for use in the evaluation. For the dietary exposure assessment, it was assumed that 100% of the TOS from the enzyme preparation remains in the final food. The Committee noted that the enzyme is either removed or inactivated during the processing of food ingredients and will have no function in the final food.

### Evaluation

The Committee concluded that dietary exposure to this α-amylase enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 660 mg TOS/kg bw per day, the highest dose tested in a 13-week oral toxicity study in rats. When this NOAEL is compared with the dietary exposure estimate of 0.08 mg TOS/kg bw per day, a MOE of more than 8000 can be calculated. Based on this MOE and the lack of concern about genotoxicity, the Committee established a temporary ADI "not specified"[2] for α-amylase (JECFA95-2) from *G. stearothermophilus* expressed in *B. licheniformis*, when used in the applications specified, at the levels of use specified and in accordance

[2] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications.

A toxicological monograph with a dietary exposure assessment was prepared.

A new tentative specifications monograph and a chemical and technical assessment were prepared.

### Recommendations

The Committee requested the following information, by the end of 2023, to complete the safety assessment:

- validated method of analysis to determine α-amylase activity, including the validation report;
- unit definition for α-amylase activity based on the method of assay; and
- analytical data using the validated method for at least five different batches of commercially available products.

## References


1. Report of the Forty-seventh Session of the Codex Committee on Food Additives. Xi'an, China, 23–27 March 2015. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2015 (REP15/FA).
2. Chapter 9: Principles related to specific groups of substances. In: Environmental health criteria 240. Principles and methods for the risk assessment of chemicals in food. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; International Programme on Chemical Safety (IPCS); 2009 (updated 2020) (https://inchem.org/documents/ehc/ehc/ehc240_chapter9.pdf, accessed 22 June 2022).
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12. UniProt [online database]. UniProt Consortium; 2022 (http://www.uniprot.org, accessed June 2022).

### 3.1.3 α-Amylase (JECFA95-3) from *Rhizomucor pusillus* expressed in *Aspergillus niger*

#### Explanation

At the request of the CCFA at its Forty-eighth session *(1)*, the Committee evaluated the safety of α-amylase (Enzyme Commission No. 3.2.1.1; CAS No. 9000-90-2) from *Rhizomucor pusillus* expressed in A*spergillus niger*. The Committee has not previously evaluated this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-3 to distinguish it from similarly named enzyme preparations. The Committee has previously evaluated several α-amylases from other sources, for which an ADI "not specified"[1] was established (e.g. Annex 3, reference 94).

The term "α-amylase" refers to the α-amylase enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product containing the enzyme of interest, which is used in the toxicity studies; and the term "enzyme preparation" refers to the product formulated for commercial use.

The Committee has previously assessed the safety of enzyme preparations derived from *A. niger* (e.g. asparaginase, Annex 3, reference 190; 3-phytase, Annex 3, reference 211), and established an ADI "not specified" for these enzyme preparations. On this basis, the present Committee considered that α-amylase (JECFA95-3) from *R. pusillus* expressed in *A. niger* met the criteria of a Class 1, Type iii enzyme, as described in EHC 240 *(2)*. A Class 1, Type iii enzyme preparation is produced by a Safe Food Enzyme Production Strain or a Presumed

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted data.

The enzyme catalyses the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units, which results in the degradation of starch and related polysaccharides. The enzyme preparation is intended for use as a processing aid in starch processing, beverage alcohol (distilling) processes, and baking and cereal-based processes.

The Committee conducted literature searches in PubMed and EBSCO. Searches were conducted with the linked search terms "α-amylase" AND "*Rhizomucor pusillus*" AND ("safety" OR "toxic" OR "allergy"). No studies relevant to the safety evaluation were found.

### Genetic background

The production organism, *A. niger*, is a non-pathogenic, filamentous fungus that is ubiquitous in the environment. It has a history of use in the production of enzymes intended for use in food processing and citric acid production *(3)*.

The non-toxigenic, non-pathogenic *A. niger* production strain was constructed through a combination of chemical mutagenesis and genetic modifications of the parental strain. The production strain was obtained by inactivation of several genes encoding for major secreted proteins, deletion of a gene encoding a protease, and disruption of genes responsible for the production of oxalic acid and fumonisin. The expression plasmid containing the α-amylase gene from *R. pusillus*, an optimized *A. niger* promoter, a transcriptional terminator and a selection marker were integrated by targeted homologous recombination. The stability of the integration and the absence of any genes of concern were confirmed by Southern blot hybridization. The production strain is deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany.

### Chemical and technical considerations

This α-amylase enzyme is produced by controlled fermentation of a pure culture of the *A. niger* production strain. The manufacture of the α-amylase enzyme preparation includes fermentation, recovery and formulation. After fermentation, the broth containing the α-amylase enzyme is recovered from the biomass by multiple filtration and concentration steps. The resulting concentrate is formulated into a powder preparation. The entire process is performed in accordance with current GMP and with food-grade raw materials. The enzyme concentrate is tested to be free from the production organism and any antibiotic activity.

The primary sequence of α-amylase enzyme produced by *A. niger* consists of 583 amino acids; its molecular weight calculated from the determined amino acid sequence is 63.5 kDa. The α-amylase produced by *A. niger* is not expected to have any secondary or subsidiary activities.

A unit definition of activity was not provided. The activity of α-amylase is determined spectrophotometrically (405 nm) by measuring the hydrolysis of a 4,6-ethylidene(G7)-*p*-nitrophenyl(G1)-α-D-maltoheptaoside (ethylidene-G7PNP) substrate by the enzyme, compared with a proprietary enzyme standard. The activity was expressed in units relative to an enzyme of a declared strength. The mean activity of α-amylase from four batches of enzyme concentrate was 57.2 units/g.

α-Amylase catalyses the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units. The enzyme preparation is intended for use as a processing aid in starch processing, beverage alcohol (distilling) processes, and baking and cereal-based processes at a maximum level of 407 mg TOS/kg of starch-based raw material. The TOS includes the enzyme of interest and residues of organic materials (e.g. proteins, peptides and carbohydrates) from the production organism during manufacture.

The α-amylase enzyme is inactivated by heat or removed during processing, and is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

Potential immunological cross-reactivity with known allergens was assessed by the sponsor in 2012 according to recommended criteria *(4)*. The amino acid sequence of α-amylase was compared with those of known allergens in two databases *(5,6)*. Three homology analyses were performed: (i) more than 35% identity over the full length of the alignment; (ii) more than 35% identity using a sliding window of 80 amino acids and a suitable gap penalty; and (iii) as for (ii) but with scaling enabled, in order to identify windows shorter than 80 amino acids but with high identity. No biologically relevant matches were found in either database.

The Committee repeated the comparison of the amino acid sequence of the enzyme with known allergens based on the recommended bioinformatics criteria *(4,7)* and as outlined in EHC 240 *(2)*. The amino acid sequence of the enzyme was compared with those of known allergens in the current version of the FARRP AllergenOnline database *(5)*. A search for matches with more than 35% identity in a sliding window of 80 amino acids, a search for exact matches over contiguous stretches of eight amino acids and a full-length FASTA sequence search did not identify homology to any allergens.

No information concerning the digestibility of this α-amylase in SGF or SIF was available.

The Committee concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity.

### Toxicological studies

A 13-week oral toxicity study of this α-amylase concentrate was conducted in rats *(8)*. The enzyme concentrate was administered by gavage at doses up to 1400 mg TOS/kg bw per day. No treatment-related adverse effects were observed. The Committee identified a NOAEL of 1400 mg TOS/kg bw per day, the highest dose tested.

An enzyme concentrate of this α-amylase was not genotoxic in either a bacterial reverse mutation assay *(9)* or an in vitro micronucleus induction assay in human peripheral blood lymphocytes *(10)*. The Committee had no concerns about potential genotoxicity of this α-amylase enzyme concentrate.

A comparison of the amino acid sequence of the enzyme with those of known protein toxins *(11)* revealed no biologically relevant homology. The Committee therefore concluded that the enzyme was unlikely to be a toxin.

### Assessment of dietary exposure

The Committee evaluated two estimates of dietary exposure to TOS from this α-amylase enzyme preparation, one submitted by the sponsor and the other estimated by the Committee. Both estimates were derived with the budget method, a screening method based on maximum physiological levels of consumption of solid foods and non-milk beverages, and on maximum use levels of the enzyme preparation. A maximum use level of 407 mg TOS/kg starch-based raw material for both solid foods and non-milk beverages was used. It was assumed that 50% of solid foods and 25% of non-milk beverages are processed, and that processed solid foods contain 25% starch (or starch-derived) dry matter, and processed beverages contain 10% starch hydrolysates. Different assumptions were made about the amount of solid foods consumed. The resulting TMDIs from solid foods and non-milk beverages were 2.29 mg TOS/kg bw per day by the sponsor and 4 mg TOS/kg bw per day by the Committee. The Committee concluded that the dietary exposure estimate of 4 mg TOS/kg bw per day was appropriate for use in the evaluation. For the dietary exposure assessment, it was assumed that 100% of the TOS of the enzyme preparation remains in the final food. The Committee noted that the enzyme is either removed or inactivated during the processing of food ingredients, and will have no function in the final food.

### Evaluation

The Committee concluded that dietary exposure to this α-amylase enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1400 mg TOS/kg bw per day, the highest dose tested in a 13-week oral toxicity study in rats. When this NOAEL is compared with the dietary exposure estimate of 4 mg TOS/kg bw per day, a MOE of more than 350 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified"[2] for α-amylase (JECFA95-3) from *R. pusillus* expressed in *A. niger*, when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications.

A toxicological monograph with a dietary exposure assessment was prepared.

A new tentative specifications monograph and a chemical and technical assessment were prepared.

### Recommendations

The Committee requested the following information, by the end of 2023, to complete the safety assessment:

- validated method of analysis to determine α-amylase activity, including the validation report;
- unit definition for α-amylase activity based on the method of assay; and
- analytical data using the validated method for at least five different batches of commercially available products.

## References

1. Report of the Forty-eighth Session of the Codex Committee on Food Additives. Xi'an, China, 14–18 March 2016. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2016 (REP16/FA).

2. Chapter 9: Principles related to specific groups of substances. In: Environmental health criteria 240. Principles and methods for the risk assessment of chemicals in food. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; International Programme

[2] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

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### 3.1.4 Amyloglucosidase (JECFA95-4) from *Rasamsonia emersonii* expressed in *Aspergillus niger*

#### Explanation

At the request of the CCFA at its Forty-eighth Session *(1)*, the Committee evaluated the safety of amyloglucosidase (Enzyme Commission No. 3.2.1.3; CAS No. 9032-08-0) from *Rasamsonia emersonii* (formerly *Talaromyces emersonii*) expressed in *Aspergillus niger*. The Committee had not previously considered this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-4 to this enzyme preparation. The Committee had previously evaluated amyloglucosidase from *A. niger* (Annex 3, reference 77), for which an ADI "not specified"[1] was established.

The term "amyloglucosidase" refers to the enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

containing the enzyme of interest, which is used in the toxicity studies; and the term "enzyme preparation" refers to the product formulated for commercial use.

The Committee has previously assessed the safety of enzyme preparations derived from *A. niger* (e.g. asparaginase, Annex 3, reference 190; phytase, Annex 3, reference 211) and established an ADI "not specified" for these enzyme preparations. On this basis, the present Committee considered that this amyloglucosidase (JECFA95-4) from *R. emersonii* expressed in *A. niger* met the criteria of a Class 1, Type iii enzyme, as described in EHC 240 *(2)*. A Class 1, Type iii enzyme preparation is produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted data.

Amyloglucosidase hydrolyses (1→4)-α as well as (1→6)-α linkages in starch. The enzyme preparation is intended for use as a processing aid in starch processing.

The Committee conducted a literature search in Google Scholar with the linked search terms "amyloglucosidase", "*Rasamsonia emersonii*" and "*Aspergillus niger*". A total of 112 hits were found, but none was considered relevant to this safety evaluation. A second literature search was conducted in Google Scholar with the linked search terms "amyloglucosidase", "*Talaromyces emersonii*" and "*Aspergillus niger*". This search identified 108 references. None of the identified publications provided additional toxicity data relevant to this evaluation.

### Genetic background

The production organism, *A. niger*, is a non-pathogenic filamentous fungus that is ubiquitous in the environment. It has a history of use in the production of enzymes intended for use in food processing and citric acid production *(3,4)*.

The non-toxigenic and non-pathogenic production strain was constructed through a combination of chemical mutagenesis and genetic modifications of the parental strain. The expression plasmid contained a promoter sequence obtained from *A. niger* BO-1, the amyloglucosidase gene obtained from *R. emersonii*, a terminator sequence obtained from *A. niger* BO-1 and an antibiotic marker. The stability of the introduced amyloglucosidase gene was confirmed by Southern blot hybridization. The production strain is deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany.

### Chemical and technical considerations

Amyloglucosidase is produced by controlled fermentation of a pure culture of the *A. niger* production strain. The manufacture of the amyloglucosidase enzyme preparation includes fermentation, recovery and formulation. After fermentation,

the broth containing the amyloglucosidase enzyme is separated from the biomass by sedimentation followed by several filtration steps. The resulting concentrate is formulated into a liquid preparation. The entire process is performed in accordance with current GMP and with food-grade raw materials. The enzyme concentrate is tested to be free from the production organism and any antibiotic activity.

The primary sequence of amyloglucosidase produced by *A. niger* consists of 591 amino acids; its molecular weight by calculation from the determined amino acid sequence is 62.8 kDa. The amyloglucosidase produced by *A. niger* is not expected to have any secondary or subsidiary activity.

The activity of amyloglucosidase is determined spectrophotometrically (340 nm) by measuring the hydrolysis of maltose, compared with a proprietary enzyme standard with activity expressed in units per gram. A unit definition of activity was not provided. The mean activity of amyloglucosidase from four batches of the liquid enzyme concentrate was 400 units/g.

Amyloglucosidase catalyses the hydrolysis of (1→4)-α and (1→6)-α glycosidic linkages in starch. The amyloglucosidase enzyme preparation is intended for use as a processing aid in starch/carbohydrate processing, baking, brewing/cereal beverage processing and potable alcohol production. The amyloglucosidase enzyme preparation is used at a maximum level of 931 mg TOS/kg starch-based raw material for solid foods and 1090 mg TOS/kg starch-based raw material for non-milk beverages. The TOS includes the enzyme of interest and residues of organic materials (e.g. proteins, peptides and carbohydrates) derived from the production organism during the manufacturing process. The amyloglucosidase enzyme is inactivated by heat or removed during processing, and is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

The amino acid sequence of the amyloglucosidase was compared with the sequences of allergens *(5–7)*, consistent with the recommended criteria *(8)*. The databases each identified a single match to a respiratory allergen, the amyloglucosidase Sch c 1 found in *Schizophyllum commune* (split-gill mushroom) *(9,10)*. The allergen showed 60–84% identity with amyloglucosidase over a sliding window of 80 amino acids; 47.9% identity over the full length of the protein; and 10 matches based on a 100% identity over eight contiguous amino acids. *S. commune* is consumed as food in India, Mexico and other countries, and there are no reports of it being associated with food allergy. There are no reports of food allergy associated with similar amyloglucosidases.

The Committee noted the significant amino acid sequence homology of this amyloglucosidase with the known respiratory allergen Sch c 1, and that

the respiratory allergen is associated with occupational asthma in bakers *(11)*. In the absence of digestibility data, the Committee was unable to complete the assessment of the potential for allergenicity from dietary exposure to this amyloglucosidase.

### Toxicological studies

A 13-week oral toxicity study in rats was conducted *(12)*. The test material was an amyloglucosidase concentrate not derived from the production strain that is the subject of this evaluation, but from a previous production strain. The Committee considered it to be relevant to the current evaluation because of the similarity of the lineage of the two organisms, and the absence of any significant differences between the two enzyme concentrates with respect to chemical purity. The enzyme concentrate was administered by gavage at doses up to 1470 mg TOS/kg bw per day. A dose-related increase in the incidence and severity of cortical vacuolation in the adrenal cortex of males was observed when compared with control animals. The incidence and severity of the lesions were consistent with the historical control values. Further, it was not associated with other histopathological changes such as inflammation, necrosis or other signs of histopathology, and was confined to one sex. The Committee concluded that this finding was not toxicologically relevant. In the absence of any relevant adverse effect, a NOAEL of 1500 mg TOS/kg bw per day was identified (rounded by the Committee from 1470 mg TOS/kg bw per day), the highest dose tested.

The enzyme concentrate yielded negative results when tested in an in vitro bacterial reverse mutation assay *(13)* and an in vitro micronucleus induction assay *(14)*. The Committee had no concerns about potential genotoxicity of this amyloglucosidase concentrate.

A comparison of the amino acid sequence of the enzyme with those of known protein toxins was conducted *(7,15)*, which revealed no biologically relevant homology. The Committee concluded that the enzyme is unlikely to be a toxin.

### Assessment of dietary exposure

The Committee evaluated two estimates of dietary exposure to the TOS from this amyloglucosidase enzyme preparation, one submitted by the sponsor and the other estimated by the Committee. Both estimates were derived with the budget method, a screening method based on maximum physiological levels of consumption of solid foods and non-milk beverages, and on maximum use levels of the enzyme preparation. Maximum use levels of 931 mg TOS/kg starch-based raw material for solid foods and 1090 mg TOS/kg starch-based raw material for

non-milk beverages were used. These TOS values were derived from a batch of the test material with a higher proportion of TOS compared with that of the commercial product; the TMDI would therefore be lower than what has been calculated. It was assumed that 50% of solid foods and 25% of non-milk beverages are processed and would contain the enzyme preparation, and that processed solid foods contain 25% starch (or starch-derived) dry matter and processed beverages contain 10% starch hydrolysates. Different assumptions were made about the amount of solid foods consumed. The resulting TMDIs from solid food and non-milk beverages were 5.63 mg TOS/kg bw per day by the sponsor and 9 mg TOS/kg bw per day by the Committee. The Committee concluded that the dietary exposure estimate of 9 mg TOS/kg bw per day was appropriate for use in the evaluation. For the dietary exposure assessment, it was assumed that 100% of the TOS from the enzyme preparation remains in the final food. The Committee noted that the enzyme is either removed or inactivated during the processing of food ingredients and will have no function in the final food.

### Evaluation

The Committee noted that amyloglucosidase may pose a risk as a respiratory allergen. In the absence of any information regarding its stability within the gastrointestinal tract, the Committee could not complete the assessment of the risk for allergenicity from dietary exposure to this amyloglucosidase enzyme preparation.

The Committee identified a NOAEL of 1500 mg TOS/kg bw per day in a 13-week study of oral toxicity in rats. When this NOAEL, the highest dose tested, is compared with the conservative dietary exposure estimate of 9 mg TOS/kg bw per day, a MOE of more than 160 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified"[2] for amyloglucosidase (JECFA95-4) from *R. emersonii* expressed in *A. niger* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications and the inability to complete the allergenicity assessment.

A toxicological monograph with a dietary exposure assessment was prepared.

A new tentative specifications monograph and a chemical and technical assessment were prepared.

[2] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

### Recommendations

The Committee requested the following information, by the end of 2023, to complete the safety assessment:

- digestibility data in order to complete the allergenicity assessment;
- validated method of analysis to determine amyloglucosidase activity, including the validation report;
- unit definition for amyloglucosidase activity based on the method of assay; and
- analytical data using the validated method for at least five different batches of commercially available products.

## References


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### 3.1.5 Asparaginase (JECFA95-5) from *Pyrococcus furiosus* expressed in *Bacillus subtilis*

#### Explanation

At the request of the CCFA at its Forty-seventh Session *(1)*, the Committee evaluated the safety of asparaginase (Enzyme Commission No. 3.5.1.1; CAS No. 9015-68-3) from *Pyrococcus furiosus* expressed in *Bacillus subtilis*. The Committee has not previously evaluated this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-5 to this enzyme preparation. The Committee had previously evaluated several other asparaginases (e.g. an asparaginase from *Aspergillus oryzae* expressed in *A. oryzae* (Annex 3, reference 187) and an asparaginase from *A. niger* expressed in *A. niger* (Annex 3, reference 190) at its Sixty-eighth and Sixty-ninth meetings, respectively), for which an ADI "not specified"[1] was established.

The term "asparaginase" refers to the asparaginase enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product containing the enzyme of interest which is used in the toxicity studies; and the term "enzyme preparation" refers to the product formulated for commercial use.

The Committee previously evaluated several food enzymes from *B. subtilis*, such as an α-amylase (Annex 3, reference 94) and a mixed carbohydrase and protease (Annex 3, reference 26), and established an ADI "not specified" or

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

ADI "not limited"[2], respectively, for these enzyme preparations. On this basis, the present Committee considered that asparaginase (JECFA95-5) from *P. furiosus* expressed in *B. subtilis* met the criteria of a Class 1, Type iii enzyme, as described in EHC 240 *(2)*. A Class 1, Type iii enzyme is produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted data.

The enzyme catalyses the hydrolysis of free asparagine to aspartic acid and ammonia. The enzyme preparation is intended for use as a processing aid in baking and the production of breakfast cereal dry materials, in potato processing, and in coffee and cocoa processing.

The Committee conducted a literature search in PubMed (all fields) with the linked search terms "Asparaginase" AND "*Pyrococcus furiosus*", and "Asparaginase" AND "*Bacillus subtilis*", which identified 10 and 35 references, respectively. None of the identified references was relevant to the toxicological evaluation of this enzyme preparation.

### Genetic background

The production organism, *B. subtilis*, is a non-pathogenic and non-toxigenic bacterium *(3)*. It is ubiquitous in nature, and occurs as a soil- and plant-living saprophyte *(3)*. *B. subtilis* has a history in the production of enzymes intended for use in food processing (Annex 3, references 26 and 94).

The production strain, *B. subtilis*, was developed from the parental strain (deposited as *B. subtilis* ATCC 6051a) by inactivation of genes encoding several proteases, surfactin and sporulation. The gene encoding asparaginase from *P. furiosus* was optimized and transferred into *B. subtilis*. The donor strain was deposited in the ATCC as *P. furiosus* ATCC 43587. The expression plasmid containing the optimized asparaginase gene, a hybrid *Bacillus* promoter, a terminator from *B. licheniformis* and marker genes were integrated by targeted homologous recombination. The stability of the insert and the absence of genes encoding for antibiotic resistance were confirmed by Southern blot analysis *(4,5)*.

### Chemical and technical considerations

Asparaginase is produced by controlled fermentation of a pure culture of the *B. subtilis* production strain. Manufacture of the asparaginase enzyme preparations includes fermentation recovery and formulation. After fermentation, the broth containing the asparaginase enzyme is separated from the biomass; this is followed by several filtration steps and concentration. The resulting concentrate

[2] The expression ADI "not limited" is no longer used by JECFA and has been replaced by ADI "not specified".

is formulated into either a liquid or a powder preparation. The entire process is performed under current GMP and with food-grade raw materials. The enzyme concentrate contains no major food allergens from the fermentation medium, and is free from the production organism and any antibiotic activity.

The asparaginase enzyme produced by *B. subtilis* strain is a dimer and each monomer consists of 326 amino acids; the molecular weight of the asparaginase monomer by calculation from the determined amino acid sequence is 35.8 kDa. The asparaginase produced by *B. subtilis* is not known to have any significant subsidiary or secondary enzymatic activities.

The activity of asparaginase is determined spectrophotometrically at 340 nm by condensing the ammonia produced by the enzymatic hydrolysis of asparagine with α-ketoglutarate and measuring the amount of nicotinamide adenine dinucleotide (NADH, $H^+$) utilized. The asparaginase activity is expressed in units/g relative to a proprietary asparaginase standard. A definition for the unit of activity was not provided. The mean activity of asparaginase from three batches of the enzyme concentrate was 54 267 units/g.

Asparaginase catalyses the hydrolysis of free asparagine to aspartic acid and ammonia. Asparaginase enzyme preparations are intended for use as processing aids in baking and the production of breakfast cereal dry materials, in potato processing, and in coffee and cocoa processing.

Asparaginase enzyme preparation is used at a maximum level of 15 000 units/kg of final food, which is equivalent to 27.8 mg TOS/kg of final food. The TOS includes the enzyme of interest and residues of organic materials (e.g. proteins, peptides and carbohydrates) derived from the production organism during the manufacturing process.

The asparaginase enzyme is inactivated or removed by heat treatment during processing, and is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

The Committee evaluated the potential for allergenicity of the enzyme preparation based on the recommended bioinformatics criteria *(6,7)* and as outlined in EHC 240 *(2)*. The amino acid sequence of the enzyme was compared with those of known allergens in two online databases *(8,9)*. A search for matches with more than 35% identity in a sliding window of 80 amino acids, a search for exact matches over contiguous stretches of eight amino acids and a full-length FASTA sequence search did not identify a homology to any known allergens. No data on the digestibility of the enzyme preparation were available. Based on the results of the bioinformatic analysis, the Committee concluded that dietary exposure to the enzyme is not anticipated to pose a risk for allergenicity.

### Toxicological studies

In a 13-week study of oral toxicity in rats *(10)*, the enzyme concentrate was mixed in water and administered by gavage at dose levels up to 1207 mg TOS/kg bw per day. The only effect that was dose- and possibly treatment-related was a decrease in mean cholesterol levels in both sexes, but the observed changes were within the historical control data range. The Committee therefore concluded that this finding was not toxicologically relevant. Based on the absence of any adverse effects, the Committee identified a NOAEL of 1207 mg TOS/kg bw per day, the highest dose tested.

The enzyme concentrate was negative in a bacterial reverse mutation test *(11)* and in an in vitro mammalian cell micronucleus assay *(12)*. The Committee therefore had no concerns about potential genotoxicity of the enzyme concentrate.

A comparison of the amino acid sequence of the enzyme with those of known protein toxins *(13)* revealed no biologically relevant homology (~ 17%). The Committee therefore concluded that the enzyme was unlikely to be a toxin.

### Assessment of dietary exposure

The Committee considered one estimate of dietary exposure to TOS from this asparaginase enzyme preparation, which was submitted by the sponsor. The standard budget method calculation, a screening method based on maximum physiological levels of consumption of solid foods and non-milk beverages, and on maximum use levels of the enzyme preparation, was used. The resulting TMDIs of TOS from the enzyme preparation were estimated by the sponsor to be 0.348 mg TOS/kg bw per day for solid foods and 0.035 mg TOS/kg bw per day for non-milk beverages, for a total of 0.383 mg TOS/kg bw per day or 0.4 mg/kg bw per day after rounding. The sponsor noted that young children could consume biscuits including ingredients that were prepared using the asparaginase enzyme preparation, and prepared an estimate for children using the budget method. The TMDI of the TOS from the enzyme preparation for biscuits consumed by young children was estimated to be 0.133 mg TOS/kg bw per day. The Committee concluded that the higher estimate of dietary exposure from the overall budget method, 0.4 mg TOS/kg bw per day, was appropriate for use in the evaluation. For the dietary exposure assessment, it was assumed that 100% of the TOS from the enzyme preparation remains in the final food. The Committee noted that the enzyme is inactivated or removed during the processing of food ingredients, and will have no function in the final food.

### Evaluation

The Committee concluded that dietary exposure to this asparaginase enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee

identified a NOAEL of 1207 mg TOS/kg bw per day, the highest dose tested, in a 13-week study of oral toxicity in rats. When this NOAEL is compared with the dietary exposure estimate of 0.4 mg TOS/kg bw per day, a MOE of more than 3000 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified"[3] for asparaginase (JECFA95-5) from *P. furiosus* expressed in *B. subtilis* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications.

A toxicological monograph with a dietary exposure assessment was prepared.

A new tentative specifications monograph and a chemical and technical assessment were prepared.

### Recommendations

The Committee requested the following information, by the end of 2023, to complete the safety assessment:

- validated method of analysis to determine asparaginase activity, including the validation report;
- unit definition for asparaginase activity based on the method of assay; and
- analytical data using the validated method for at least five different batches of commercially available products.

## References

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### 3.1.6 β-Amylase (JECFA95-6) from *Bacillus flexus* expressed in *Bacillus licheniformis*

#### Explanation

At the request of the CCFA at its Forty-eighth Session *(1)*, the Committee evaluated the safety of β-amylase (Enzyme Commission No. 3.2.1.2; CAS No. 9000-91-3) from *Bacillus flexus* expressed in *B. licheniformis*. The Committee has not previously evaluated any β-amylase enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-6 to this enzyme preparation.

The term "β-amylase" refers to the β-amylase enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product containing the enzyme of interest, which is used in the toxicity studies; and the term "enzyme preparation" refers to the product formulated for commercial use.

The Committee previously evaluated several food enzymes from *B. licheniformis* – for example, a carbohydrase (α-amylase) (Annex 3, reference 70) and an α-amylase containing a genetically engineered α-amylase gene from *B. licheniformis* (Annex 3, reference 166) at its Twenty-ninth and Sixty-first meetings, respectively – and established an ADI "not specified"[1] for these enzyme preparations. On this basis, the present Committee considered that β-amylase (JECFA95-6) from *B. flexus* expressed in *B. licheniformis* met the criteria of a Class 1, Type iii enzyme, as described in EHC 240 *(2)*. A Class 1, Type iii enzyme is produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted information.

The enzyme catalyses the hydrolysis of the (1→4)-α-D-glucosidic linkages in polysaccharides. The enzyme preparation is intended for use as a processing aid, typically for starch processing in the manufacture of food products, such as syrups, containing maltose.

The Committee conducted a literature search in PubMed (all fields) with the linked search terms "beta-amylase" AND "*Bacillus flexus*", and "beta-amylase" AND "*Bacillus licheniformis*", which identified 3 and 52 references, respectively. No additional toxicity data were identified.

### Genetic background

The production organism, *B. licheniformis*, is a non-pathogenic and non-toxigenic bacterium *(3)*. It is ubiquitous in nature, and occurs as a soil- and plant-living saprophyte *(3)*. *B. licheniformis* has a history in the production of enzymes intended for use in food processing (Annex 3, references 70 and 166).

The production strain NZYM-JA *(4,5)* was developed from *B. licheniformis* by inactivation of the genes encoding α-amylase gene and several other genes. The β-amylase gene was from a *B. flexus* donor. An expression cassette containing the β-amylase gene, the chaperone-encoding gene and other regulatory elements was used in the transformation. Southern blot analysis was performed to confirm the number and position of the copies inserted in the production strain. Plasmids containing genes of concern were subsequently removed from the strain, which was demonstrated by genome sequence analysis, and a loss of antibiotic resistance. The production strain is deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany.

### Chemical and technical considerations

β-Amylase is produced by pure culture fermentation of the *B. licheniformis* production strain. Manufacture of the enzyme preparation includes fermentation, recovery and formulation. After fermentation, the broth containing the β-amylase enzyme is separated from the biomass, then concentrated by multiple filtration steps. The resulting concentrate is formulated and standardized into a liquid preparation. The entire process is performed in accordance with current GMP and with food-grade raw materials. The enzyme concentrate is tested to ensure that it is free from the production organism and any antibiotic activity.

The primary sequence of β-amylase produced by *B. licheniformis* consists of 515 amino acids; its molecular weight by calculation from the determined amino acid sequence is 57.6 kDa *(4)*. β-Amylase produced by *B. licheniformis* does not have any secondary activity.

The activity of β-amylase is determined spectrophotometrically by measuring the hydrolysis of maltohexaose at 540 nm, compared with a proprietary enzyme standard with activity expressed in β-amylase units (BAMU); 1 BAMU is defined as the amount of enzyme required to hydrolyse 1 μmol of maltohexaose per minute under the conditions of the assay. The mean activity from three batches of the enzyme concentrate was 11 000 BAMU/g.

β-Amylase catalyses the hydrolysis of the (1→4)-α-D-glucosidic linkages in polysaccharides to remove maltose units from the non-reducing ends. The enzyme preparation is intended for use as a processing aid, typically for starch processing for the production of syrups in a variety of food applications. The enzyme preparation is used at a maximum level of 99.1 mg TOS/kg of starch-based raw material. The TOS includes the enzyme of interest and residues of organic materials (e.g. proteins, peptides and carbohydrates) from the production organism during the manufacture.

The β-amylase enzyme is inactivated or removed during processing, and is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

The Committee evaluated the potential for allergenicity of the enzyme preparation using a weight-of-evidence approach including the recommended bioinformatics criteria *(6,7)* and as outlined in EHC 240 *(2)*. The amino acid sequence of the enzyme was compared with those of known allergens in two online databases *(8,9)*. A search for matches with more than 35% identity in a sliding window of 80 amino acids and a full-length FASTA sequence search identified homology to one known food allergen, *Triticum aestivum* (Tri a 17.0101). The Committee determined that Tri a 17 and β-amylase showed a highest identity of 42.47%, with an identity of 31.4% across the full alignment. In contrast, the sponsor stated that

Tri a 17 and β-amylase showed a highest identity of 44.7%, with an identity of 25.7% across the full alignment. The difference is the result of the sponsor using an older version (version 19) of the AllergenOnline database. A search for exact matches over contiguous stretches of eight amino acids generated two hits, which also indicated homology to Tri a 17. A recently published article *(10)* reported that wheat β-amylase with Tri a 17 is associated with allergic reactions upon wheat ingestion by sensitized people with a known wheat allergy.

An in vitro digestibility study *(11)* of the enzyme preparation with SGF showed that all proteins of the enzyme preparation were digested at the earliest time point of 2 minutes, indicating that the enzyme has the potential to be digested readily in the acidic conditions of the stomach. The Committee also noted that the enzyme preparation would be removed or inactivated during the manufacturing process.

Although the Committee acknowledged that the enzyme is homologous to a known food allergen, when taking into account the results of the digestibility study, the Committee concluded that dietary exposure to the enzyme was not anticipated to pose a risk for allergenicity.

### Toxicological studies

In a 13-week study of oral toxicity in rats *(12)*, the enzyme concentrate was mixed in water and administered by gavage at doses up to 1199 mg TOS/kg bw per day. The Committee did not identify any treatment-related or toxicologically relevant effects in any of the assessed parameters. Based on the absence of any adverse effects, the Committee identified a NOAEL of 1199 mg TOS/kg bw per day, the highest dose tested.

The enzyme concentrate was negative in a bacterial reverse mutation test *(13)* and in an in vitro mammalian cell micronucleus assay *(14)*. The Committee therefore had no concerns about potential genotoxicity of the enzyme concentrate.

A comparison of the amino acid sequence of the enzyme with those of known protein toxins *(15)* revealed no biologically relevant homology (~ 16%). The Committee therefore concluded that the enzyme was unlikely to be a toxin.

### Assessment of dietary exposure

The Committee evaluated two estimates of dietary exposure to TOS from this β-amylase enzyme preparation, one submitted by the sponsor and the other estimated by the Committee. Both estimates were derived with the budget method, a screening method based on maximum physiological levels of consumption of solid foods and non-milk beverages, and on maximum use levels of the enzyme preparation. A maximum use level of 99.1 mg TOS/kg starch-based raw material for both solid foods and non-milk beverages was used. It was

assumed that 50% of solid foods and 25% of non-milk beverages are processed, and that processed solid foods contain 25% starch (or starch-derived) dry matter and processed beverages contain 13% starch. Different assumptions were made about the amount of solid foods consumed. The resulting TMDIs from solid foods and non-milk beverages were 0.63 mg TOS/kg bw per day by the sponsor and 1 mg TOS/kg bw per day by the Committee. The Committee concluded that the dietary exposure estimate of 1 mg TOS/kg bw per day was appropriate for use in the evaluation. For the dietary exposure assessment, it was assumed that 100% of the TOS from the enzyme preparation remains in the final food. The Committee noted that the enzyme is either inactivated or removed during the processing of food ingredients, and will have no function in the final food.

### Evaluation

The Committee concluded that dietary exposure to this β-amylase enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1199 mg TOS/kg bw per day, the highest dose tested, in a 13-week study of oral toxicity in rats. When this NOAEL is compared with the dietary exposure estimate of 1 mg TOS/kg bw per day, a MOE of around 1200 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified"[1] for β-amylase (JECFA95-6) from *B. flexus* expressed in *B. licheniformis* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications.

A toxicological monograph with a dietary exposure assessment was prepared.

A new tentative specifications monograph and a chemical and technical assessment were prepared.

### Recommendations

The Committee requested the following information, by the end of 2023, to complete the safety assessment:

- validated method of analysis to determine β-amylase activity, including the validation report; and
- analytical data using the validated method for at least five different batches of commercially available products.

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

# References


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### 3.1.7 Lipase (JECFA95-7) from *Thermomyces lanuginosus* and *Fusarium oxysporum* expressed in *Aspergillus oryzae*

#### Explanation

At the request of the Codex Committee on Food Additives at its Forty-eighth Session *(1)*, the Committee evaluated the safety of a lipase (triacylglycerol lipase; Enzyme Commission No. 3.1.1.3; CAS No. 9001-62-1) from *Thermomyces lanuginosus* and *Fusarium oxysporum* expressed in *Aspergillus oryzae*. The Committee had not previously evaluated this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-7 to this enzyme preparation. The Committee had previously evaluated several other lipases, including lipase from *A. oryzae* at its Eighteenth meeting for which an ADI "not specified"[1] was established (Annex 3, reference 35). At its Eighty-ninth meeting, the Committee noted the specifications for lipase from *A. oryzae* var. had been withdrawn at its Fifty-fifth meeting because the requested data had not been submitted (Annex 3, reference 145). The consequences of the withdrawal of specifications on the ADI were never addressed and, as a result, the Committee decided to withdraw the ADI of "not specified" for lipase from *A. oryzae* var. at its Eighty-ninth meeting and recommended the reconsideration of the ADI at a future meeting (Annex 3, reference 246).

The term "lipase" refers to the triacylglycerol lipase enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product containing the enzyme of interest, which is used in the toxicity studies; and the term "enzyme preparation" refers to the formulated product for commercial use.

The Committee previously assessed the safety of enzyme preparations derived from *A. oryzae* (e.g. asparaginase and phospholipase A1; Annex 3, reference 187) and established an ADI "not specified" for these enzyme preparations. On this basis, the present Committee considered that lipase (JECFA95-7) from *T. lanuginosus* and *F. oxysporum* expressed in *A. oryzae* met the criteria of a Class 1, Type iii enzyme as described in EHC 240 *(2)*. A Class 1, Type iii enzyme is produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted data.

The enzyme hydrolyses ester linkages of triacylglycerides and phospholipids. The enzyme preparation is intended to be used as a processing aid in baking and in the processing of cereal-based foods. The Committee conducted

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

a literature search in Google Scholar with the linked search terms "lipase" AND "*Aspergillus oryzae*" AND "*Thermomyces lanuginosus*" OR "*Humicola lanuginose*" AND "safety" OR "tox". A total of 480 hits were found. One reference *(3)* was identified that summarized a series of toxicity studies conducted with lipase from *Humicola lanuginosa* (now known as *T. lanuginosus*) expressed in *A. oryzae*; however, not enough information was available to adequately compare the lipase used in these studies to the lipase described in the present monograph. This reference was therefore not considered relevant for the present toxicological evaluation. Two additional references *(4,5)* were identified in the literature search that described the same studies summarized by Greenough et al. *(3)*; for the reason described above, neither reference was considered relevant to the present toxicological evaluation.

### Genetic background

The production organism, *A. oryzae*, is a non-pathogenic, filamentous fungus found in cereals, cereal grains and spoiled foods *(6)*. It has a history of use as a source organism in the production of food enzymes *(6–8)*.

The non-toxigenic, non-pathogenic *A. oryzae* production strain was obtained by a combination of chemical mutagenesis and genetic modifications of the parental strain. The parental strain was obtained from the Institute for Fermentation, Osaka, Japan. The production strain was obtained by inactivation of genes encoding for a major secreted protein, deletion of genes encoding for three proteases, and disruption of genes responsible for production of kojic acid and mycotoxins. The expression plasmid used in the transformation contained a lipase gene created from portions of lipase genes from *T. lanuginosus* and *F. oxysporum*, an optimized *A. niger* promoter, an *A. niger* terminator and a selectable marker. The stability of the integration, and the absence of any genes of concern in the production strain, were confirmed by Southern blot hybridization.

### Chemical and technical considerations

Lipase is produced by controlled submerged fermentation of a pure culture of the *A. oryzae* production strain. Manufacture of the enzyme preparation includes fermentation, recovery and formulation. After fermentation, the broth containing the lipase enzyme is separated from the biomass via a series of filtration steps, and concentrated. The resulting concentrate is formulated into a powder preparation. The entire process is performed in accordance with current GMP using food-grade raw materials. The final enzyme preparation contains no major food allergens from the fermentation medium and is free from the production organism and any antibiotic activity.

The primary sequence of lipase enzyme produced by *A. oryzae* consists of 317 amino acids; its molecular weight calculated from the determined amino acid sequence is 35 kDa. The lipase produced by *A. oryzae* is not known to have any significant subsidiary or secondary activity.

The activity of lipase expressed in lipase units (LU) is determined by measuring the rate of butyric acid released from tributyrin; 1 LU is defined as the amount of enzyme required to liberate 1 µmol of titratable butyric acid per minute under the conditions of the assay. The mean activity of lipase from four batches of the enzyme concentrate was 10 450 LU/g.

Lipase catalyses the hydrolysis of ester linkages in triacylglycerol and phospholipids. The enzyme preparation is intended for use as a processing aid in baking and in the processing of cereal-based foods up to a maximum level of 20 mg TOS/kg flour. The TOS includes the enzyme of interest and residues of organic materials (e.g. proteins, peptides and carbohydrates) derived from the production organism during the manufacturing process.

The lipase enzyme is inactivated by heat during processing. It is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

Two bioinformatics analyses were submitted by the sponsor *(9,10)* comparing the amino acid sequence of this lipase with the sequences of known allergens in two online databases *(11,12)*. A search for amino acid sequence matches with more than 35% identity in a sliding window of 80 amino acids, a search for sequence identity of eight contiguous amino acids and a full-length FASTA sequence search were conducted in both databases and did not identify any matches. The Committee repeated the comparison of the amino acid sequence of this enzyme with known allergens in two online databases *(13,14)* based on bioinformatics criteria recommended by FAO/WHO *(15)*, Codex Alimentarius *(16)* and JECFA (Annex 3, reference 223), and did not identify any matches. No data on the digestibility of this enzyme in the gastrointestinal tract were submitted. The Committee considered that dietary exposure to this lipase is not anticipated to pose a risk for allergenicity.

### Toxicological studies

In a 13-week study of oral toxicity in rats *(17)*, the enzyme concentrate was administered by gavage at doses up to 1080.2 mg TOS/kg bw per day. No toxicologically relevant treatment-related effects were observed in any of the evaluated parameters. The Committee identified a NOAEL of 1080 mg TOS/kg bw per day (rounded from 1080.2 mg TOS/kg bw per day) for the enzyme concentrate, which was the highest dose tested.

The enzyme concentrate was not genotoxic in a bacterial reverse mutation assay *(18)* or in vitro micronucleus assay under test conditions *(19)*. The Committee had no concerns about potential genotoxicity of the enzyme concentrate.

A comparison of the amino acid sequence of this lipase with those of known protein toxins (UniProt database) revealed no biologically relevant homology (< 18%) *(10)*. The Committee therefore concluded that this lipase is unlikely to be a toxin.

### Assessment of dietary exposure

The Committee evaluated one estimate of dietary exposure to TOS from this lipase enzyme preparation, submitted by the sponsor. It combined a high estimate of daily consumption of baked goods (flour at 7.64 g/kg bw per day) with the maximum use level to treat flour (20 mg TOS/kg flour). The TMDI was calculated as 0.153 mg TOS/kg bw per day (0.2 mg TOS/kg bw per day after rounding). For the dietary exposure assessment, it was assumed that 100% of the TOS from the enzyme preparation remains in the final food. As stated above, the Committee noted that the enzyme is inactivated during the processing of food ingredients and will have no function in the final food.

### Evaluation

The Committee concluded that dietary exposure to this lipase enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1080 mg TOS/kg bw per day, the highest dose tested, in the 13-week study of oral toxicity in rats. When this NOAEL is compared with the dietary exposure estimate of 0.2 mg TOS/kg bw per day, a MOE of more than 5000 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the Committee established an ADI "not specified"[2] for lipase (JECFA95-7) from *T. lanuginosus* and *F. oxysporum* expressed in *A. oryzae* when used in the applications specified, at the levels of use specified and in accordance with current GMP.

A toxicological monograph with a dietary exposure assessment was prepared.

A new specifications monograph and a chemical and technical assessment were prepared.

[2] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

# References


1. Report of the Forty-eighth Session of the Codex Committee on Food Additives. Xi'an, China, 14–18 March 2016. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2016 (REP16/FA).
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19. Lipopan Xtra. Induction of micronuclei in cultured human peripheral blood lymphocytes. Unpublished report (study no. 1974/51) by Covance Laboratories Ltd; 2007.

### 3.1.8 Phospholipase A2 (PLA2; JECFA95-8) from porcine pancreas expressed in *Aspergillus niger*

At the request of the CCFA at its Forty-seventh meeting *(1)*, the Committee evaluated the safety of phospholipase A2 (PLA2) (Enzyme Commission No. 3.1.1.4; CAS No. 9001-84-7) from porcine pancreas expressed in *Aspergillus niger*. The Committee had not previously evaluated this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-8 to this enzyme preparation. The Committee previously evaluated a number of PLA2 enzyme preparations, including PLA2 from *Streptomyces violaceoruber* expressed in *S. violaceoruber* for which an ADI "not specified"[1] was established (Annex 3, reference 248).

PLA2 hydrolyses *sn*-3-phospholipids at the *sn*-2 position. This enzyme preparation is intended to be used as a processing aid in egg processing, vegetable oil degumming, vegetable lecithin modification and baking applications.

Because of the late submission of highly relevant toxicological data, other missing information and time constraints, the Committee was unable to complete this evaluation.

The Committee at its present meeting drafted a toxicological monograph with a dietary exposure assessment, a new tentative specifications monograph, and a chemical and technical assessment for phospholipase (PLA2; JECFA95-8) from porcine pancreas expressed in *A. niger* from the data submitted by the sponsor, but these were not finalized for publication.

#### Recommendations

The Committee recommends that the evaluation of this enzyme preparation is completed at a future meeting. The Committee requires that the following

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

information is submitted before the enzyme preparation can be considered for review at a future meeting:

- additional data to clarify the genotoxic potential of the PLA2 enzyme concentrate;
- digestibility data for enzyme preparations containing both glucoamylase and PLA2;
- results from five different batches of all types of PLA2 enzyme preparations using the assay to determine PLA2 activity provided in the dossier;
- validation information of the alternative method of analysis used to determine PLA2 activity (this should include the method description in English);
- unit definition for the PLA2 activity based on the alternative method of assay; and
- analytical data using the alternative validated method for at least five different batches of all types of commercially available products.

## Reference

1. Report of the Forty-seventh Session of the Codex Committee on Food Additives, Xi'an, China, 23–27 March 2015. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2015 (REP15/FA).

### 3.1.9 Xylanase (JECFA95-9) from *Bacillus licheniformis* expressed in *Bacillus licheniformis*

#### Explanation

At the request of the CCFA at its Fifty-first Session *(1)*, the Committee evaluated the safety of xylanase (endo-1,4-β-xylanase, Enzyme Commission No. 3.2.1.8; CAS No. 9025-57-4) from *Bacillus licheniformis* expressed in *B. licheniformis*. The Committee had not previously evaluated this enzyme preparation. The present Committee allocated the unique JECFA enzyme identifier JECFA95-9 to this enzyme preparation. The Committee had previously evaluated several other xylanases, including xylanase from *B. subtilis* expressed in *B. subtilis*, and xylanase

[1] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

from *Thermomyces lanuginosus* expressed in *Fusarium venenatum* (Annex 3, references 167 and 174), for which an ADI "not specified"[1] was established.

The term "xylanase" refers to the endo-1,4-β-xylanase enzyme and its amino acid sequence; the term "enzyme concentrate" refers to the fermentation product containing the enzyme of interest, which is used in the toxicity studies; and the term "enzyme preparation" refers to the product formulated for commercial use.

The Committee previously assessed the safety of enzyme preparations derived from *B. licheniformis* (e.g. pullulanase, Annex 3, reference 205; serine protease, Annex 3, reference 211) and established an ADI "not specified" for these enzyme preparations. On this basis, the present Committee considered that xylanase (JECFA95-9) from *B. licheniformis* expressed in *B. licheniformis* met the criteria of a Class 1, Type iii enzyme as described in EHC 240 *(2)*. A Class 1, Type iii enzyme preparation is produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain. Although toxicity data and a dietary exposure assessment are not required for Class 1, Type iii enzymes, the Committee evaluated the submitted data.

Xylanase catalyses the endohydrolysis of (1→4)-β-D-xylosidic linkages in xylans including arabinoxylans into oligosaccharides of variable lengths. The enzyme is intended for use as a processing aid in the manufacture of baked goods and cereal-based products.

The Committee conducted a literature search in Google Scholar with the linked search terms "xylanase" AND "*Bacillus licheniformis*", which resulted in 8270 references. None of the identified references were relevant to the toxicological evaluation of this enzyme preparation.

### Genetic background

The production organism, *B. licheniformis*, is a non-pathogenic and non-toxigenic bacterium. It is common in foods including agricultural products such as cereals, and has a history in the production of enzymes intended for use in food processing *(3–8)* (Annex 3, references 72 and 167).

The production strain NZYM-CE was obtained from *B. licheniformis* BW302 *(9,10)*. Two copies of the expression cassette, consisting of the *xylanase* gene from *B. licheniformis* and other regulatory elements, were inserted at two loci *(10)*. The stability of the inserts was confirmed by Southern blot analysis. Southern blot and genome sequence analyses were performed on the production strain to verify the absence of relevant genes of concern, including antibiotic resistance markers. The production strain is deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany.

### Chemical and technical considerations

Xylanase is produced by pure culture fermentation of the *B. licheniformis* production strain. Manufacture of the xylanase enzyme preparation includes fermentation, recovery and formulation. After fermentation, the broth containing the xylanase enzyme is separated from the biomass via multiple filtration steps, then concentrated. The resulting concentrate is formulated and standardized into either a liquid or a powder preparation. The entire process is performed in accordance with current GMP and with food-grade raw materials. The enzyme concentrate is tested to ensure that it is free from the production organism and antibiotic activity.

The primary sequence of xylanase produced by *B. licheniformis* consists of 407 amino acids; its molecular weight calculated from the determined amino acid sequence is 45.4 kDa *(9)*. The xylanase produced by *B. licheniformis* is not expected to have any secondary or subsidiary activities.

Xylanase activity is determined spectrophotometrically (405 nm) by measuring the formation of complexes of 4-hydroxybenzoic acid hydrazide and carbohydrates released by the hydrolysis of wheat arabinoxylan. The xylanase activity is expressed in units/g relative to a proprietary xylanase standard. A definition for the unit of activity was not provided. The mean activity from three batches of the enzyme concentrate was 4377 units/g.

Xylanase catalyses the endohydrolysis of (1→4)-β-D-xylosidic linkages in xylans including arabinoxylans, which results in the generation of (1→4)-β-D-xylan oligosaccharides of variable length. The enzyme preparation is intended for use as a processing aid in the manufacture of baked goods and cereal-based products at a maximum use level of 1 mg TOS/kg flour.

The xylanase enzyme is either removed or inactivated by heat during processing, and is not expected to have any technological function in the final food.

### Assessment of potential allergenicity

Xylanase from *B. licheniformis* expressed in *B. licheniformis* was evaluated by the sponsor in 2022 for potential allergenicity by the bioinformatics criteria recommended by the European Food Safety Authority (EFSA) *(11)* and FAO/WHO *(12)*. The amino acid sequence of the enzyme was compared with those of known allergens in two online databases *(13,14)*. A search for matches with more than 35% identity in a sliding window of 80 amino acids, a search for exact matches over contiguous stretches of eight amino acids and a full-length FASTA sequence search did not identify a homology to any known allergens. No biologically relevant homology was found between the xylanase and any of the allergens in the databases mentioned above. No data were available on the digestibility of

xylanase in the gastrointestinal tract. The Committee concluded that dietary exposure to xylanase from *B. licheniformis* expressed in *B. licheniformis* is not anticipated to pose a risk for allergenicity.

### Toxicological studies

In a 13-week study of general toxicity in rats, no toxicologically relevant treatment-related effects were observed when the enzyme concentrate was administered by gavage at doses up to 1020 mg TOS/kg bw per day *(15)*. A NOAEL was identified as the highest dose tested (i.e. 1020 mg TOS/kg bw per day).

The enzyme concentrate was negative in a bacterial reverse mutation assay *(16)* and an in vitro mammalian micronucleus assay *(17)*. The Committee therefore had no concerns about potential genotoxicity of the xylanase enzyme concentrate.

### Assessment of dietary exposure

The Committee evaluated estimates of dietary exposure to TOS in this xylanase enzyme preparation. The Committee used the budget method to estimate the TMDI of xylanase in solid food, based on a maximum use level of 1 mg TOS/kg food (assuming that all solid foods contain the maximum use level for flour), a consumption value of 0.025 kg/kg bw per day, and the assumption that 50% of solid food may contain the enzyme. The Committee noted that the method provided a conservative estimate of dietary exposure, and the resulting TMDI of xylanase was estimated to be 0.01 mg TOS/kg bw per day for solid foods. The sponsor submitted a more refined dietary exposure estimate for xylanase based on the summarized intake of grains and grain-based products given in the EFSA Comprehensive European Food Consumption Database. However, the Committee noted that this estimate has been included in those that EFSA estimated in 2019 *(9)*; the Committee also noted and concluded that the highest estimate of dietary exposure to xylanase of 0.01 mg TOS/kg bw per day for infants at the 95th percentile, calculated with maximum use levels recommended for the respective food processes and individual data from the EFSA Comprehensive European Food Consumption Database, should be considered in the evaluation. For the dietary exposure assessment, it was assumed that 100% of the TOS from the enzyme preparation remains in the final food. The Committee noted that the enzyme is inactivated during the processing of food ingredients, and will have no function in the final food.

### Evaluation

The Committee concluded that dietary exposure to this xylanase enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee

identified a NOAEL of 1020 mg TOS/kg bw per day, the highest dose tested, in the 13-week study of oral toxicity in rats. When this NOAEL is compared with the dietary exposure estimate of 0.01 mg TOS/kg bw per day, a MOE of more than 100 000 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the Committee allocated a temporary ADI "not specified"[2] for xylanase (JECFA95-9) from *B. licheniformis* expressed in *B. licheniformis* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications.

A toxicological monograph with a dietary exposure assessment was prepared.

A new tentative specifications monograph and a chemical and technical assessment were prepared.

### Recommendations

The Committee requested the following information, by the end of 2023, to complete the safety assessment:

- validated method of analysis to determine xylanase activity, including the full validation report;
- unit definition for xylanase activity based on the method of assay; and
- analytical data using the validated method for at least five different batches of commercially available products.

## References

1. Report of the Fifty-first Session of the Codex Committee on Food Additives. Jinan, China, 25–29 March 2019. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2019 (REP19/FA).

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[2] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting (Annex 3, reference 243) for clarification of the term ADI "not specified".

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## 3.2 Revision of specifications

### 3.2.1 Spirulina extract

Spirulina extract was on the agenda of the Committee at its Eighty-sixth meeting. A specifications monograph was prepared and made tentative by the Committee (Annex 3, reference 242) pending submission of the following information:

- full compositional characterization of commercial products in both liquid and powder forms;
- full compositional characterization of the aqueous extract before formulation/standardization;
- validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data); and
- validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).

The present Committee evaluated the compositional data and analytical methods received in response to the above requests.

The tentative specifications were revised and the tentative status was removed. The temporary status of the ADI "not specified" determined at the Eighty-sixth meeting of the Committee was also removed. The chemical and technical assessment was revised.

# 4. Flavouring agents

## 4.1 Safety evaluation

### 4.1.1 Alicyclic ketones, secondary alcohols and related esters

#### Introduction

At the request of the CCFA at its Fifty-first session *(1)*, the Committee evaluated an additional two flavouring agents in the group of alicyclic ketones, secondary alcohols and related esters for the first time. In addition, the Committee considered new data for 10 previously evaluated flavouring agents in this group and data on three structurally related substances: the formyl and acetate esters of 4-*tert*-butylcyclohexanol and 2-*tert*-butylcyclohexanone.

The Committee evaluated 25 members of this group at its Fifty-ninth meeting (Annex 3, reference 160) and 12 members of this group at its Seventy-third meeting (Annex 3, reference 202). The Committee concluded that none of the 37 flavouring agents was a safety concern at the estimated dietary exposures.

The additional flavouring agents in this group are *trans*-4-*tert*-butylcyclohexanol (No. 2263) and caryophylla-3(4),8-dien-5-ol (No. 2264) (mixture of CAS No. 38284-26-3 and CAS No. 34298-31-2). Both flavouring agents have been reported to occur naturally; No. 2263 occurs in white wine and No. 2264 occurs in clary sage, clove bud, pepper and Scotch spearmint oil *(2–4)*.

The two additional members of this group were evaluated according to the revised Procedure for the Safety Evaluation of Flavouring Agents (Annex 3, reference 230).

A literature search for toxicological data was performed in Google Scholar, PubMed, Embase and Web of Science using the names and CAS numbers of the flavouring agents under evaluation in this group; no additional relevant references were identified.

#### Assessment of dietary exposure

The total annual volume of production of the two flavouring agents in the group of alicyclic ketones, secondary alcohols and related esters is 0.2 kg in the USA *(5,6)*. No production volumes were reported for Japan, Europe or Latin America.

Dietary exposures were estimated with both the single portion exposure technique (SPET) and the maximized survey-derived intake (MSDI) method; the higher of the two values for each flavouring agent is reported in Table 1. The estimated daily dietary exposure is higher for *trans*-4-*tert*-butylcyclohexanol (No. 2263) (6000 µg/day, SPET value). For the other flavouring agent, caryophylla-3(4),8-dien-5-ol (mixture) (No. 2264), the estimated daily dietary exposure ranged from 0.01 to 300 µg/day, with the SPET yielding the higher estimate.

### Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination (ADME) of the flavouring agents in the group of alicyclic ketones, secondary alcohols and related esters was provided in the monographs from the Fifty-ninth and Seventy-third meetings (Annex 3, references 160 and 202). Specific information on one of the additional flavouring agents (No. 2263) evaluated at this meeting has become available.

Metabolic studies on alicyclic ketones indicate that they are converted to the corresponding secondary alcohols, which can be further oxidized to ketones or form conjugates with glucuronic acid or sulfate prior to urinary elimination.

### Application of the revised Procedure for the Safety Evaluation of Flavouring Agents

**Step 1.** There are no structural alerts for genotoxicity for the additional two flavouring agents (Nos 2263 and 2264) in this group. Chemical-specific genotoxicity data on flavouring agents previously evaluated in this group and on the additional flavouring agents do not indicate any genotoxic potential.

**Step 2.** In applying the revised Procedure for the Safety Evaluation of Flavouring Agents to the additional two flavouring agents, the Committee assigned both flavouring agents (Nos 2263 and 2264) to structural class I *(7)*.

**Step 3.** Dietary exposures were estimated with both the MSDI method and the SPET, and are presented in Table 1.

**Step 4.** The highest estimated dietary exposure to one flavouring agent (No. 2264) in structural class I is below the threshold of toxicological concern for the class (i.e. 1800 µg/person per day). The Committee therefore concluded that this flavouring agent (No. 2264) is not a safety concern at the current estimated dietary exposure. The highest estimated dietary exposure of the other flavouring agent (No. 2263) in structural class I is above the threshold of toxicological concern for that class (i.e. 1800 µg/person per day). Evaluation of this flavouring agent therefore proceeded to Step 5.

**Step 5.** For *trans*-4-*tert*-butylcyclohexanol (No. 2263), the NOAEL of 240 mg/kg bw per day in a 13-week gavage study in male and female rats *(8)* provides an adequate MOE (2400) relative to the SPET estimate of 6000 µg/day (or 100 µg/kg bw per day for a 60-kg person).

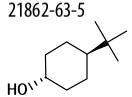
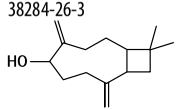
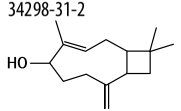
Table 1 summarizes the evaluations of the two flavouring agents in the group of alicyclic ketones, secondary alcohols and related esters that were considered at the present meeting (Nos 2263 and 2264).

### Consideration of combined intakes from use as flavouring agents

The Committee previously considered the potential combined intake for this group of alicyclic ketones, secondary alcohols and related esters and identified

Table 1

**Summary of the results of safety evaluations of alicyclic ketones, secondary alcohols and related esters used as flavouring agents[a,b,c,d]**

| Flavouring agent | No. | CAS No. and structure | *Step 4* Does intake exceed the threshold of toxicological concern?[e] | *Step 5* Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate MOE?[f] | Comments on predicted metabolism | Conclusion based on current estimated dietary exposure |
|---|---|---|---|---|---|---|
| **Structural Class I** | | | | | | |
| *trans*-4-*tert*-Butylcyclohexanol | 2263 | 21862-63-5  | Yes; SPET: 6000 µg/day | Yes. The NOAEL of 240 mg/kg bw per day for *trans*-4-*tert*-butylcyclohexanol in a 13-week study in rats *(8)* is 2400 times the estimated dietary exposure of No. 2263 when used as a flavouring agent. | –[g] | No safety concern |
| Caryophylla-3(4),8-dien-5-ol (mixture) | 2264 | 38284-26-3  34298-31-2  | No; SPET: 300 µg/day | NA | –[g] | No safety concern |

CAS, Chemical Abstracts Service; MOE, margin of exposure; NA, not applicable; NOAEL, no-observed-adverse-effect level; SPET, single portion exposure technique.

[a] Thirty-seven flavouring agents in this group were previously evaluated by the Committee (Annex 3, references 160 and 202).

[b] Step 1: There are no structural alerts for genotoxicity for the additional two flavouring agents (Nos 2263 and 2264), and data on genotoxicity of No. 2263 do not indicate potential for genotoxicity.

[c] Step 2: Both flavouring agents are in structural class I.

[d] Step 3: Dietary exposures were estimated by both the SPET and MSDI method, and the higher of the two values for each flavouring agent is reported. SPET gave the higher estimate for each flavouring agent. All dietary exposure values are expressed in µg/day.

[e] Step 4: The threshold for human intake for structural class I is 1800 µg/day.

[f] The margin of exposure was calculated based on the highest daily per capita intake calculated either by the SPET or MSDI method for a body weight of 60 kg.

[g] The substances are detoxicated by glucuronic acid conjugation and excretion in the urine.

no safety concerns. The two additional flavouring agents in this group (Nos 2263 and 2264) have a low MSDI value (0.01 μg/day). According to the screening assessment for combined intake recommended by the Committee at its Seventy-third meeting (Annex 3, reference 202), the Committee concluded that consideration of combined intakes is not necessary because the additional flavouring agents would not contribute significantly to the combined intake of this group.

### Consideration of additional data on previously evaluated flavouring agents

The Committee considered additional data on 10 of the 37 previously evaluated flavouring agents in this group. Studies of short-term toxicity (Nos 1109, 1114 and 2053), reproductive and developmental toxicity (Nos 1109, 1114 and 2053), and genotoxicity (Nos 1093, 1099, 1109, 1111, 1114, 1115, 2051–2053 and 2057) were available. These new toxicological data support the conclusions of the previous evaluations that these flavouring agents would not give rise to safety concerns.

### Conclusions

In the previous evaluations of 37 substances in this group of alicyclic ketones, secondary alcohols and related esters, studies of ADME, acute toxicity, short- and long-term toxicity, and genotoxicity were evaluated (Annex 3, references 160 and 202). None raised safety concerns.

Studies of ADME, acute toxicity, short-term toxicity, developmental toxicity and genotoxicity were available for one of the two additional flavouring agents (No. 2263). For the structurally related substance 4-*tert*-butylcyclohexyl acetate, studies of short-term toxicity, reproductive and developmental toxicity, and genotoxicity were available. In addition, studies of genotoxicity were available for the structurally related substances 2-*tert*-butylcyclohexanone and the formate ester of 4-*tert*-butylcyclohexanol.

The Committee concluded that the two additional flavouring agents (Nos 2263 and 2264) would not give rise to safety concerns at the current estimated dietary exposures.

The Committee also concluded that the additional data presented do not give rise to safety concerns and further support the safety of the 37 previously evaluated flavours in this group.

An addendum to the monograph was prepared.

# References


1. Report of the Fifty-first Session of the Codex Committee on Food Additives. Jinan, China, 25–29 March 2019. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2019 (REP19/FA).

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5. 2015 IOFI Global Poundage Survey. Geneva: International Organization of the Flavor Industry; 2018. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium.

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8. Sieber M. BAS 102: 90-Day oral toxicity (gavage) study in the Wistar rat with 28-day recovery. Unpublished report (C84918) from Harlan Laboratories Ltd, Itingen, Switzerland; 2011. Submitted to the Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium.


## 4.2 Specifications of identity and purity

### 4.2.1 New specifications

At its Eighty-ninth meeting (Annex 3, reference 246), the Committee prepared tentative specifications for two flavouring agents (Nos 2263 and 2264) for which a safety evaluation was not completed. At the current meeting, the evaluation of the two flavouring agents was completed as part of the safety evaluation for the group of alicyclic ketones, secondary alcohols and related esters. Full specifications were prepared.

# 5. Future work and recommendations

As reported elsewhere (see Section 2.4), the Committee expressed its frustration that many of the current data submissions were either inconsistent with key aspects of the guidelines published by the Committee, or else inadequate or incomplete. To be able to complete the safety evaluations of the food additives assessed at this meeting, the Committee recommends that the information listed in Table 2 below be provided.

Table 2

**Information requested by the Committee to be able to complete safety evaluations of the food additives discussed at the Ninety-fifth JECFA**

| Report item | Recommendation |
|---|---|
| 3.1.1. α-Amylase (JECFA95-1) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis* | The Committee requested the following information, by the end of 2023, to complete the safety assessment:<br>• validated method of analysis to determine α-amylase activity, including the validation report;<br>• unit definition for α-amylase activity based on the method of assay; and<br>• analytical data using the validated method for at least five different batches of commercially available products. |
| 3.1.2. α-Amylase (JECFA95-2) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis* | The Committee requested the following information, by the end of 2023, to complete the safety assessment:<br>• validated method of analysis to determine α-amylase activity, including the validation report;<br>• unit definition for α-amylase activity based on the method of assay; and<br>• analytical data using the validated method for at least five different batches of commercially available products. |
| 3.1.3. α-Amylase (JECFA95-3) from *Rhizomucor pusillus* expressed in *Aspergillus niger* | The Committee requested the following information, by the end of 2023, to complete the safety assessment:<br>• validated method of analysis to determine α-amylase activity, including the validation report;<br>• unit definition for α-amylase activity based on the method of assay; and<br>• analytical data using the validated method for at least five different batches of commercially available products. |
| 3.1.4. Amyloglucosidase (JECFA95-4) from *Rasamsonia emersonii* expressed in *Aspergillus niger* | The Committee requested the following information, by the end of 2023, to complete the safety assessment:<br>• digestibility data in order to complete the allergenicity assessment;<br>• validated method of analysis to determine amyloglucosidase activity, including the validation report;<br>• unit definition for amyloglucosidase activity based on the method of assay; and<br>• analytical data using the validated method for at least five different batches of commercially available products. |
| 3.1.5. Asparaginase (JECFA95-5) from *Pyrococcus furiosus* expressed in *Bacillus subtilis* | The Committee requested the following information, by the end of 2023, to complete the safety assessment:<br>• validated method of analysis to determine asparaginase activity, including the validation report;<br>• unit definition for asparaginase activity based on the method of assay; and<br>• analytical data using the validated method for at least five different batches of commercially available products. |
| 3.1.6. β-Amylase (JECFA95-6) from *Bacillus flexus* expressed in *Bacillus licheniformis* | The Committee requested the following information, by the end of 2023, to complete the safety assessment:<br>• validated method of analysis to determine β-amylase activity, including the validation report; and<br>• analytical data using the validated method for at least five different batches of commercially available products. |

Table 2 (continued)

| Report item | Recommendation |
|---|---|
| 3.1.8. Phospholipase A2 (PLA2; JECFA95-8) from porcine pancreas expressed in *Aspergillus niger* | The Committee recommends that the evaluation of this enzyme preparation is completed at a future meeting. The Committee requested the JECFA Secretariat to urge the sponsor and Codex Members to ensure that the following additional information is available for evaluation prior to requesting inclusion of this enzyme preparation in the CCFA JECFA Priority List:<br>• additional data to clarify the genotoxic potential of the PLA2 enzyme concentrate;<br>• digestibility data for enzyme preparations containing both glucoamylase and PLA2;<br>• results from five different batches of all types of PLA2 enzyme preparations using the assay to determine PLA2 activity provided in the dossier;<br>• validation information of the alternative method of analysis used to determine PLA2 activity (this should include the method description in English);<br>• unit definition for the PLA2 activity based on the alternative method of assay; and<br>• analytical data using the alternative validated method for at least five different batches of all commercially available products. |
| 3.1.9. Xylanase (JECFA95-9) from *Bacillus licheniformis* expressed in *Bacillus licheniformis* | The Committee requested the following information, by the end of 2023, to complete the safety assessment:<br>• validated method of analysis to determine xylanase activity, including the full validation report;<br>• unit definition for xylanase activity based on the method of assay; and<br>• analytical data using the validated method for at least five different batches of commercially available products. |

# Corrigenda

The Committee discussed the tentative errata. One request was for the amendment of the name of the microorganism *Geobacillus stearothermophilus* used for the Glucosyl Steviol Glycosides (GSG) production (Steviol Glycosides Framework, annex 4) by replacing the existing name of the microorganism with *Anoxybacillus caldiproteoliticus* (or adding it as an alternative name). The Committee decided to refer this request to a later meeting in order to allow for a more careful evaluation of possible implications of this requested name change.

The other requests for corrections (Table 3), reported to the JECFA Secretariat, were evaluated by the Committee and found to be necessary. However, these corrections will only be made in the electronic versions available in the online database.

Table 3

**Requests for revisions and additions reported to the JECFA Secretariat**

| Substance | Original text | Revised text | Additional information |
|---|---|---|---|
| Saccharin | An ADI of 0–5 mg/kg bw for saccharin and its Ca, K, Na salts was established at 41st JECFA. | A group ADI of 0–5 mg/kg bw for saccharin and its Ca, K, Na salts, expressed as Na saccharin, was established at 41st JECFA. | The reporting basis for saccharins should be revised as "For saccharin and its Ca, K, Na salts, expressed as Na saccharin" |
| Paprika oleoresin<br>Monograph | Functional uses: colour, flavouring agent | Functional uses: flavouring agent | Correct functional class |
| Lysozyme<br>Monograph | Functional uses: preservative (mainly to prevent the late blowing of cheese caused by *Clostridium tyrobutyricum*) | Functional uses: processing aid for cheese production | Correct functional class |
| β-carotene, synthetic INS 160a(i)<br>Monograph | In the "purity test", "procedure" section, the impurity at relative retention time of 0.85 currently reads<br>all-trans-□-carotene (0.85) | It should read "all-trans-γ-carotene". | |
| | In the "purity test", "calculation" section, the formula is wrong; the multiplication sign should be replaced by a subtraction sign | $\frac{A_{\text{total}} - A_{\beta\text{-carotenes}}}{A_{\text{total}}} \times 100$ | |
| Jagua blue | Synonyms: Jagua blue | Add synonyms.<br>Synonyms: Jagua blue, Genipapo, huito blue, huito, jagua. | Transcription errors |
| Monograph | Name: "Jagua (genipin-glycine) blue (Jagua blue)" | Name: jagua (genipin-glycine) blue | |
| Steviol glycosides<br>Framework | "Reagents" section (page 11)<br>- Mobile phase A: Deionized water, HPLC or LC-MS grade, filtered using a 0.2-µm filter, with 0.1% formic acid or acetic acid. (Note: If only UV detection will be used, 20 mM sodium phosphate buffer at pH 2.6 or 0.01% trifluoroacetic acid may be used.) | "Reagents" section (page 11) to be amended to read: "0.01% formic acid or acetic acid." | Transcription error |

Table 3 (continued)

| Substance | Original text | Revised text | Additional information |
|---|---|---|---|
| Steviol glycosides | The Molecular Weight RRF values in table 2: | The Molecular Weight RRF values located in table 2 (pages 13, 14) to be amended: | Transcription error |
| Framework | Rebaudioside B: 0.82<br>Steviolbioside: 0.83 | Rebaudioside B: 0.83<br>Steviolbioside: 0.66 | |
| Steviol glycosides<br>Framework | Calculate the concentration of minor steviol glycosides:<br>Conc. (% w/w) = CX x MX x 100 / MA x Csample | Conc. (% w/w) = CX x MX x 100 / (MA x Csample) | Transcription error |
| Steviol glycosides<br>Framework | "Equilibration"<br>Powdered samples should be equilibrated in the lab not less than 12 hours before assaying. | "Equilibration"<br>"Powdered samples and powdered standards should be equilibrated in the lab not less than 12 hours before assaying."<br>Addition of Note:<br>"An unopened reference standard with moisture listed on a Certificate of Analysis may be used without equilibrating." | Item for discussion: request for amendment to and addition of a note to the "Equilibration" section. |
| Steviol glycosides<br>Framework | "Equilibration"<br>The loss on drying of the equilibrated sample should be determined concurrently with performing the assay using the conditions in Annexes 1–4 (Vol. 4). | "Equilibration"<br>"The loss on drying of the equilibrated sample should be determined concurrently with performing the assay using the conditions in Annexes 1–4 (Vol. 4). Karl Fischer titration may be used as an alternative to loss on drying for determining moisture of equilibrated samples and standards when performing the assay." | Item for discussion: request for addition of Karl Fischer titration as alternative |

# Annex 1

## Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

**Ninety-fifth JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)**
**6–17 and 22 June 2022**

**Virtual meeting: 12:00–16:00 (CET)**

1. Opening

2. Declarations of Interests (information by the Secretariat on any declared interests and discussion, update by experts)

3. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs

4. Adoption of the agenda

5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives

6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full Committee)

7. Food additives other than flavouring agents

    7.1. Safety evaluations
        7.1.1 α-Amylase (JECFA95-1) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*
        7.1.2 α-Amylase (JECFA95-2) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*
        7.1.3 α-Amylase (JECFA95-3) from *Rhizomucor pusillus* expressed in *Aspergillus niger*
        7.1.4 Amyloglucosidase (JECFA95-4) from *Rasamsonia emersonii* expressed in *Aspergillus niger*
        7.1.5 Asparaginase (JECFA95-5) from *Pyrococcus furiosus* expressed in *Bacillus subtilis*
        7.1.6 β-amylase (JECFA95-6) from *Bacillus flexus* expressed in *Bacillus licheniformis*

7.1.7 Lipase (JECFA95-7) from *Thermomyces lanuginosu*s and *Fusarium oxysporum* expressed in *Aspergillus oryzae*
7.1.8 Phospholipase A2 (PLA2; JECFA95-8) from porcine pancreas expressed in *Aspergillus niger*
7.1.9 Xylanase (JECFA95-9) from *Bacillus licheniformis* expressed in *Bacillus licheniformis*

7.2. Revision of specifications
7.2.1 Spirulina extract

8. Flavouring agents
8.1 Alicyclic ketones, secondary alcohols and related esters

9. Other matters to be considered (general considerations)

10. Other matters as may be brought forth by the Committee during discussions at the meeting

11. Adoption of the report

# Annex 2

# Toxicological information and information on specifications

*Food additives evaluated toxicologically and assessed for dietary exposure*

| Food additive | JECFA enzyme identifier | Specifications | ADIs and other conclusions on toxicology and dietary exposure |
|---|---|---|---|
| α-Amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis* | JECFA95-1 | N, T | The Committee concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 67 mg TOS/kg bw per day, the highest dose tested in a 13-week oral toxicity study in rats. When this NOAEL is compared with the dietary exposure estimate of 0.2 mg TOS/kg bw per day, a MOE of more than 330 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified"[a] for α-amylase (JECFA95-1) from *G. stearothermophilus* expressed in *B. licheniformis*, when used in the applications specified, at the levels of use specified and in accordance with current GMP. This ADI "not specified" was made temporary because of the tentative nature of the specifications. |
| α-Amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis* | JECFA95-2 | N, T | The Committee concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 660 mg TOS/kg bw per day, the highest dose tested in a 13-week oral toxicity study in rats. When this NOAEL is compared with the dietary exposure estimate of 0.08 mg TOS/kg bw per day, a MOE of more than 8000 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified" for α-amylase (JECFA95-2) from *G. stearothermophilus* expressed in *B. licheniformis*, when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications. |
| α-Amylase from *Rhizomucor pusillus* expressed in *Aspergillus niger* | JECFA95-3 | N, T | The Committee concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1400 mg TOS/kg bw per day, the highest dose tested in a 13-week oral toxicity study in rats. When this NOAEL is compared with the dietary exposure estimate of 4 mg TOS/kg bw per day, a MOE of more than 350 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified" for α-amylase (JECFA95-3) from *R. pusillus* expressed in *A. niger*, when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications. |

| Food additive | JECFA enzyme identifier | Specifications | ADIs and other conclusions on toxicology and dietary exposure |
|---|---|---|---|
| Amyloglucosidase from *Rasamsonia emersonii* expressed in *Aspergillus niger* | JECFA95-4 | N, T | The Committee noted that amyloglucosidase may pose a risk as a respiratory allergen. In the absence of any information regarding its stability within the gastrointestinal tract, the Committee could not complete the assessment of the risk for allergenicity from dietary exposure to this enzyme. The Committee identified a NOAEL of 1500 mg TOS/kg bw per day in a 13-week study of oral toxicity in rats. When this NOAEL, the highest dose tested, is compared with the conservative dietary exposure estimate of 9 mg TOS/kg bw per day, a MOE of more than 160 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified" for amyloglucosidase (JECFA95-4) from *R. emersonii* expressed in *A. niger* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications and the inability to complete the allergenicity assessment. |
| Asparaginase from *Pyrococcus furiosus* expressed in *Bacillus subtilis* | JECFA95-5 | N, T | The Committee concluded that dietary exposure to the enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1207 mg TOS/kg bw per day, the highest dose tested, in a 13-week study of oral toxicity in rats. When this NOAEL is compared with dietary exposure estimate of 0.4 mg TOS/kg bw per day, a MOE of more than 3000 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified" for asparaginase (JECFA95-5) from *P. furiosus* expressed in *B. subtilis* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications. |
| β-Amylase from *Bacillus flexus* expressed in *Bacillus licheniformis* | JECFA95-6 | N, T | The Committee concluded that dietary exposure to the enzyme preparation is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1199 mg TOS/kg bw per day, the highest dose tested, in a 13-week study of oral toxicity in rats. When this NOAEL is compared with the dietary exposure estimate of 1 mg TOS/kg bw per day, a MOE of around 1200 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee established a temporary ADI "not specified" for β-amylase (JECFA95-6) from *B. flexus* expressed in *B. licheniformis* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications. |

| Food additive | JECFA enzyme identifier | Specifications | ADIs and other conclusions on toxicology and dietary exposure |
|---|---|---|---|
| Lipase from *Thermomyces lanuginosus* and *Fusarium oxysporum* expressed in *Aspergillus oryzae* | JECFA95-7 | N | The Committee concluded that dietary exposure to this lipase is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1080 mg TOS/kg bw per day, the highest dose tested in the 13-week study of oral toxicity in rats. When this NOAEL is compared with the dietary exposure estimate of 0.2 mg TOS/kg bw per day, a MOE of more than 5000 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee established an ADI "not specified" for lipase (JECFA95-7) from *T. lanuginosus* and *F. oxysporum* expressed in *A. oryzae* when used in the applications specified, at the levels of use specified and in accordance with current GMP. |
| Phospholipase A2 (PLA2) from porcine pancreas expressed in *Aspergillus niger* | JECFA95-8 | No[b] | Because of the late submission of highly relevant toxicological data, other missing information and time constraints, the Committee was unable to complete this evaluation. The Committee recommended that the evaluation of this enzyme preparation is completed at a future meeting. |
| Xylanase from *Bacillus licheniformis* expressed in *Bacillus licheniformis* | JECFA95-9 | N, T | The Committee concluded that dietary exposure to this xylanase is not anticipated to pose a risk for allergenicity. The Committee identified a NOAEL of 1020 mg TOS/kg bw per day, the highest dose tested, in the 13-week study of oral toxicity in rats. When this NOAEL is compared with the dietary exposure estimate of 0.01 mg TOS/kg bw per day, a MOE of more than 100 000 can be calculated.<br><br>Based on this MOE and the lack of concern for genotoxicity, the Committee allocated a temporary ADI "not specified" for xylanase (JECFA95-9) from *B. licheniformis* expressed in *B. licheniformis* when used in the applications specified, at the levels of use specified and in accordance with current GMP. The ADI "not specified" was made temporary because of the tentative nature of the specifications. |

ADI: acceptable daily intake; GMP: Good Manufacturing Practices; MOE: margin of exposure; N: new specification; NOAEL: no-observed-adverse-effect limit; T: tentative specification; TOS: total organic solids.

[a] The reader is referred to the Technical Report of the Eighty-seventh JECFA meeting for clarification of the term ADI "not specified".

[b] No specifications were prepared. Information is required to prepare specifications (see Section 5).

## *Food additives considered for specifications only*

| Food additive | Specifications |
|---|---|
| Spirulina extract (INS 134) | R |

R: revised specifications.

### *Flavouring agents evaluated by the revised Procedure for the Safety of Evaluation of Flavouring Agents*

#### *Alicyclic ketones, secondary alcohols and related esters*

| Flavouring agent[a] | No. | Specifications | Conclusion based on current estimated dietary exposure |
|---|---|---|---|
| Trans-4-*tert*-butylcyclohexanol | 2263 | N | No safety concern |
| Caryophylla-3(4),8-dien-5-ol | 2264 | N | No safety concern |

N: new specification.

[a] Both flavouring agents are in structural class I.

# Annex 3

## Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).

2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).

3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).

4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).

5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).

6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).

7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).

8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).

9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).

10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.

13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.

15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.

16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.

17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.

18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.

19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.

20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.

21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.

22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.

24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.

25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.

26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some

antioxidants (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.

28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.

29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.

30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.

32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).

33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.

34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.

35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.

37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.

38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.

39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.

40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.

42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.

43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.

44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.

45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.

46. Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.

47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.

48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.

49. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 7, 1978.

50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.

51. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 14, 1980.

52. Specifications for identity and purity of food colours, flavouring agents, and other food additives. FAO Food and Nutrition Paper, No. 12, 1979.

53. Evaluation of certain food additives (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.

54. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 15, 1980.

55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.

56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.

57. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 16, 1981.

58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.

59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.

60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.

61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.

63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.

64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.

65. Guide to specifications – General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.

66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.

67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.

68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.

69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.

70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.

71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.

72. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.

73. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.

74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.

75. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 37, 1986.

76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.

77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.

78. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.

79. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 38, 1988.

80. Evaluation of certain veterinary drug residues in food (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.

82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988.

83. Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.

84. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.

85. Evaluation of certain veterinary drug residues in food (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.

86. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 25, 1990.

87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.

88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.

89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.

90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.

91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.

92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.

93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.

94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.

95. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, 1991.

96. Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).

97. Evaluation of certain veterinary drug residues in food (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.

98. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 29, 1991.

99. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, 1991.

100. Guide to specifications – General notices, general analytical techniques, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.

101. Evaluation of certain food additives and naturally occurring toxicants (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.

102. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series, No. 30, 1993.

103. Compendium of food additive specifications: addendum 1. FAO Food and Nutrition Paper, No. 52, 1992.

104. Evaluation of certain veterinary drug residues in food (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.

105. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 31, 1993.

106. Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper, No. 41/5, 1993.

107. Evaluation of certain food additives and contaminants (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.

108. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 32, 1993.

109. Compendium of food additive specifications: addendum 2. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.

110. Evaluation of certain veterinary drug residues in food (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.

111. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 33, 1994.

112. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/6, 1994.

113. Evaluation of certain veterinary drug residues in food (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.

114. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 34, 1995.

115. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/7, 1995.

116. Evaluation of certain food additives and contaminants (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.

117. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 35, 1996.

118. Compendium of food additive specifications: addendum 3. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.

119. Evaluation of certain veterinary drug residues in food (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.

120. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 36, 1996.

121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.

122. Evaluation of certain food additives and contaminants (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.

123. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 37, 1996.

124. Compendium of food additive specifications, addendum 4. FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.

125. Evaluation of certain veterinary drug residues in food (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.

126. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 38, 1996.

127. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/9, 1997.

128. Evaluation of certain veterinary drug residues in food (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.

129. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 39, 1997.

130. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/10, 1998.

131. Evaluation of certain food additives and contaminants (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.

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## Evaluation of certain food additives

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, including flavouring agents, to identify safety concerns and to prepare specifications for the identity and purity of the food additives.

The first part of the report includes guidance on the naming and identification of enzyme preparations for evaluation by JECFA. Discussions of data submission issues, including late or incomplete supporting information, are also described.

This is followed by summaries of the Committee's evaluations of technical, toxicological and dietary exposure data for eight specific food additives: α-amlyase (JECFA95-1) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*; α-amlyase (JECFA95-2) from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*; α-amlyase (JECFA95-3) from *Rhizomucor pusillus* expressed in *Aspergillus niger*; amyloglucosidase (JECFA95-4) from *Rasamsonia emersonii* expressed in *Aspergillus niger*; asparaginase (JECFA95-5) from *Pyrococcus furiosus* expressed in *Bacillus subtilis*; β-amylase (JECFA95-6) from *Bacillus flexus* expressed in *Bacillus licheniformis*; lipase (JECFA95-7) from *Thermomyces lanuginosus* and *Fusarium oxysporum* expressed in *Aspergillus oryzae*; and xylanase (JECFA95-9) from *Bacillus licheniformis* expressed in *Bacillus licheniformis*. The Committee was unable to complete their evaluation of phospholipase A2 (PLA2; JECFA95-8) from porcine pancreas expressed in *Aspergillus niger* because of the late submission of highly relevant toxicological data. Specifications for the food additive spirulina extract were revised.

Summaries are also provided of the safety evaluations of one group of flavouring agents (alicyclic ketones, secondary alcohols and related esters).

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