Appendix II

ACTION REQUIRED AS A RESULT OF CHANGES IN THE ACCEPTABLE DAILY INTAKE (ADI) STATUS AND OTHER RECOMMEDATIONS ARISING FROM THE 92ND AND 95TH JECFA

(For information and action)

PART A: From 92ND JECFA Meeting

Table 1. Food additives evaluated toxicologically and/or considered for specifications at the 92ND JECFA meeting

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommendation of CCFA53
210 211 212 213	Benzoic acid, its salts and derivatives	The 92nd JECFA evaluated a new extended one-generation reproductive toxicity study on benzoic acid. This study showed no treatment-related adverse effects, indicating a NOAEL of 1000 mg/kg bw per day, the highest dose tested. Applying a chemical specific adjustment factor of 2 for interspecies toxicokinetics variation instead of the default factor of 4.0, **the 92nd JECFA established a group ADI of 0–20 mg/kg bw**, which applies to benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents. **The 92nd JECFA withdrew the previous group ADI of 0–5 mg/kg bw.** The 92nd JECFA noted that the high dietary exposure estimate, expressed as benzoic acid, of 7.1 mg/kg bw per day for children aged 3–9 years does not exceed the group ADI of 0–20 mg/kg bw.	Note the JECFA conclusion that the new data that have become available since the previous evaluation of benzoic acid, its salts and derivatives give reason to revise the ADI. Note that JECFA withdrew the previous group ADI of 0–5 mg/kg bw benzoic acid, its salts and derivatives and established a new group ADI of 0–20 mg/kg bw. The new group ADI applies to benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents. Note the new specifications for benzoic acid, its salts and derivatives (see CX/FA 23/53/4).
	Collagenase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*	Negative results were observed in genotoxicity studies with a powdered enzyme concentrate. The 92nd JECFA identified a NOAEL of 940 mg TOS/kg bw per day (rounded from 939.6), the highest dose tested in a 13-week study of oral toxicity in rats. The 92nd JECFA identified a NOAEL of 940 mg TOS/kg bw per day, the highest dose tested in a 13-week study of oral toxicity in rats. Comparison of this NOAEL with the estimated dietary exposure of 0.43 mg TOS/kg bw per day gave a margin of exposure (MOE) of > 2100. In view of this MOE and the lack of concern about genotoxicity, the **92nd JECFA established an ADI "*not***	Note that JECFA established an ADI "*not specified*" for collagenase from *S. violaceoruber*, when used in the applications specified and in accordance with good manufacturing practice. Note the new JECFA specifications for collagenase from *Streptomyces violaceoruber* expressed in *S. violaceoruber* (see CX/FA 23/53/4).

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommendation of CCFA53
		***specified*"[1] for collagenase from *S. violaceoruber*,** when used in the applications specified and in accordance with good manufacturing practice.	
	β-Glucanase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*	The 92nd JECFA noted negative results in studies of genotoxicity and in studies of oral toxicity in rats. The 92nd JECFA identified a NOAEL of 950 mg TOS/kg bw per day (rounded by the 92nd JECFA from 953.3), the highest dose tested. Comparison of this NOAEL with the estimated dietary exposure of 0.15 mg TOS/kg bw per day gave an MOE >6300. On the basis of this MOE and the lack of concern about genotoxicity, the 92nd **JECFA established an ADI "*not specified*"[3] for β-glucanase from *S. violaceoruber*,** for the proposed uses and in accordance with good manufacturing practice.	Note that JECFA established an ADI "*not specified*" for β-glucanase from *S. violaceoruber* for the proposed uses and in accordance with good manufacturing practice. Note the new JECFA specifications for β-Glucanase from *Streptomyces violaceoruber* expressed in *S. violaceoruber* (see CX/FA 23/53/4).
	Phospholipase A2 from *Streptomyces violaceoruber* expressed in *S. violaceoruber*	The 92nd JECFA noted negative results were obtained in genotoxicity tests. In a 13-week study of oral toxicity in rats, small effects were seen at low incidence at the high dose of 956 mg TOS/kg bw per day, which might have been related to treatment. The 92nd JECFA therefore identified a NOAEL of 190 mg TOS/kg per day (rounded by the 92nd JECFA from 191 mg TOS/kg bw per day). A comparison of the estimated dietary exposure of 0.25 mg TOS/kg bw per day with the NOAEL of 190 mg TOS/kg bw per day from the oral toxicity study gives a MOE of 760. On this basis and in the absence of concern about genotoxicity, the 92nd **JECFA established an ADI "*not specified*"[3] for the phospholipase A2 enzyme preparation from *S. violaceoruber*** when used in the applications specified and in accordance with good manufacturing practice.	Note that JECFA established an ADI "*not specified*" for the phospholipase A2 enzyme preparation from *S. violaceoruber* when used in the applications specified and in accordance with good manufacturing practice. Note the existing specifications for phospholipase A2 enzyme preparation from *Streptomyces violaceoruber expressed in S. violaceoruber* were revised (see CX/FA 23/53/4).
101(iv)	Riboflavin from *Ashbya gossypii*	The 92nd JECFA noted that riboflavin from *A. gossypii* has low acute toxicity and does not raise concern for genotoxicity. The NOAEL from a 90-day oral toxicity study in rats was 3000 mg/kg bw per day, the highest dose tested. Comparison of this NOAEL with	Note that JECFA established a group ADI "*not specified*" for riboflavin, riboflavin-5´-phosphate, riboflavin from *B. subtilis* and riboflavin from *A. gossypii*, expressed as riboflavin.

[1] The reader is referred to the Technical Report of the 87th JECFA meeting for clarification of the term "*ADI not specified*".

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommendation of CCFA53
		the estimated dietary exposure of 3.6 mg/kg bw per day, based on maximum reported use levels, resulted in an MOE > 800. **The 92nd JECFA established a group ADI "*not specified*" [3] for riboflavin, riboflavin- 5´-phosphate, riboflavin from *B. subtilis* and riboflavin from *A. gossypii*, expressed as riboflavin**. The 92nd JECFA withdrew the previous group ADI of 0–0.5 mg/kg bw. The 92nd JECFA noted that in view of information received implies that riboflavin is no longer produced synthetically for use as a food additive, the 92nd JECFA recommends that the CCFA reconsider the requirement for specifications for synthetically produced riboflavin. The 92nd JECFA noted that for future work that the previously established specifications for riboflavin and riboflavin from *B. subtilis*, JECFA proposes to: • Rename "riboflavin" as "riboflavin, synthetic"; • Replace the existing method for determination of lumiflavin in both specifications to avoid use of chloroform; and • Delete the functional use of "nutrient supplement" from the specifications monograph on riboflavin from B. subtilis, as the Codex food additive definition does not include nutrients.	Note that JECFA withdrew the previous group ADI of 0–0.5 mg/kg bw. Note the new JECFA specifications for Riboflavin from *Ashbya gossypii* (see CX/FA 23/53/4). Note that JECFA remarked that riboflavin is no longer produced synthetically for use as a food additive and recommends that the CCFA reconsider the requirement for specifications for Riboflavin, synthetic (INS 101(i)).
	Ribonuclease P from *Penicillium citrinum*	The 92nd JECFA identified a NOAEL of 980 mg TOS/kg bw per day (the highest dose tested) in a 13-week study in which rats were treated with ribonuclease P concentrate from *P. citrinum* AE-RP by gavage. A comparison of the estimated dietary exposure of 1.3 mg TOS/kg bw per day with the NOAEL of 980 mg TOS/kg bw per day gives an MOE > 750. On the basis of this MOE and the lack of concern for genotoxicity, **the 92nd JECFA established an ADI "*not specified*" [3] for the ribonuclease P enzyme preparation from *P. citrinum* AE-RP**, used in the applications specified and in accordance with good manufacturing practice. The 92nd JECFA noted that ribonuclease P can also be produced by	Note that JECFA established an ADI "*not specified*" for the ribonuclease P enzyme preparation from *P. citrinum* AE-RP, used in the applications specified and in accordance with good manufacturing practice. Note the new JECFA specifications for the ribonuclease P from *P. citrinum* AE-RP (see CX/FA 23/53/4).

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		P. citrinum RP-4, but insufficient information was available on the enzyme concentrate produced from this strain. To evaluate the safety of ribonuclease P from *P. citrinum* RP-4, toxicological studies with well-characterized enzyme concentrate are required.	

PART B: From 95th JECFA Meeting

Table 1. Food additives evaluated toxicologically and/or considered for specifications at the 95th JECFA meeting

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommended action by CCFA
	α-Amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*	The 95th JECFA concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity. The 95th JECFA 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. Based on this MOE and the lack of concern for genotoxicity, **the 95th JECFA 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. The 95th JECFA 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.	Note that JECFA established a temporary ADI "*not specified*" for α-amylase 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 was made temporary because of the tentative nature of the specifications. Note the new tentative specifications for α-Amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis* (see CX/FA 23/53/4). Note the JECFA request for technical information by the end of 2023, to complete the safety assessment.

[2] The reader is referred to the Technical Report of the 87th JECFA meeting for clarification of the term "*ADI not specified*".

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommended action by CCFA
	α-Amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*	The 95th JECFA concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity. The 95th JECFA identified a NOAEL of 660mg 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 0.08mg TOS/kg bw per day, a MOE of more than 8000 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the 95th JECFA 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. The 95th JECFA 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.	Note that JECFA 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. This ADI was made temporary because of the tentative nature of the specifications. Note the new tentative specifications for **α-Amylase from *Geobacillus stearothermophilus* expressed in *Bacillus licheniformis*** (see CX/FA 23/53/4). Note the JECFA request for technical information by the end of 2023, to complete the safety assessment.
	α-Amylase from *Rhizomucor pusillus* expressed in *Aspergillus niger*	The 95th JECFA concluded that dietary exposure to this α-amylase is not anticipated to pose a risk for allergenicity. The 95th JECFA 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 95th JECFA established a temporary ADI "*not specified*"[4] 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. The 95th JECFA requested the following information, by the end of 2023, to complete the safety assessment:	Note that JECFA 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. This ADI was made temporary because of the tentative nature of the specifications. Note the new tentative specifications for α-Amylase from *Rhizomucor pusillus* expressed in *Aspergillus niger* (see CX/FA 23/53/4).

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommended action by CCFA
		• 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.	Note the JECFA request for technical information by the end of 2023, to complete the safety assessment.
	Amyloglucosidase from *Rasamsonia emersonii* expressed in *Aspergillus niger*	The 95th JECFA 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 95th JECFA could not complete the assessment of the risk for allergenicity from dietary exposure to this enzyme. The 95th JECFA 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 **95th JECFA established a temporary ADI "*not specified*" [4] 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. The 95th JECFA 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.	Note that JECFA 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. Note the new tentative specifications for *R* Amyloglucosidase from *Rasamsonia emersonii* expressed in *Aspergillus niger* (see CX/FA 23/53/4). Note the JECFA request for technical information by the end of 2023, to complete the safety assessment.

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommended action by CCFA
	Asparaginase from *Pyrococcus furiosus* expressed in *Bacillus subtilis*	The 95th JECFA concluded that dietary exposure to the enzyme preparation is not anticipated to pose a risk for allergenicity. The 95th JECFA 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. Based on this MOE and the lack of concern for genotoxicity, **the 95th JECFA established a temporary ADI "*not specified*" [4] 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. The 95th JECFA 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 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.	Note that JECFA 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. This ADI was made temporary because of the tentative nature of the specifications. Note the new tentative specifications for Asparaginase from *Pyrococcus furiosus* expressed in *Bacillus subtilis* (see CX/FA 23/53/4). Note the JECFA request for technical information by the end of 2023, to complete the safety assessment.
	β-Amylase from *Bacillus flexus* expressed in *Bacillus licheniformis*	The 95th JECFA concluded that dietary exposure to the enzyme preparation is not anticipated to pose a risk for allergenicity. The 95th JECFA 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 1mg 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 95th JECFA established a temporary ADI "*not specified*" [4] 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.	Note that JECFA established a temporary ADI "*not specified*" for β-amylase 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. This ADI was made temporary because of the tentative nature of the specifications. Note the new tentative specifications for β-amylase from *B. flexus* expressed in *B. licheniformis* (see CX/FA 23/53/4). Note the JECFA request for technical information by the end of 2023, to

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommended action by CCFA
			complete the safety assessment.
	Lipase from *Thermomyces lanuginosus* and *Fusarium oxysporum* expressed in *Aspergillus oryzae*	The 95th JECFA concluded that dietary exposure to this lipase is not anticipated to pose a risk for allergenicity. The 95th JECFA 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 **95th JECFA established an ADI "*not specified*" [4] 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.	Note that JECFA established an ADI "*not specified*" for lipase 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. Note the new specifications for Lipase from *Thermomyces lanuginosus* and *Fusarium oxysporum* expressed in *Aspergillus oryzae* (see CX/FA 23/53/4).
	Phospholipase A2 (PLA2) from porcine pancreas expressed in *Aspergillus niger*	**Because of the late submission of highly relevant toxicological data, other missing information and time constraints, the 95th JECFA was unable to complete this evaluation.** The 95th JECFA recommended that the evaluation of this enzyme preparation is completed at a future meeting. **The 95th JECFA 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:** • 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 commercially available products.	Note that JECFA was unable to complete the evaluation due to late submission of relevant data. Note the JECFA request for the JECFA Secretariat to urge the sponsor and Codex Members to ensure that the additional data requested by JECFA is available for evaluation prior to requesting inclusion of this enzyme preparation in the CCFA JECFA Priority List.

INS Number	Food additive	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information	Recommended action by CCFA
	Xylanase from *Bacillus licheniformis* expressed in *Bacillus licheniformis*	The 95th JECFA concluded that dietary exposure to this xylanase is not anticipated to pose a risk for allergenicity. The 95th JECFA identified a NOAEL of 1020mg 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 0.01mg TOS/kg bw per day, a MOE of more than 100000 can be calculated. Based on this MOE and the lack of concern for genotoxicity, the 95th JECFA 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. The 95th JECFA requested the following information, by the end of 2023, to complete the safety assessment: • validated method of analysis to determine xylanase 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.	Note that JECFA established 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 was made temporary because of the tentative nature of the specifications. This ADI was made temporary because of the tentative nature of the specifications. Note the new tentative specifications for Xylanase from *Bacillus licheniformis* expressed in *Bacillus licheniformis* (see CX/FA 23/53/4). Note the JECFA request for technical information by the end of 2023, to complete the safety assessment.

Table 2. Flavouring agents evaluated at the 95th JECFA meeting

The flavouring agents were evaluated by the revised Procedure for the Safety Evaluation of Flavouring Agents.

Alicyclic ketones, secondary alcohols and related esters

Flavouring agent[3]	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

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