WHO Technical Report Series

1007

Evaluation of certain food additives

Eighty-fourth 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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This report contains the collective views of an international groups of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization

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Rome, 6–15 June 2017

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List of abbreviations

ADI	acceptable daily intake
bw	body weight
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CCFA49	Forty-ninth Session of the Codex Committee on Food Additives
CIFOCOss	FAO/WHO Chronic individual food consumption database – Summary statistics
CITREM	citric and fatty acid esters of glycerol
CYP	cytochrome P450
CSAF	chemical-specific adjustment factor
EFSA	European Food Safety Authority
F_0	parental generation
F_1	first filial generation
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GLP	good laboratory practice
GSFA	(Codex) General Standard for Food Additives
HPLC	high-performance liquid chromatography
HPLC–UV	high-performance liquid chromatography with ultraviolet detection
IC_{50}	half maximal inhibitory concentration
INS	International Numbering System for Food Additives
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
NOAEL	no-observed-adverse-effect level
no./No.	number
OECD	Organisation for Economic Co-operation and Development
Panx1	pannexin 1
UV	ultraviolet
USA	United States of America
USFDA	United States Food and Drug Administration
WHO	World Health Organization

Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Evaluation of certain food additives. WHO Food Additives Series, No. 75, 2018.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 20, 2018.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome from 6 to 15 June 2017. The meeting was opened on behalf of Director-General Graciano da Silva of the Food and Agriculture Organization of the United Nations (FAO) by Dr Ren Wang, Assistant Director-General, FAO Agriculture and Consumer Protection Department.

Dr Wang preceded his opening remarks by welcoming Dr Yongxiang Fan, Vice-Chairperson of the Codex Committee on Food Additives, and all other meeting participants. Summarizing the mandate of the Codex Alimentarius Commission as simultaneously protecting the health of consumers, ensuring fair practices in the food trade and promoting coordination of all food standards work undertaken by international governmental and nongovernmental organizations, Dr Wang reminded the meeting that Codex standards are supported by scientifically sound, globally relevant yet independent safety assessments provided by experts and specialists in a wide range of disciplines. Noting that the FAO and WHO advisory bodies provide a focal point for food-related safety assessments through a number of joint expert committees for a large variety of food safety topics including food additives – the topic of this JECFA meeting – Dr Wang emphasized that participants had been invited not as representatives of their employer or country, but to provide sound and independent scientific advice to generate food standards designed to be health-protective for all consumers and trade-inclusive for all regions and countries.

Dr Wang pointed out that several people at the meeting had also been present in 2016, for JECFA's 60th anniversary, as well as in preceding years, thus contributing their continued guidance and providing the necessary stability. Dr Wang welcomed them as well as participants present at JECFA for their first time. He emphasized that, as the sciences evolve, JECFA needs everyone to add to the pool of knowledge, helping to update processes, procedures and approaches to incorporate new scientific insights and consider more data and more diverse studies and inputs in JECFA deliberations.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the eighty-fourth meeting had completed declaration of interest forms. No conflicts of interest were identified.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 *(1)*, there have been 83 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the eighty-second meeting (Annex 1, reference *230*). The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives (section 2);
- review and prepare specifications for certain food additives (sections 3 and 4 and Annex 2); and
- undertake safety evaluations of certain food additives (sections 3 and 4 and Annex 2).

2.1 Report from the Forty-ninth Session of the Codex Committee on Food Additives (CCFA)

Dr Yongxiang Fan, Vice-Chair of CCFA, provided the Committee with an update on the work of CCFA since the eighty-second meeting of JECFA (Annex 1, reference *230*).

The Forty-ninth Session of the CCFA (CCFA49) noted the conclusions of the eighty-second meeting of JECFA on the safety of 12 substances *(2)*. CCFA49 agreed to include lutein esters from *Tagetes erecta* (International Numbering System for Food Additives [INS] No. 161b(iii)) and octenyl succinic acid–modified gum arabic (INS No. 423) in Table 3 ("Additives Permitted for Use in Food in General, Unless Otherwise Specified, in Accordance with Good Manufacturing Practice") of the *Codex General Standard for Food Additives* (GSFA) (CODEX STAN 192–1995) (*3*). CCFA49 solicited members to provide more information or data to JECFA to complete the evaluation for carob bean gum (INS No. 410) and cassia gum (INS No. 427) and noted that no action was necessary for other substances.

CCFA49 finalized work on more than 400 provisions of the GSFA and forwarded specifications for the identity and purity of 15 food additives (one new specification and 14 revised specifications) and 29 flavourings (23 new specifications and six revised specifications) prepared by the eighty-second meeting of JECFA and recommended to the Fortieth Session of the Codex Alimentarius Commission for adoption. CCFA49 agreed to amend the introduction of the *List of Codex Specifications for Food Additives* (http://www.codexalimentarius.net/gsfaonline/foods/index.html) to address the concerns on the reference made to secondary additives in the specifications. In addition to adding functional classes and technological purposes to two food additives,

new INS numbers were also assigned to five food additives. CCFA49 agreed on a revised priority list of substances for evaluation (or re-evaluation) by JECFA, which includes 62 substances and 70 flavourings. CCFA49 also agreed to remove the asterisked (*) note indicating those substances high on the CCFA priority list, as the working group had not discussed this matter.

CCFA49 also established an electronic working group that will address the concerns for the use of nitrates and nitrites as food additives. The working group will analyse which issues CCFA can address and clarify the scope of the question(s) that JECFA or another appropriate FAO/WHO scientific advice body can address by taking into consideration the feasibility and data availability for such advice.

CCFA49 continued work on aligning food additive provisions in the Codex standards and the corresponding provisions of the GSFA. CCFA49 agreed that the Chairs of the working groups on GSFA, alignment, INS and JECFA priority, working with China (the host of CCFA), develop a discussion paper on future strategies for CCFA, to be considered at the Fiftieth Session of the CCFA. This paper will also consider aspects of prioritization of substances on the priority list that relate to JECFA evaluation.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, the Committee took into consideration the principles established and contained in the 2009 publication *Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food* (EHC 240)*(4)*.

2.2.1 Information requirements for submissions on products derived from natural sources

The Committee noted that, at the current meeting, a number of food additives were evaluated that were derived from natural sources. The Committee recalled that at previous meetings the need for sponsors to provide sufficient data for chemical, technical, dietary exposure and toxicological evaluation was stressed. At its thirty-first meeting, the Committee emphasized that "A full understanding of the source and chemical nature of such products was considered essential for an evaluation of their safety-in-use" (Annex 1, reference *77*). At the sixty-eighth meeting, the Committee provided considerations on "Extensions of an existing ADI to substances obtained from different sources and/or by different manufacturing processes" (Annex 1, reference *187*).

The Committee recognized that a component of interest (e.g. carotenes) may be present in the product of commerce at a low percentage relative to other components either because it is extracted together with components of similar polarity or solubility or because of subsequent standardization in the final product formulation. The Committee also recognized that some substances (e.g. gums or tannins) are complex mixtures and their components are affected to varying degrees, depending on their source or through processing. It is important to fully characterize all components of the final product, taking care to also provide the detailed manufacturing process as well as information on the carryover of substances from the starting material to the final product.

The present Committee again stressed that a full characterization of the products in commerce and a relevant set of biochemical and toxicological data on such products are essential for the Committee to develop a specifications monograph and the related safety assessment. It is not possible to complete the evaluation of a food additive if its composition cannot be compared to the substances tested biochemically and toxicologically. This is particularly important where the submission relies on literature data.

The Committee encourages CCFA to consider the above information requirements before accepting proposals for food additive evaluations to be included in the CCFA priority list.

2.2.2 Update on activities relevant to JECFA

The Committee was provided with an update of work in the WHO International Programme on Chemical Safety (IPCS). The WHO Chemical Risk Assessment Network and its activities were described, including the work on a review of how chemical-specific adjustment factors (CSAFs) are being used in regulatory and non-regulatory risk assessments.

The Secretariat informed the Committee about ongoing activities on risk assessment methodology and update of certain chapters of EHC 240: *Principles and methods for the risk assessment of chemicals in food (4)*. In particular, more detailed guidance on the interpretation and evaluation of genotoxicity studies will be developed; as well, the guidance on dose–response modelling and application of the benchmark dose approach will be updated. The chapter on exposure assessment will be updated, taking all recent developments into account. Further guidance will also be developed on the evaluation of enzyme preparations.

The Committee was also informed that the JECFA guidance for setting acute reference doses for veterinary drugs is now available online: http://www.who.int/foodsafety/chem/jecfa/Guidance-document-ARfD-2017.pdf?ua=1.

WHO recently published a distance learning tool on how to access and analyse the food contamination data submitted to the Global Environment Monitoring System – Food Contamination Monitoring and Assessment

Programme (GEMS/Food) database. This tool was developed in collaboration with the Chulabhorn Research Institute (Bangkok, Thailand), a WHO Collaborating Centre. A password-protected access to the learning tool is available upon request from: vergerp@who.int.

2.3 Food additive specifications and analytical methods

2.3.1 Corrigenda for specifications monographs

The following requests for corrections in JECFA Food Additives Specifications Monographs were received by the JECFA Secretariat (see Table 1). The Committee at the current meeting evaluated the information provided and made the following corrections. These corrections will be published in the electronic versions and in the online database of JECFA Food Additives Specifications Monographs. The information is provided here to make interested parties aware of these changes.

Table 1
Corrections in JECFA Food Additives Specifications Monographs

Food additive	Original text	New text	Additional explanations
Carob bean gum (clarified) (JECFA 82, FAO JECFA Monographs 19, 2016)	Heading: Carob bean gum	Heading: Carob bean gum (clarified)	In the original publication of FAO JECFA Monographs 19, the monograph heading omitted ("(clarified)"), while the specifications referred to the clarified carob bean gum
Carob bean gum (JECFA 82, FAO JECFA Monographs 19, 2016)	None Specifications have been prepared and adopted at JECFA 82 for carob bean gum but were not published in the FAO JECFA Monographs 19.	Please refer to http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/detail/en/c/484/	
CITREM (JECFA 82, FAO JECFA Monographs 19, 2016)	Lead (Vol. 4) Not more than 2 mg/kg. (Not more than 0.1 mg/kg for use in infant formula and formula for special medical purposes intended for infants).	Lead (Vol. 4) Not more than 2 mg/kg. (Not more than **0.5** mg/kg for use in infant formula and formula for special medical purposes intended for infants).	Transcription error
Diammonium hydrogen phosphate (JECFA 59, FAO JECFA Monographs 1, 2006)	CAS 7783-54-0	CAS 7783-28-0	
Dimethyl dicarbonate (JECFA 63, FAO JECFA Monographs 1, 2006)	CAS 004-525-33-1	CAS 4525-33-1	
Ferrous sulfate (JECFA 53, FAO JECFA Monographs 1, 2006)	CAS 7720-78-7	CAS 7782-63-0	
Ferrous sulfate, dried (JECFA 53, FAO JECFA Monographs 1, 2006)	No CAS number	CAS 7720-78-7	
Paprika extract (JECFA 79, FAO JECFA Monographs 16, 2014)	Preamble: An ADI of 0–1.5 mg/kg bw was allocated at the 79th JECFA (2014).	Preamble: An ADI of 0–1.5 mg/kg bw **(expressed as total carotenoids)** was allocated at the 79th JECFA (2014).	
Paprika oleoresin (JECFA 59, FAO JECFA Monographs 1, 2006)	INS160c	INS160c(i)	
L-Malic acid (flavouring)	Optical rotation: −0.23 (25 °C)	Optical rotation: −2.3 (8.5 g/100 mL water at 20 °C)	The magnitude and direction of the optical rotation are dependent on solvent, temperature and concentration of L-malic acid.

ADI: acceptable daily intake; bw: body weight; CAS: Chemical Abstracts Service; CITREM: citric and fatty acid esters of glycerol; FAO: Food and Agriculture Organization of the United Nations; JECFA: Joint FAO/WHO Expert Committee on Food Additives
Bolding for clarity only.

3. Specific food additives

The Committee evaluated the safety of nine food additives and revised the specifications for five other food additives. Information on the safety evaluations and specifications is summarized in Annex 2.

3.1 Safety evaluations[1]

3.1.1 Brilliant Blue FCF

Explanation

Brilliant Blue FCF (Chemical Abstracts Service [CAS] No. 3844-45-9; INS No. 133) is a dye with a triphenylmethane base structure permitted as a food colour in the European Union, Japan, the United States of America (USA) and other regions. It is used for colouring breakfast cereals, cakes and cupcakes, candies, chewing gum, dairy products, decorations for baking, flavoured water and frozen treats.

The Committee previously evaluated the use of Brilliant Blue FCF as a food colour at the thirteenth meeting in 1969 (Annex 1, reference *19*). The specifications for Brilliant Blue FCF were prepared at the twenty-eighth JECFA meeting in 1984 and revised for metal specifications at the fifty-ninth meeting in 2002 (Annex 1, references *66* and *160*). An acceptable daily intake (ADI) of 0–12.5 mg/kg body weight (bw) was established by the Committee in 1969 (Annex 1, reference *19*). The ADI was based on a no-observed-adverse-effect level (NOAEL) of 5% (equivalent to 2500 mg/kg bw per day) derived from a chronic dietary toxicity study in rats (Hansen et al., 1966), with no explanation for the 200-fold uncertainty factor. More recent studies, including studies on absorption and excretion, biochemical effects, long- and short-term toxicity, carcinogenicity, genotoxicity, reproductive and developmental toxicity and allergenicity as well as studies on neurobehavioural effects and interaction with the membrane protein pannexin 1 (Panx1), have since become available.

Brilliant Blue FCF has been evaluated by the present Committee at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016). Almost all of the new data were provided by the sponsor. Only a few additional publications were identified in a literature search. The pre-1969 studies described below were considered by the Committee at the thirteenth meeting in 1969 (Annex 1, reference *19*).

Chemical and technical considerations

Brilliant Blue FCF consists mainly of disodium 3-[*N*-ethyl-*N*-[4-[[4-[*N*-ethyl-*N*-(3-sulfobenzyl)amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadiene-

[1] Numbered references cited in the subsections of section 3.1 are provided at the end of each subsection.

1-ylidene]ammoniomethyl]benzenesulfonate and its isomers, together with subsidiary colouring matters, as well as sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by condensing 2-formylbenzenesulfonic acid with a mixture of 3-[(*N*-ethyl-*N*-phenylamino) methyl]benzenesulfonic acid and its 2- and 4-isomers to form the leuco base precursor. Oxidation of the leuco base precursor with either chromium- or manganese-containing compounds produces the dye, which is isolated as the disodium salt. The dye contains not less than 85% total colouring matters. Impurities include unreacted starting material and reaction by-products (~2%), subsidiary colouring matters (≤6%), residual leuco base precursor (≤5%), unsulfonated primary aromatic amines (≤0.01% calculated as aniline), lead (≤2 mg/kg), chromium (≤50 mg/kg) and manganese (≤100 mg/kg).

Biochemical aspects

When Brilliant Blue FCF was administered orally to rats, almost the entire dose was excreted unchanged in the faeces within 40 hours. The colour was also found in the bile of rats, rabbits and dogs after oral administration. Only 5% of the dose administered was excreted in the bile of dogs (Hess & Fitzhugh, 1953, 1954, 1955). In other studies, absorption of Brilliant Blue FCF was about 0.5% in rats (Brown et al., 1980; Phillips et al., 1980), with more than 99% of total intake excreted in the faeces and less than 1% recovered in the urine. Results of thin-layer chromatography (TLC) of urine and bile samples 24 hours after ingestion showed that about 95% of excreted radioactivity was unaltered ^{14}C-radiolabelled Brilliant Blue FCF and that about 5% was unidentified metabolite(s) or degradation product(s). Mass spectrometric analysis was, however, not used.

An ex vivo porcine tongue system showed that about 0.2% of Brilliant Blue FCF diffused through the surface oral mucosa layers (Lucová et al., 2013).

Equilibrium dialysis methods have demonstrated that Brilliant Blue FCF binds to rat plasma protein (Iga, Awazu & Nogami, 1971; Iga et al., 1971). The extent of binding of Brilliant Blue FCF with plasma protein was 65% after 160 hours of dialysis at 37 °C.

In an in vitro study in which the Xenopus oocyte expression system was used for pharmacological investigations on purinergic P2 receptors that interact with the membrane channel protein Panx1 in inflammasome signalling, Brilliant Blue FCF was shown to be a selective inhibitor of Panx1 channels, with a half maximal inhibitory concentration (IC_{50}) of 0.27 µmol/L; no significant effect on the P2X7R receptor was observed at concentrations as high as 100 µmol/L (Wang, Jackson & Dahl, 2013). The Committee was aware that Panx1 activation/inhibition is one of several signalling pathways involved in various physiological processes at the cellular level (e.g. immune function) and that there are many exogenous and endogenous modulators of these pathways. Interactions of

substances with P2 receptors and Panx1 are an active area of research, particularly in development of drug treatments for diverse chronic diseases. The Committee noted that a similar pattern of channel inhibition was observed with Fast Green FCF, and further research may clarify if the inhibition of Panx1 observed in an in vitro system has any relevance for the safety assessment for substances in food.

Toxicological studies

The acute toxicity of Brilliant Blue FCF is low. The median lethal dose (LD_{50}) in mice (Sasaki et al., 2002) and rats (Lu & Lavallee, 1964) was higher than 2000 mg/kg bw.

In a 1-year dietary study, 12 dogs were fed Brilliant Blue FCF (purity not reported) at 0%, 1% or 2%. No clinical signs, gross lesions or microscopic pathological findings were attributed to exposure to Brilliant Blue FCF (Hansen et al., 1966).

The long-term toxicity of Brilliant Blue FCF was investigated in three studies in mice and five in rats.

No evidence of treatment-related carcinogenicity was found when male and female mice were fed Brilliant Blue FCF at a dose of 1 mg/kg bw per day over 500–700 days (Waterman & Lignac, 1958).

The administration of Brilliant Blue FCF to male and female mice for up to 80 weeks in the diet at concentrations of 0%, 0.015%, 0.15% or 1.5% (equivalent to 0, 20, 200 and 2000 mg/kg bw per day, respectively) resulted in slight reduction in weight gain and increased incidence of foam cells in the liver at the highest dose (Rowland et al., 1975). The NOAEL was 0.15% (equivalent to 200 mg/kg bw per day).

In a long-term toxicity study in which Brilliant Blue FCF was fed to male and female mice for 24 months (104 weeks) at dietary concentrations of 0%, 0.5%, 1.5% or 5% (equal to 0, 661, 2064 and 7354 mg/kg bw per day for males and 0, 819, 2562 and 8966 mg/kg bw per day for females, respectively), the NOAEL was 5% (equal to 7354 mg/kg bw per day), the highest concentration tested (IRDC, 1981a; Borzelleca, Depukat & Hallagan, 1990). The Committee noted that the survival at the end of the study was about 50% in both control and treated groups.

When Brilliant Blue FCF was fed to male and female rats at a dietary level of 4% for 600 days, there were no treatment-related tumours (Willheim & Ivy, 1953). In another long-term toxicity study in which rats were fed a diet containing 0.1% Brilliant Blue FCF over their lifetime (daily intake 10–15 mg), no treatment-related tumours were found (Klinke, 1955). Similarly, when male and female rats were fed diets containing 0%, 0.3% or 3% Brilliant Blue FCF for 75 weeks, no treatment-related adverse effects were observed on tumour incidence, growth or haematological findings (Mannell, Grice & Allmark, 1962).

In its previous evaluation at the thirteenth meeting (Annex 1, reference *19*), the Committee established an ADI of 0–12.5 mg/kg bw based on a 2-year toxicity study in which male and female rats were fed a diet containing 0%, 0.5%, 1.0%, 2.0% or 5.0% Brilliant Blue FCF. The NOAEL was 5.0% (equivalent to 2500 mg/kg bw per day), the highest concentration tested (Hansen et al., 1966).

In a long-term toxicity study that included an in utero exposure phase, Brilliant Blue FCF was fed to the F_0 rats for up to 17 weeks at levels of 0%, 0.1%, 1% or 2% (calculated to provide doses of 0, 50, 514 and 1073 mg/kg bw per day for males and 0, 62, 631 and 1318 mg/kg bw per day for females, respectively). The F_1 animals were administered Brilliant Blue FCF at the same dose levels for up to 116 weeks for males and 111 weeks for females. The NOAEL was 1% (equal to 631 mg/kg bw per day), based on 15% decreased mean terminal body weight and decreased survival of female rats at the highest dose level (IRDC, 1981b; Borzelleca, Depukat & Hallagan, 1990).

The Committee concluded from these studies in mice and rats that there is no concern with respect to carcinogenicity of Brilliant Blue FCF.

No mutagenic activity has been observed with Brilliant Blue FCF in several in vitro mutagenicity studies conducted in *Salmonella typhimurium*, *Bacillus subtilis* and *Escherichia coli*. Positive findings were reported in two in vitro chromosomal aberration assays, one in vitro micronucleus assay and one in vitro comet assay in mammalian cells, but these studies had a number of shortcomings (Kawachi et al., 1980; Ishidate et al., 1984; Kus & Eroglu, 2015; Pandir, 2016). In contrast, negative results were obtained in an in vivo micronucleus assay in bone marrow (Hayashi et al., 1988) and a comet assay in the stomach, colon, liver, kidney, bladder, lung, brain and bone marrow of mice (Sasaki et al., 2002). Based on the available data, the Committee concluded that there is no concern with respect to genotoxicity of Brilliant Blue FCF.

No treatment-related adverse reproductive effects were found in a single-generation study in male and female rats fed Brilliant Blue FCF at doses up to 1318 or 1073 mg/kg bw per day, respectively (IRDC, 1981b; Borzelleca, Depukat & Hallagan, 1990). Similarly, no treatment-related adverse effects were seen in a three-generation study in rats treated with Brilliant Blue FCF at doses up to 1000 mg/kg bw per day (BioDynamics Inc., 1971). In developmental toxicity studies, no adverse effects were reported in rats treated with Brilliant Blue FCF at doses up to 2000 mg/kg bw per day (BioDynamics Inc., 1972a) or in rabbits at doses up to 200 mg/kg bw per day (BioDynamics Inc., 1972b).

Other studies have reported no evidence for allergenicity (Kreindler, Slutsky & Haddad, 1980), skin irritation (BIBRA, 1990), dermal sensitization (BIBRA, 1990) or skin cancer (Carson, 1984) as a result of treatment with Brilliant Blue FCF.

In a one-generation study on neurobehavioural development in mice (Tanaka et al., 2012), Brilliant Blue FCF was given in the diet at concentrations of 0%, 0.08%, 0.24% or 0.72% (equal to 0, 111–407, 347–1287 and 1032–3856 mg/kg bw per day, respectively, exposure depending on gestational age). The high dose of Brilliant Blue FCF resulted in a few statistically significant effects on neurobehavioural development (exploratory behaviour and surface righting response). However, the Committee noted that the effects on exploratory behaviour were inconsistent and that there were no effects from exposure to Brilliant Blue at any dose in several other neurobehavioural tests in this study. The Committee concluded that the findings were not robust enough to be used in the safety assessment.

Observations in humans

Case reports describe the use of Brilliant Blue FCF in enteral feeding solutions associated with discoloration of skin, urine and serum and toxicity, including 12 deaths (WHO, 2003; Maloney & Brand, 2016). The Committee noted that these case reports relate to seriously ill patients, particularly those with increased gut permeability (e.g. patients with sepsis), and that a causal relationship with Brilliant Blue FCF has not been established.

Assessment of dietary exposure

Estimates of dietary exposure to Brilliant Blue FCF published by the European Food Safety Authority (EFSA) (EFSA, 2010), Food Standards Australia New Zealand (FSANZ) (FSANZ, 2012), the United States Food and Drug Administration (USFDA) (Doell et al., 2016), India (Dixit et al., 2011), Kuwait (Husain et al., 2006) and the Republic of Korea (Ha et al., 2013) were available to the Committee. The estimate of dietary exposure to Brilliant Blue FCF calculated by EFSA (4.8 mg/kg bw per day for children at the 95th percentile) was much higher than those of the USFDA and FSANZ (both 0.2 mg/kg bw per day for children at the 90th percentile) and the Republic of Korea (0.03 mg/kg bw per day for the whole population at the 95th percentile). Estimates from India and Kuwait were also lower than the EFSA estimates, but higher than the estimates from the USFDA and FSANZ. The Committee considered that the higher values in the EFSA estimates were due to the use of maximum reported use levels, whereas the other studies used mean analysed levels. The Committee concluded that the use of the more conservative EFSA estimate of 5 mg/kg bw per day should be considered in the safety assessment for Brilliant Blue FCF.

Evaluation

The Committee concluded that the available data support the revision of the ADI for Brilliant Blue FCF and that the study on long-term toxicity in rats should be

considered as the pivotal study (IRDC, 1981b; Borzelleca, Depukat & Hallagan, 1990). In this study, a NOAEL of 631 mg/kg bw per day was identified, based on a 15% decrease in mean terminal body weight and decreased survival of females at 1318 mg/kg bw per day. The Committee established an ADI of 0–6 mg/kg bw based on this NOAEL by applying an uncertainty factor of 100 for interspecies and intraspecies differences.

The Committee noted that the conservative dietary exposure estimate of 5 mg/kg bw per day (95th percentile for children) is less than the upper limit of the ADI of 0–6 mg/kg bw established for Brilliant Blue FCF and concluded that dietary exposure to Brilliant Blue FCF for children and all other age groups does not present a health concern.

The previous ADI of 0–12.5 mg/kg bw was withdrawn.

A toxicological and dietary exposure monograph was prepared.

At the present meeting, the existing specifications for Brilliant Blue FCF were revised, and a maximum limit for manganese was added. High-performance liquid chromatography (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water or aqueous ammonium acetate.

The specifications were revised, and a Chemical and Technical Assessment was prepared.

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3.1.2 β-Carotene-rich extract from *Dunaliella salina*

Explanation

β-Carotene-rich extract from *Dunaliella salina* is a natural orange food colour. It is used as a colour in a wide range of food and beverages, including cider, malt beverages, water-based flavoured drinks, margarines, cheeses, cake fillings, custards, yogurts, processed nuts, precooked pastas and noodles and other products. Intended use levels of the product range from 20 mg/kg to 1200 mg/kg, depending on the food item or category.

Carotenes from natural sources (including carotenes from *D. salina*) were reviewed at the thirty-first, thirty-fifth and forty-first meetings of the Committee (Annex 1, references *77*, *88* and *107*). At the thirty-first meeting, the Committee concluded that the group ADI of 0–5 mg/kg bw established for the sum of the synthetic carotenoids β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters by the eighteenth Committee was not applicable to natural carotenes as they did not comply with the specifications for β-carotene. At the thirty-fifth and forty-first meetings, the Committee considered the available data inadequate to establish an ADI for the dehydrated algal carotene preparations or for the vegetable oil extract of *D. salina*. At the fifty-seventh meeting, the group ADI for synthetic β-carotene was extended to include β-carotene from Blakeslea trispora (Annex 1, reference *154*).

The Committee was asked by the Forty-eighth Session of the CCFA (FAO/WHO, 2016) to evaluate carotenes from *D. salina*. New short-term animal studies as well as studies on genotoxicity and developmental toxicity were submitted.

A comprehensive literature search was conducted on carotenes from *D. salina*. The Committee also considered a limited number of publications on β-carotene from other sources that became available since the previous evaluation. In light of the information submitted, the Committee limited the assessment to a vegetable oil preparation of a β-carotene-rich d-limonene extract of *D. salina*, hereafter referred to as *D. salina* d-limonene extract.

Chemical and technical considerations

β-Carotene-rich d-limonene extract of *D. salina* is produced from *D. salina*, an extreme halotolerant alga that inhabits natural and human-made salt lakes and ponds. The carotene-rich alga is harvested and concentrated, and the carotenoids are extracted using an essential oil rich in d-limonene. The resulting extract is saponified, purified, centrifuged, evaporated and finally mixed with a vegetable oil to obtain a commercial product with a carotene content of about 30% by weight. β-Carotene accounts for more than 95% of the carotene content of the extracted material as a mixture of *trans* and *cis* isomers in a ratio of approximately 2:1 by weight. The remainder of the carotene content includes α-carotene, lutein, zeaxanthin and cryptoxanthin. In addition to the colour pigments and vegetable oil used for standardization, d-limonene extracts of *D. salina* contain lipids and other fat-soluble components naturally occurring in the source material, such as fatty acids, long-chain alcohols, alkenes and waxes. The composition of these fat-soluble components is primarily a mixture of fatty acids common to vegetable oils used in foods.

Carotenoids are naturally occurring pigments that are responsible for the bright colours of various fruits and vegetables, including citrus fruits, carrots and tomatoes. β-Carotene, a provitamin A, is the most common of these carotenoids, consisting of an unsaturated chain containing identical substituted ring structures at each end.

Biochemical aspects

β-Carotene is absorbed and detected in human serum and liver when consumed as an extract from *D. salina* or as synthetic β-carotene (Redlich et al., 1996). Peak levels of β-carotene in human serum occur between 24 and 48 hours after ingestion (Stahl, Schwarz & Sies, 1993). Absorption appears to be linear when doses up to 30 mg are ingested, but the degree of absorption decreases at higher concentrations (Woutersen et al., 1999). Absorption of β-carotene varies between 10% and 90% in humans and is dependent on various conditions, such as the food matrix and nutritional status of the individual (Wang et al., 1993; von Laar et al., 1996; Woutersen et al., 1999). In humans, the major storage sites for carotenoids are the liver and adipose tissue, and hepatic and adipose tissue levels tend to correlate with serum levels (Gaziano et al., 1995; Redlich et al., 1996). In

human serum, most of the β-carotene is present as the all-*trans* isomer, in spite of significant intake of the 9-*cis* isomer (Stahl, Schwarz & Sies, 1993; Rock, 1997; Woutersen et al., 1999).

In contrast to humans, mice, rats, hamsters and rabbits have very low levels of serum and tissue β-carotene due to the very high activity of intestinal β-carotene-15,15′-dioxygenase that efficiently converts β-carotene to retinal (During, Albaugh & Smith, 1998; During et al., 2001; Woutersen et al., 1999). On this basis, the Committee concluded that these species are not suitable models for the evaluation of β-carotene in humans.

The toxicokinetics of β-carotene in ferrets and preruminant calves have been shown to be similar to the absorption of β-carotene in humans. Ferrets that consumed 18 μmol/L β-carotene as a suspension in water for 16 days after a β-carotene elimination period of 10 days were shown to accumulate β-carotene in serum, liver and adrenal tissue (White et al., 1993). β-Carotene was also significantly increased in liver, spleen, lung and serum of preruminant calves fed a single oral dose of 20 mg. Serum levels were still elevated 264 hours post dosing (Poor et al., 1992).

Toxicological studies

At the present meeting, the Committee evaluated two new 90-day studies in rats, in vitro and in vivo genetic toxicity assays and a developmental toxicity study in rats conducted using a *D. salina* d-limonene extract. The Committee deemed these studies useful for evaluating the toxicity of the non-β-carotene portion of the extract.

In a 90-day study submitted to the Committee for this evaluation, rats were treated by gavage with *D. salina* d-limonene extract (containing 31% carotenes) at doses of 0, 318, 954 or 3180 mg/kg bw per day (calculated from doses of 0, 100, 300 and 1000 mg/kg bw per day carotenes using a correction factor of 3.18). Superficial erosion of the mucosa with infiltration of neutrophilic granulocytes and haemorrhages in the fundus region of the stomach were observed in one female and five males in the high-dose group (Leuschner, 2006a). The Committee concluded that the findings in the fundus were most likely due to a local effect of the high concentration of the test material given as a bolus, and identified the NOAEL to be 3180 mg/kg bw per day of *D. salina* d-limonene extract, the highest dose tested.

In another 90-day study, rats were fed diets containing 0%, 0.63%, 1.25%, 2.5% or 5% of an oil extract of carotenes from Dunaliella alga (species not specified). The Committee noted that although the test substance was not specified, based upon the reported percentage of β-carotene (31.4%) and the description of the material, this is likely a *D. salina* d-limonene extract. The average doses of the Dunaliella carotene extract were reported as 0, 352, 696,

1420 and 2750 mg/kg bw per day for males and 0, 370, 748, 1444 and 2879 mg/kg bw per day for females (Kuroiwa et al., 2006). Although the authors identified a NOAEL of 1.25% based on a 6% reduction of body weight gain in males at 2.5% and 5%, the Committee considered this not to be a toxicologically relevant effect. The Committee concluded that the NOAEL was 5% (2750 mg/kg bw per day) of Dunaliella extract, the highest concentration tested.

No long-term toxicity and carcinogenicity studies were available for *D. salina* d-limonene extract.

The *D. salina* d-limonene extract tested negative in genotoxicity assays, including the bacterial reverse mutation assay in five strains of *S. typhimurium*, the forward gene mutation assay in cultured mammalian cells ($TK^{+/-}$ L5178Y) with and without metabolic activation and an in vivo mouse bone marrow micronucleus test. No concerns for genotoxicity were identified (Leuschner, 2006b,c; Stien, 2006).

No reproductive toxicity studies were available for the *D. salina* d-limonene extract.

D. salina d-limonene extract (carotene content 31%) was administered to pregnant rats from gestation days 6 to 19 by oral gavage at doses of 0, 318, 954 or 3180 mg/kg bw per day of the extract (calculated from doses of 0, 100, 300 and 1000 mg/kg bw per day carotenes using a correction factor of 3.18). No maternal or developmental toxicity was observed (Leuschner, 2007).

Observations in humans

No studies were available on the *D. salina* d-limonene extract.

The Committee noted two independent trials of heavy smokers (at least 1 package/day for 36 years on average) who received β-carotene supplements. In the first study, participants received β-carotene (20 mg/day) supplementation, with or without α-tocopherol supplementation (Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group, 1994). In the second study, participants received β-carotene (30 mg/day) + retinol (25 000 International Units of vitamin A) (Omenn et al., 1996a,b). Both studies showed increased, rather than the hypothesized decreased, incidence of lung cancer. A subsequent systematic review of nine randomized clinical trials showed no increase in the incidence of lung cancer in heavy smokers at supplemental doses of β-carotene varying from 6 to 15 mg/day for about 5–7 years (Druesne-Pecollo et al., 2010).

Assessment of dietary exposure

The Committee considered dietary exposure to β-carotene from *D. salina* d-limonene extract assuming its uses as a food additive in the same food categories and at the same maximum use levels (β-carotene basis) as previously evaluated β-carotene additives. The Committee concluded that dietary exposure

to β-carotene would not change, as the extract will provide β-carotene at a level equivalent to that from other β-carotene food additives.

The Committee therefore reviewed dietary exposures to β-carotene reported in the literature. Estimates of dietary exposure from the following regions/countries were included in this review: Australia (Hodge et al., 2009), China (Chen et al., 2015; Wang et al., 2014), the Czech Republic (Stepaniak et al., 2016), "Europe" (Elmadfa, 2009; referenced in EFSA, 2012), France (Lassale et al., 2016), Italy (Sette et al., 2010), Japan (Yabuta et al., 2017), Republic of Korea (Kim et al., 2016), Poland (Kopeć et al., 2013; Stepaniak et al., 2016), Russian Federation (Stepaniak et al., 2016) and Spain (Beltrán-de-Miquel et al., 2015). As chemical analyses of β-carotene in food cannot distinguish β-carotene added to food from that occurring naturally, these dietary exposure estimates reflect total dietary exposure to β-carotene.

Overall, mean or median dietary exposures to β-carotene ranged from 1.4 to 11 mg/day in adults. For children, data from Europe showed a maximum mean exposure of 7.3 mg/day; globally, few data were available for children's dietary exposures. For high percentile consumers of foods containing β-carotene, dietary exposures were as high as 13.7 mg/day (adults in Europe).

The Committee concluded that a high daily dietary exposure to β-carotene of 15 mg (0.25 mg/kg bw for a 60 kg individual) is appropriate for use in safety assessment. Using this dietary exposure estimate and the assumptions that all the β-carotene in the diet comes from this extract and that the extract contains 30% β-carotene, 35% algal lipids (upper level of a range of 20–35%) and 0.3% d-limonene (maximum amount), dietary exposure to the other toxicologically relevant constituents of this extract would be 18 mg/day (0.3 mg/kg bw per day for a 60 kg individual) for algal lipids and 0.2 mg/day (0.003 mg/kg bw per day for a 60 kg individual) for d-limonene.

Evaluation

The Committee noted that the total dietary exposure to β-carotene is not expected to increase when *D. salina* d-limonene extract is used as a food colour.

The Committee has also considered the basis for the ADI established for the group of carotenoids by the Committee at the eighteenth meeting. The group ADI (0–5 mg/kg bw) was derived using a four-generation study in rats with a NOAEL for β-carotene of 50 mg/kg bw per day with application of a safety factor of 10 because of the natural occurrence of carotenoids in the human diet and the low toxicity observed in animal studies. This ADI applies to the use of β-carotene as a colouring agent and not to its use as a food supplement.

Data that have become available since the previous evaluation show large differences in absorption of β-carotene between rodent species and humans. Specific β-carotene-15,15′-dioxygenase activity with β-carotene as substrate

in the intestine of rodents is nearly 1 million-fold higher than that of humans. The Committee considered that rodents are inappropriate animal models for establishing an ADI for β-carotene because of the virtual absence of systemic absorption in rodents.

The Committee noted that the toxicity of the other components of the *D. salina* d-limonene extract can be evaluated using the results of rodent studies. The *D. salina* d-limonene extract used in the toxicological studies contained β-carotene at approximately 30%, algal lipids at 20–35% and diluent vegetable oil at 35–50%. The *D. salina* d-limonene extract did not show genotoxicity in the evaluated studies. Short-term toxicity studies in rats give a NOAEL equal to 3180 mg/kg bw per day, the highest dose tested. No effects were observed in a developmental toxicity study in rats. No long-term toxicity or reproductive studies have been conducted with the *D. salina* d-limonene extract. Correction of the dose used to derive the NOAEL for *D. salina* d-limonene extract of 3180 mg/kg bw per day for the percentage of the algal component (20–35%) gives an adjusted NOAEL of 636–1113 mg/kg bw per day for the algal lipid component of the test substance. The margin of exposure for the algal lipid component in the *D. salina* d-limonene extract is 2120–3710 using a dietary exposure of 18 mg/day (0.3 mg/kg bw per day). The Committee concluded that dietary exposure to the algal component of the extract does not pose a health concern.

The Committee concluded that there was no health concern for the use of β-carotene-rich extract from *D. salina* when used as a food colour and in accordance with the specifications established at this meeting. This conclusion was reached because total dietary exposure to β-carotene will not increase and there are no toxicity concerns for the non-carotene components of the extract. The Committee emphasized that this conclusion applies to the use of this extract as a food colour, not as a food supplement.

A toxicological and dietary exposure monograph was prepared.

A specifications monograph and a Chemical and Technical Assessment were prepared.

Recommendations

The Committee recommends that the group ADI for the sum of carotenoids, including β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters, be re-evaluated in light of evidence that shows very low absorption of β-carotene in rodents and rabbits in contrast to humans.

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3.1.3 Fast Green FCF

Explanation

Fast Green FCF (CAS No. 2353-45-9; INS No. 143) is a dye with a synthetic triphenylmethane base structure permitted as a food colour in Japan, the USA and other regions. It is used for colouring breakfast cereals, cakes and cupcakes, drink mixers and frozen treats.

The Committee previously evaluated Fast Green FCF at its thirteenth, twenty-fifth, twenty-ninth and thirtieth meetings (Annex 1, references *19*, *56*, *70* and *73*). At the thirteenth meeting, the Committee established an ADI of 0–12.5 mg/kg bw based on a long-term feeding study in rats. At the twenty-fifth meeting, the ADI of 0–12.5 mg/kg bw was made temporary pending the results of adequate long-term feeding studies and a multigeneration reproduction/developmental study. At the twenty-ninth meeting, two long-term toxicity and carcinogenicity studies and a three-generation reproductive study were available for review. It was noted that a mouse oral carcinogenicity study was negative, but that an increased incidence of urothelial hyperplasia and/or neoplasia of the bladder was observed at the highest dose in the rat study. The biological significance of observed differences in benign and malignant tumours at other sites was considered questionable since, apart from the bladder, complete histopathological examinations were not performed on the low- and intermediate-dose animals. The temporary ADI was extended to permit complete histopathological examination of all groups of rats and biometric examination of the data. At the thirtieth meeting, the Committee reviewed histopathological data from the rat oral carcinogenicity study and concluded that Fast Green FCF was noncarcinogenic in rats and established an ADI of 0–25 mg/kg bw, based on a long-term study of toxicity in rats.

At the present meeting, the Committee re-evaluated this food colour at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016).

A toxicological dossier was submitted that included new studies on genotoxicity and neurological effects, and a search of the literature was

conducted that yielded one additional study relevant for the present evaluation. This Committee also considered studies evaluated at previous meetings of the Committee (before 1986).

Chemical and technical considerations

Fast Green FCF consists mainly of disodium 3-[*N*-ethyl-*N*-[4-[[4-[*N*-ethyl-*N*-(3-sulfobenzyl)amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]ammoniomethyl]-benzenesulfonate and its isomers, together with subsidiary colouring matters, as well as sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by condensing 2-formylhydroxybenzenesulfonic acid with a mixture of 3-[(*N*-ethyl-*N*-phenylamino)methyl]benzenesulfonic acid and its 2- and 4-isomers to form the leuco base precursor. Oxidation of the leuco base precursor with either chromium- or manganese-containing compounds produces the dye, which is isolated as the disodium salt. The dye contains not less than 85% total colouring matters. Impurities include unreacted starting material and reaction by-products (approximately 2%), subsidiary colouring matters (≤6%), residual leuco base precursor (≤5%), unsulfonated primary aromatic amines (≤0.01% calculated as aniline), lead (≤2 mg/kg), chromium (≤50 mg/kg) and manganese (≤100 mg/kg).

Biochemical aspects

Absorption of orally administered Fast Green FCF was shown to be less than 5%; almost all the administered colour was excreted unchanged in the faeces of the rats (Hess & Fitzhugh, 1953, 1954, 1955).

In an in vitro study, in which the *Xenopus* oocyte expression system was used for pharmacological investigations on purinergic P2 receptors that interact with the membrane channel protein pannexin 1 (Panx1) in inflammasome signalling, Fast Green FCF was shown to be a selective inhibitor of pannexin 1 (Panx1) channels, with an IC_{50} of 0.27 μmol/L, and did not significantly inhibit the P2X7R receptor (Wang, Jackson & Dahl, 2013). The Committee was aware that Panx1 activation/inhibition is one of several signalling pathways involved in various physiological processes at the cellular level (e.g. immune function) and that there are many exogenous and endogenous modulators of these pathways. Interactions of substances with P2 receptors and Panx1 are an active area of research, particularly in development of drug treatments for diverse chronic diseases. The Committee noted that a similar pattern of channel inhibition was observed with Brilliant Blue FCF, and further research may clarify if the inhibition of Panx1 observed in an in vitro system has any relevance for the safety assessment for substances in food.

Toxicological studies

Fast Green FCF has low oral acute toxicity in rats (Lu & Lavallee, 1964) and dogs (Radomski & Deichman, 1956).

A short-term study of toxicity revealed no compound-related effects in dogs fed Fast Green FCF at 0%, 1.0% or 2.0% of the diet (equal to 0, 269 and 695 mg/kg bw per day, respectively) for 2 years (Hansen et al., 1966).

Two previously reviewed long-term studies of oral toxicity showed no compound-related effects in mice and rats. The NOAEL was 2% Fast Green FCF in the diet (equivalent to 3000 mg/kg bw per day) in mice and 5.0% (equivalent to 2500 mg/kg bw per day) in rats (Hansen et al., 1966).

No treatment-related increase in tumour incidence was found in a mouse carcinogenicity study (Hogan & Knezevich, 1981). At the twenty-ninth meeting, the Committee concluded that the NOAEL was 5% Fast Green FCF in the diet, the highest dose tested. The present Committee noted that the mean body weights of females in the 5% dose group were consistently lower than those of controls after the commencement of the study (−10% compared with relevant controls at termination of the study). The Committee considered this decrease in body weights to be a treatment-related adverse effect and concluded that the NOAEL was 1.5% Fast Green FCF (equal to 3392 mg/kg bw per day), based on the lower body weights observed at 5% (equal to 11 805 mg/kg bw per day) in females.

A carcinogenicity study in rats reported an increased incidence of urothelial hyperplasia and/or neoplasia of the bladder (Knezevich & Hogan, 1981). However, a peer review of the histopathological data showed that Fast Green FCF is noncarcinogenic in this species (Dua, Chowdury & Moch, 1982; O'Donnell, 1982; USFDA, 1982a,b). The previous Committee agreed with this conclusion at its thirtieth meeting and concluded that the NOAEL in this dietary study was 5% Fast Green FCF (equal to 3184 mg/kg bw per day), the highest dose tested (Annex 1, reference *73*). The present Committee concurred with this conclusion.

Whereas 10 of the 18 available genotoxicity tests were negative, four in vitro and four in vivo studies yielded positive results. Given that all of the studies with positive test outcomes had several limitations in experimental design and reporting, whereas an in vivo mouse bone marrow micronucleus assay (Hayashi et al., 1988) and an in vivo mouse tissue comet assay (Sasaki et al., 2002) were clearly negative, the Committee concluded that there is no concern with respect to genotoxicity of Fast Green FCF.

No reproductive toxicity was reported at doses up to 1000 mg/kg bw per day over three generations of rats (Smith, 1973).

No developmental toxicity studies were available. However, information on the developmental toxicity of the structurally related substance Brilliant Blue

FCF, which differs from Fast Green FCF by a single hydroxyl group, was available. No developmental toxicity was reported in rats treated with Brilliant Blue FCF at doses up to 2000 mg/kg bw per day or in rabbits at up to 200 mg/kg bw per day (BioDynamics Inc. 1972a,b). Based on these findings, the Committee concluded that there is no concern for developmental toxicity for Fast Green FCF.

Observations in humans

No data were available.

Assessment of dietary exposure

Estimates of dietary exposure to Fast Green FCF published by the Republic of Korea (Ha et al.. 2013) and the USFDA (Doell et al., 2016) were available. Because the estimates were based on only a few findings in a limited number of food groups, the Committee conducted a conservative assessment using the FAO/WHO Chronic individual food consumption database – Summary statistics (CIFOCOss) database and Codex maximum levels.

Dietary exposure to Fast Green FCF was estimated to be 12 mg/kg bw per day for adolescents, the age group with the highest exposure, at the 95th percentile. This estimate was much higher than those of both the USFDA (0.09 mg/kg bw per day for children at the 90th percentile) and the Republic of Korea (0.003 mg/kg bw per day for the whole population at the 95th percentile). The Committee concluded that these differences were due to the use of Codex maximum levels, in contrast to the estimates from the USFDA and the Republic of Korea, which used mean analysed levels for all foods.

The Committee concluded that the conservative estimate of 12 mg/kg bw per day, prepared using CIFOCOss data, should be considered in the safety assessment for Fast Green FCF.

Evaluation

The Committee at previous meetings concluded that Fast Green FCF is not carcinogenic. The evidence newly available at this meeting indicates that there is no concern with respect to genotoxicity of Fast Green FCF. The ADI of 0–25 mg/kg bw established previously by the Committee was based on a long-term rat dietary study in which a NOAEL of 5% Fast Green FCF (equivalent to 2500 mg/kg bw per day), the highest concentration tested, was identified (Hansen et al., 1966).

The Committee concluded that the new data that had become available since the previous evaluation gave no reason to revise the ADI and confirmed the ADI of 0–25 mg/kg bw. The Committee noted that the conservative dietary exposure estimate for Fast Green FCF of 12 mg/kg bw per day (95th percentile for adolescents) was below the upper bound of the ADI. The Committee concluded

that dietary exposures to Fast Green FCF for adolescents and all other age groups do not present a health concern.

A toxicological and dietary exposure monograph was prepared.

At the present meeting, the existing specifications for Fast Green FCF were revised, and a maximum limit for manganese was added. HPLC methods were added to determine subsidiary colouring matters and organic compounds other than colouring matters. The assay method was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water or aqueous ammonium acetate.

The specifications monograph was revised, and a Chemical and Technical Assessment was prepared.

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3.1.4 Gum ghatti

Explanation

Gum ghatti (CAS No. 9000-26-6), also known as Indian gum, ghatti gum or gum ghati, is the dried gummy exudate from wounds in the bark of *Anogeissus latifolia* Wallich (family Combretaceae), a large tree native to India and Sri Lanka (Al-Assaf, Phillips & Amar, 2009). Gum ghatti is used as a thickener and stabilizer. It is permitted as a food additive in Japan and the USA.

Gum ghatti was previously evaluated at the twenty-sixth and twenty-ninth JECFA meetings (Annex 1, references *59* and *70*). Heavy metal specifications were revised at the fifty-seventh JECFA meeting (Annex 1, reference *154*). No ADI could be established at the twenty-sixth or twenty-ninth meetings because of insufficient data, but the Committee did not make specific recommendations for further studies; no monographs were prepared.

At the present meeting, the Committee evaluated gum ghatti at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016). A toxicological dossier was submitted. Two new 90-day rat studies as well as genotoxicity studies

have become available since the previous evaluations. To address any data gaps for gum ghatti, the safety data on other polysaccharide-based gums were considered based on their similar general structure, chemical and functional properties, technical uses, lack of absorption as intact substances and their metabolism in the lower gastrointestinal tract.

A comprehensive literature search up to April 2017 was performed in PubMed and TOXLINE. Although the search resulted in five additional papers, these did not add further relevant data to those submitted to the Committee for this meeting.

Chemical and technical considerations

Unprocessed gum ghatti occurs as both amorphous "tears" of various sizes and as broken irregular pieces. It is light to dark brown in colour, has little or no odour and is available commercially in the form of brown tears or grey to reddish-grey powder. The product in commerce is manufactured by collecting the dried translucent exudate as tears, partially dissolving these in water and filtering. The final product is sterilized and dried to a gummy, lump form or spray-dried to a powder form.

Gum ghatti consists mainly of calcium (or occasionally magnesium) salts of high molecular weight and water-soluble complex polysaccharides. The hydrolysis of the polysaccharide yields L-arabinose, D-galactose, D-glucuronic acid and D-mannose, and small amounts of D-xylose and L-rhamnose. The reported average molar ratio of the various units is L-arabinose:D-galactose:D-glucuronic acid:D-mannose:D-xylose:L-rhamnose = 40:25:20:7:1:1 (Sakai et al., 2013). Gum ghatti also contains protein-bound arabinogalactan units, tannins and moisture. The weight average molecular weight of gum ghatti is in the order of several hundred kDa (Kang et al., 2015).

Biochemical aspects

Absorption, distribution, metabolism and excretion data on gum ghatti were not available. However, similar to other gums and dietary fibres, gum ghatti is unlikely to be significantly digested or absorbed in the stomach or small intestine. Based on its chemical composition, gum ghatti is expected to be enzymatically degraded and fermented by the microflora in the large intestine to hydrogen gas, carbon dioxide and short-chain fatty acids, which can be absorbed and metabolized (Ali, Ziada & Blunden, 2009).

Toxicological studies

In an acute toxicity study in male rats (Newell & Maxwell, 1972), no deaths were reported at 10 000 mg/kg bw, the highest dose tested.

Two new 90-day studies of the toxicity of gum ghatti (purity 85%) have been performed in rats.

In the first study (Davis & Lea, 2011), rats were fed a basal diet (AIN-93M) containing 0%, 0.5%, 1.5% or 5% gum ghatti (equal to 0, 337, 1018 and 3044 mg/kg bw for males and 0, 396, 1149 and 3308 mg/kg bw per day for females, respectively). Although haematological and clinical chemistry effects were observed, they were not dose related, not found in both sexes and/or not correlated with any histopathological findings.

Increased caecal weights were observed in the male and female rats at 5% gum ghatti. In addition, in 6 out of 10 high-dose males, minimal to mild mucosal hyperplasia and/or minimal to mild crypt elongation were observed in the caecum, whereas no lesions were found in the caecum of female rats. In 2 out of 10 high-dose females, ulcerative colitis was observed in the colon; no significant lesions were observed in the colon of male rats.

In order to evaluate the relevance of the ulcerative colitis, the possible role of the AlN-93M diet and the possibility that intrinsically susceptible littermates had been randomly assigned to the same group, a second study tested two different basal diets (AIN-93M and NIH-07) containing 0% or 5% gum ghatti (equal to 0 and 3671 mg/kg bw per day for rats fed the AIN-93M diet and 0 and 3825 mg/kg bw per day for rats fed the NIH-07 diet) (Davis, 2012). This study deviated from Organisation for Economic Co-operation and Development (OECD) Test Guideline 408, as only one dose was tested in only one sex (female) and the histopathological examination did not include the full range of recommended organs, because the aim of this second study was to follow up on the observations reported in female rats at the highest dose in the first study.

Increased empty caecal weights were observed in animals exposed to gum ghatti in both diets. Full caecal weights (absolute and relative) were also increased in rats exposed to gum ghatti in the AIN-93M diet but not the NIH-07 diet. Focal lymphoid hyperplasia of the colon was observed in all study groups, but there was no association with the dietary exposure to gum ghatti, and the authors concluded that these findings were incidental and not treatment related. A pathology working group subsequently concluded that the ulcerative colitis observed in the colon of the female rats was a sporadic event not associated with the dietary exposure to gum ghatti and that the caecal changes in male rats could not be confirmed as caecal crypt hyperplasia/crypt elongation (Maronpot et al., 2013).

The Committee noted that the effects on caecal weights observed in both sexes at 5% gum ghatti have also been reported in other toxicity studies of poorly digestible polysaccharides and gum products (Tulung, Rémésy & Demigné, 1987; Wyatt et al., 1988; Levrat et al., 1991; Doi et al., 2006; Ali, Ziada & Blunden, 2009; Hagiwara et al., 2010). The increase in caecal weight is considered to be the result

of microbial fermentation of undigested and unabsorbed gum in the lower large intestine (Newberne, Conner & Estes, 1988). The Committee considered this increase in caecal weight at 5% dietary gum ghatti to be an adaptive, rather than adverse, response. Based on the results of the new 90-day studies (Davis & Lea, 2011; Davis, 2012), the Committee identified a NOAEL of 3044 mg/kg bw per day (equal to 2590 mg/kg bw per day, corrected for purity), the highest dose tested.

No long-term studies of the toxicity or carcinogenicity of gum ghatti were available.

In vitro and in vivo genotoxicity studies of gum ghatti have recently been conducted. These, together with earlier in vitro and in vivo studies, showed no evidence for a genotoxic potential of gum ghatti. The Committee concluded that there were no genotoxicity concerns for gum ghatti.

No reproductive toxicity studies were available for gum ghatti.

Studies on developmental toxicity of gum ghatti administered by oral gavage were performed in mice, rats, hamsters and rabbits (Food and Drug Research Laboratories, Inc., 1972a). In mice and hamsters dosed at 0, 17, 80, 370 or 1700 mg/kg bw per day, there were no treatment-related adverse effects on the dams. There were also no treatment-related adverse effects on the numbers of implantations, resorptions or live and dead fetuses or on the frequency of external, soft tissue or skeletal abnormalities.

In the rat, there were four, zero, one, one and five maternal deaths at 0, 17, 80, 370 and 1700 mg/kg bw per day, respectively. Severe diarrhoea and urinary incontinence with anorexia were observed in the 2–3 days prior to death. Petechial haemorrhage was observed in the mucosa of the small intestine of the dams that died. There were no treatment-related adverse embryo-fetal effects at any dose, including in those rats that survived at the highest dose tested.

In the rabbit study, there were 15 animals per dose group. There were 3, 0, 3, 5 and 10 maternal deaths in the 0, 7, 33, 150 and 700 mg/kg bw per day dose groups, respectively. As with rats, severe diarrhoea and urinary incontinence with anorexia were observed in the 2–3 days prior to death. In addition, all animals aborted prior to death. Petechial haemorrhage was observed in the mucosa of the small intestine of the does that died. There were no treatment-related adverse embryo-fetal effects at any dose, including in the two pregnant rabbits that survived at the highest dose tested.

These developmental toxicity studies were performed prior to OECD guidelines or good laboratory practice (GLP) standards and do not comply with several modern standards/guidelines: the purity of the substance was not stated; the treatment period covered the major phase of organogenesis but did not extend to the end of gestation; and the rationale for dose selection in all four studies was not presented. In addition, none of the study reports presented the clinical

observations, feed consumption, gravid uteri weights or statistical analyses of the results. The Committee also noted that there were maternal deaths at high doses in mice, rats and, in particular, rabbits in developmental toxicity studies on other gums conducted by the same laboratory at about the same time, in which the test substance was also administered by oral gavage. The Committee considered that this may have been due to the difficulty of administering high concentrations of viscous substances by gavage. They further noted that no treatment-related adverse maternal or developmental effects were reported in surviving high-dose animals in studies on gum ghatti and other gums. Despite the deficiencies in the study methods and reporting and the occurrence of maternal deaths, there were no effects on embryo-fetal growth or development at doses up to 1700 mg/kg bw per day.

In view of the gaps in the database for gum ghatti (i.e. the absence of any long-term toxicity or carcinogenicity studies, the limitations of the developmental toxicity studies and the lack of any reproductive studies), the Committee considered data on structurally related gums. The gum most closely related to gum ghatti is gum arabic (also known as gum acacia); the two gums have similar monosaccharide profiles with respect to L-arabinose, L-rhamnose, D-galactose and D-glucuronic acid (Pitthard & Finch, 2001; Akiyama, Yamazaki & Tanamoto, 2011).

Developmental toxicity studies on other gums in mice, rats, hamsters and rabbits (Food and Drug Research Laboratories Inc., 1972b) were conducted by the same laboratory that conducted the developmental toxicity studies on gum ghatti. As such, they may have had similar limitations. A more recent combined fertility and developmental toxicity study of gum arabic in rats (Collins et al., 1987) was previously evaluated by the Committee, which considered that this study did not give cause for concern about the safety of gum arabic (Annex 1, reference *89*). EFSA (EFSA, 2017) also described more recent fertility studies in rats (Morseth & Ihara, 1989; Huynh et al., 2000) and considered that these studies did not give cause for concern about the safety of gum arabic. Based on the combined fertility and developmental toxicity study in rats (Collins et al., 1987), an overall NOAEL of 10 647 mg/kg bw per day (the highest dose tested) was identified for reproductive, developmental and parental effects. The Committee noted that reproductive and developmental toxicity studies on other gums (carob bean gum [FAS 16], cassia gum [FAS 62], gellan gum [FAS 28], guar gum [FAS 8], karaya gum [FAS 24]), tara gum [FAS 21]), tragacanth [FAS 20]), xanthan gum [FAS 21]) also previously evaluated by the Committee (Annex 1, references *57*, *197*, *95*, *39*, *84*, *74*, *72* and *74*) did not raise any health concerns for reproductive or developmental effects.

Overall, the Committee concluded that there were no health concerns for gum ghatti regarding reproductive or developmental effects.

Previously evaluated carcinogenicity studies of gum arabic in mice and rats conducted by the United States National Toxicology Program (National Toxicology Program, 1982) found no indications of any treatment-related increases in tumour incidence at dietary gum arabic concentrations of 2.5% and 5.0% (equivalent to 1250 and 2500 mg/kg bw per day for rats and 3750 and 7500 mg/kg bw per day for mice). The Committee noted that other previously evaluated chronic toxicity/carcinogenicity studies in mice and rats (carob bean gum, gellan gum, guar gum, tara gum, xanthan gum) also raised no health concerns regarding carcinogenic potential.

Observations in humans

No observations of gum ghatti in humans were available. However, three human studies on gum arabic found that daily ingestion by adults of up to 30 g (equivalent to 500 mg/kg bw per day for a 60 kg individual) over 18–21 days was well tolerated (Ross et al., 1983; Sharma, 1985; Cherbut et al., 2003). Furthermore, Ross et al. (1983) found that gum arabic could not be detected in the stool, indicating complete fermentation in the colon.

Assessment of dietary exposure

The Committee received one assessment of dietary exposure to gum ghatti from the sponsor and prepared estimates of dietary exposure based on model diets and potential use scenarios using food consumption data from the European Union and the USA.

The sponsor's submission noted that gum ghatti is used in a number of countries. The only use levels reported were from the USA. The Committee was unable to find information on the typical use levels in other countries. The submission to the Committee contained two reports outlining use levels for gum ghatti in a number of GSFA food categories. The Committee prepared estimates of dietary exposure based on these levels.

One report contains use levels for foods in GSFA categories 1.1.4 "Flavoured fluid milk drinks" and categories 14.1 and 14.2 (various beverage categories). The maximum use level for milk beverages was 150 mg/L; for non-alcoholic beverages, 100 mg/L; and for alcoholic beverages, 300 mg/L. Using food consumption data from the USA, the Committee completed a scenario assessment of dietary exposure by assuming 250 g/day consumption of milk beverages (95th percentile); 900 g/day of non-alcoholic beverages (95th percentile); and 750 g/day of alcoholic beverages (95th percentile). The estimated dietary exposure to gum ghatti would be 350 mg/day or 6 mg/kg bw per day for a 60 kg individual. The use of these maximizing assumptions in the preparation of the estimate from

these three broad food groups results in a highly conservative estimate of chronic dietary exposure to gum ghatti.

The second report contains a more extensive list of foods potentially containing gum ghatti. The Committee concluded that only consumption of noodles containing gum ghatti at a use level of 6000 mg/kg diet would result in a dietary exposure different from that in the scenario discussed above. The dietary exposure to gum ghatti from consumption of 60 g of prepared noodles containing the maximum level would be approximately 360 mg/day (6 mg/kg bw per day for a 60 kg individual), doubling the previous scenario estimate (12 mg/kg bw per day).

The Committee also used the EFSA Food Additive Intake Model (Version 1.0) with the use levels from the sponsor's report to estimate dietary exposure to gum ghatti. The estimated exposure for adults was 6 mg/kg bw per day.

The Committee considered that a dietary exposure of 12 mg/kg bw per day was suitable for use in a safety assessment of gum ghatti.

Evaluation

Because limited toxicological data on gum ghatti were available, ADIs were not established at previous meetings (Annex 1, references *59* and *70*). The present Committee evaluated two new 90-day studies in rats that did not show adverse effects at doses up to 3044 mg/kg bw per day, the highest dose tested (equal to 2590 mg/kg bw per day when corrected for purity). The Committee took into account the lack of systemic exposure to gum ghatti because of its high molecular weight and polysaccharide structure, its lack of toxicity in short-term studies, the lack of concern for genotoxicity and the absence of treatment-related adverse effects in studies of gum arabic and other polysaccharide gums with a similar profile.

The Committee concluded that gum ghatti is unlikely to be a health concern and established an ADI "not specified" for gum ghatti that complies with the specifications.

Therefore, the Committee concluded that the estimated dietary exposure to gum ghatti of 12 mg/kg bw per day does not represent a health concern.

A consolidated monograph was prepared.

The specifications were revised based on submitted information and available literature. An HPLC method for the identification of the gum constituents was added to replace the thin-layer chromatography method. One identity method, using a mercury-containing reagent, was removed. L- Rhamnose was added as one of the constituents of gum ghatti, based on current literature reports.

The specifications were revised, and a Chemical and Technical Assessment was prepared.

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3.1.5 Jagua (Genipin–Glycine) Blue

Explanation

Jagua (Genipin–Glycine) Blue (CAS No. 1314879-21-4) is the product of the reaction between stoichiometric equivalents of genipin extracted from the unripe *Genipa americana* Linne (Rubiaceae) fruit and glycine, resulting in a blue-coloured genipin–glycine polymer and dimers. This report refers to the blue-coloured genipin–glycine polymer and dimer content of Jagua (Genipin–Glycine) Blue as the "blue polymer" content. *G. americana* fruit has traditionally been used for the preparation of juices, jellies, marmalades and liquors (Ramos-de-la-Peña et al., 2015).

Jagua (Genipin–Glycine) Blue is permitted for use as a food colour in Colombia.

Jagua (Genipin–Glycine) Blue has not been evaluated previously by the Committee. It was on the agenda at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016).

The sponsor provided a dossier containing chemical, technical, dietary exposure and toxicological data, including unpublished in vitro studies, genotoxicity studies and in vivo toxicological studies in rats and dogs.

A comprehensive literature search on *G. americana* and the related colour Gardenia Blue was conducted. One long-term toxicity study on Gardenia Blue was identified and added to the toxicological data submitted to the Committee for this meeting.

The name was changed from "Jagua extract" to "Jagua (Genipin–Glycine) Blue" because the name "Jagua extract" was not adequately descriptive.

Chemical and technical considerations

G. americana L. is a small to medium-sized tree (UNCTAD, 2005) that belongs to the Rubiaceae family and is native to central and tropical South America (Djerassi, Gray & Kincl, 1960; Ueda, Iwahashi & Tokuda, 1990). The plant yields edible berries referred to as jagua fruit, chipara, guayatil, maluco, caruto or huito (Ramos-de-la-Peña et al., 2015) in Spanish and as genipap in English.

The unripe jagua fruit contains high levels of a cyclopentan-[C]-pyran skeleton class of compound, called iridoids (Dinda, Debnath & Harigaya, 2007a,b). Genipin is a unique iridoid in its ability to crosslink with primary amines present in amino acids and proteins, in the presence of oxygen, to produce high molecular weight water-soluble blue pigments (Fujikawa et al., 1987; Touyama et al., 1994a,b; Paik et al., 2001; Park et al., 2002; Cho et al., 2006; Lee, Lee & Jeong, 2009).

The deep blue/black colour of Jagua (Genipin–Glycine) Blue is obtained by treating peeled and ground pulp of unripe fruits of *G. americana* L. with water. The resulting juice is filtered and treated with a stoichiometric amount of glycine based on the concentration of genipin in the water extract; it is heated at 70 °C for 2 hours, until the blue colour is completely formed. The product is centrifuged, concentrated and/or dried. Unreacted genipin is considered an impurity of Jagua (Genipin–Glycine) Blue. The liquid product is obtained by concentrating the Jagua (Genipin–Glycine) Blue up to 20–50°Bx and formulating with food-grade glycerine or other permitted food additives. Alternatively, a powder is obtained, after concentrating the Jagua (Genipin–Glycine) Blue to 20°Bx, mixing with a food-grade carrier, then spray-drying and sieving.

The Jagua (Genipin–Glycine) Blue product in commerce contains a blue polymer (20–40%) and three blue dimers (approximately 1.5%) as colouring matters. The remaining components of the product are carbohydrates (>55%), protein (approximately 7%) and water (approximately 5%). The blue polymer composed of repeating dimers has the molecular formula $(C_{27}H_{25}O_8N_2)_n$ and an average molecular weight of 6000 Da. The molecular formulae of the three identified dimers are $C_{28}H_{28}O_8N_2$ (CAS No. 1313734-13-2), $C_{27}H_{25}O_8N_2$ (CAS

No. 104359-67-3) and $C_{27}H_{24}O_8N_2$ (CAS No. 1313734-14-3). The blue polymer and the three dimers have been identified by nuclear magnetic resonance spectroscopy (^1H, ^{13}C), infrared spectroscopy, mass spectroscopy and HPLC. The Jagua (Genipin–Glycine) Blue is stable and has no decomposition products under normal storage conditions.

Biochemical aspects

The molecular weight and chemical properties of the "blue polymer" of Jagua (Genipin–Glycine) Blue suggest that the polymer is unlikely to be absorbed intact from the gastrointestinal tract. A size distribution analysis showed that less than 1.5% of the mixture contained dimers with molecular weights of around 500 Da, which could be absorbed. An in vitro study using a Caco-2 cell intestinal barrier model showed that "blue polymer" has poor passive penetration, but there is some evidence to suggest that a small proportion of Jagua (Genipin–Glycine) Blue, possibly the smallest coloured molecular species (such as genipin–glycine dimers of molecular weight approximately 500 Da, or other coloured low molecular weight components), was actively transported (Gilbert, 2015). No "blue polymer" was detectable in the plasma of dogs in an oral gavage repeated-dose study (tested up to 338 mg/kg bw per day, limit of quantification 1 mg/mL) on day 1 or 91 following dosing with Jagua (Genipin–Glycine) Blue (Mancari, 2016). No investigations into biotransformation of the "blue polymer" were undertaken.

Toxicological studies

Results from an oral gavage acute toxicity test in the rat showed no adverse effects at the highest tested dose of 660 mg/kg bw (Allingham, 2014).

Results from oral gavage 90-day repeated-dose toxicity studies in rats and dogs showed no adverse effects at 330 mg/kg bw per day or 338 mg/kg bw per day of "blue polymer", respectively, the highest doses tested (Allingham et al., 2014; Mancari, 2016). The dog study deviated from the relevant OECD test guideline, but the Committee considered these deviations to be minor and to not affect the validity of the study. The Committee noted that in dogs, the urine was coloured green with an intensity that appeared to be in proportion to the administered dose, and there was an increase in measured serum bilirubin values attributed to the interference of the test article with the analytical method, suggesting that some of the "blue polymer" had been absorbed from the gastrointestinal tract. Green-coloured urine was not observed in rats. In all 90-day animal studies, all treated animals had faeces that were coloured blue, which is consistent with poor absorption of the high molecular weight component of the "blue polymer" from the gastrointestinal tract.

There were no long-term toxicity or carcinogenicity studies available on Jagua (Genipin–Glycine) Blue. To address the data gap, one non-GLP carcinogenicity study in rats on a structurally related genipin-based blue polymer from *Gardenia jasminoides* (Gardenia Blue) was considered. The Gardenia Blue used in the study was formed from a mixture of genipin and a protease digest of soy proteins, resulting in different amino acids attached to genipin. The Committee noted that the purity of blue polymer in the Gardenia Blue was not described. At concentrations up to 5% in the diet (equal to 2173 mg/kg bw in the males and 2533 mg/kg bw in the females), there were no treatment-related adverse effects or changes in tumour incidence (Imazawa et al., 2000).

There was no evidence of genotoxicity of Jagua (Genipin–Glycine) Blue in vitro, with bacterial reverse mutation assays and a mouse lymphoma assay, or in vivo, with a mouse micronucleus assay. The Committee concluded that there was no concern with regard to genotoxicity.

No reproductive or developmental toxicity studies on Jagua (Genipin–Glycine) Blue were available; there were also no available reproductive or developmental toxicity studies on Gardenia Blue.

Observations in humans

No relevant human studies were available.

Assessment of dietary exposure

Estimates of dietary exposure to Jagua (Genipin–Glycine) Blue prepared by the sponsor based on dietary data for the United States population, estimated use levels and use frequencies were available to the Committee. In addition, a conservative assessment using the CIFOCOss database and maximum use levels provided by the sponsor was performed by the Committee. The 95th percentile estimates of dietary exposure for Jagua (Genipin–Glycine) Blue on a "blue polymer" basis calculated by the Committee were 11 mg/kg bw per day for children and 5 mg/kg bw per day for adolescents. These estimates were much higher than those calculated by the sponsor. The difference between the sponsor's estimates and the Committee's estimates was due to the use by the sponsor of lower use levels and use frequencies.

The Committee concluded that the conservative estimate of 11 mg/kg bw per day for children and 5 mg/kg bw per day (for adolescents), prepared using the CIFOCOss model, should be considered in the safety assessment for Jagua (Genipin–Glycine) Blue on a "blue polymer" basis.

Evaluation

The Committee noted that in 90-day toxicity studies with Jagua (Genipin–Glycine) Blue in the dog and rat, no treatment-related adverse effects were found at the highest doses tested; in addition, genotoxicity tests were negative, and no treatment-related adverse effects were observed in a carcinogenicity study with the structurally related food colour, Gardenia Blue. Based on the coloration of the urine in the dogs and the increase in serum bilirubin test values, which was attributed to interference of the test article with the analytical method, the Committee concluded that some component of the Jagua (Genipin–Glycine) Blue is absorbed and excreted, most likely the dimers or other coloured low molecular weight component. (The dimers make up less than 1.5% of Jagua (Genipin–Glycine) Blue). However, the Committee noted that the highest doses tested in both 90-day studies were only 330 and 338 mg/kg bw per day (expressed on a "blue polymer" basis) in rats and dogs, respectively. The Committee was concerned that the possible effects of the low molecular weight species that could be absorbed would not have been adequately investigated.

A comparison of the dietary exposure estimate (11 mg/kg bw per day) with the NOAEL from the 90-day studies of oral toxicity in rats and dogs (approximately 330 mg/kg bw per day) gives a margin of exposure of approximately 30.

Because of the limited biochemical and toxicological database and the low margin of exposure, the Committee was unable to complete the evaluation for Jagua (Genipin–Glycine) Blue.

A toxicological and dietary exposure monograph was prepared.

A new tentative specifications monograph and a Chemical and Technical Assessment were prepared.

The Committee raised concern regarding the potential toxicity of low molecular weight fraction of the total colouring matter in Jagua (Genipin–Glycine) Blue. The Committee recommends additional biochemical and toxicological studies (e.g. absorption, distribution, metabolism and excretion studies, long-term toxicity, carcinogenicity, reproductive and developmental toxicity studies), including on the use of higher doses of the "blue polymer", including the dimers, in order to complete an evaluation of the safety of Jagua (Genipin–Glycine) Blue.

To support the above, additional information is required on:

- Characterization of the low molecular weight components of the "blue polymer";
- A validated method for the determination of the dimers; and
- Data on concentrations of dimers from five batches of the commercial product.

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3.1.6 Metatartaric acid

Explanation

Metatartaric acid (CAS No. 56959-20-7/39469-81-3; INS No. 353), a polymer of L(+)-tartaric acid, is used as a food additive in winemaking in the following countries and regions: Argentina, Australia, Brazil, Canada, Chile, the European Union, New Zealand, Norway, Paraguay, Russian Federation, South Africa, Turkey and Uruguay.

Metatartaric acid, which was not previously evaluated by the Committee, was evaluated at the request of the CCFA at its Forty-eighth Session (FAO/WHO, 2016). It is proposed for use in winemaking at a level of good manufacturing practice. The data that were submitted in response to the call for data related to its use as a food additive in winemaking only.

The safety of L(+)-tartaric acid and DL-tartaric acid and their sodium and potassium salts was evaluated at the seventeenth and twenty-first meetings of the Committee (Annex 1, references *32* and *44*). At its seventeenth meeting, the Committee established a group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid. At its twenty-first meeting, the Committee reaffirmed the ADI for the L(+)-tartrate monosodium salt and the existing specifications for L(+)-tartaric acid, but did not establish an ADI for DL-tartrate monosodium salt.

L(+)-Tartaric acid, the naturally occurring form of tartaric acid, occurs in many fruits and wines. Tartrate crystals (potassium bitartrate and calcium tartrate) develop naturally in wine and are the major cause of sediment in bottled wines. In order to prevent sedimentation, metatartaric acid has been used in wine since 1955 (OIV, 2012; Guise et al., 2014).

At the present meeting, the Committee reviewed a short-term toxicity study and a genotoxicity study of metatartaric acid. A literature search was conducted, but no studies relevant for the safety assessment of metatartaric acid were identified. However, for L(+)-tartaric acid, three other toxicity studies were

identified and evaluated. Studies on L(+)-tartaric acid from 1977 onward had not been previously reviewed by the Committee.

Chemical and technical considerations

Metatartaric acid is typically manufactured using L(+)-tartaric acid from natural sources. It is formed by the intermolecular esterification between the carboxylic group of one L-tartaric acid unit and the secondary alcohol group of another molecule of L-tartaric acid, which may be followed by further intermolecular and intramolecular esterification reactions (Sprenger et al., 2015). The primary components of metatartaric acid are the L-tartaric acid monomer, ditartrate monoester and diester, and polyester chains of varying degrees of polymerization. The average molecular weight range has been determined in commercial products to be 2.2–8.9 kDa, with a polydispersity index up to 50. Metatartaric acid is used as a stabilizer and sequestrant in wine to prevent growth and precipitation of potassium bitartrate and calcium tartrate crystals (Marchal & Jeandet, 2009). Stability studies in wine indicated that it undergoes hydrolysis to tartaric acid over time, but the rate of hydrolysis is dependent on pH and storage temperature (Ribéreau-Gayon et al., 2006; Morello, 2012).

Metatartaric acid is produced by heating L-tartaric acid from grapes at 150–170 °C under atmospheric or reduced pressure for less than 1 hour (Ribéreau-Gayon et al., 2006). This process produces a colourless liquid, which is cooled, dried and ground into an off-white powder. Variations in production temperature, pressure and time allow manufacturers to alter the degree of esterification in the final product.

Biochemical aspects

Metatartaric acid

Metatartaric acid, a polydisperse polymer of tartaric acid units linked together by ester bonds, is anticipated to undergo rapid enzyme-mediated hydrolysis to L(+)-tartaric acid once exposed to carboxylesterases in the gastrointestinal tract.

L(+)-Tartaric acid

The disposition of L(+)-tartaric acid following ingestion appears to differ markedly between most of the animal species investigated (rats, rabbits, dogs and pigs) and humans. In rats, rabbits, dogs and pigs, most of the ingested tartrate is absorbed and excreted unchanged (50–100%) in the urine (Underhill et al., 1931; Gry & Larsen, 1978). The extent of absorption and urinary excretion of unchanged tartrate in guinea-pigs (13–27%) is similar to that observed in humans (12%) (Underhill et al., 1931; Chadwick et al., 1978).

In rats, 15–22% of ingested tartrate is exhaled as carbon dioxide. Microbial fermentation was confirmed following intracaecal administration, when 66% of the administered dose (18.8 mg/kg bw) was exhaled as radiolabelled carbon dioxide, while less than 2% of the administered dose was absorbed and excreted in urine (Chasseaud, Down & Kirkpatrick, 1977; Chadwick et al., 1978). In rats, the concentration–time curve for radiolabelled L(+)-tartrate suggested a short half-life in plasma of around 3 hours (Down et al., 1977).

Apart from its excretion in urine, there is evidence of extensive microbial fermentation of L(+)-tartaric acid to carbon dioxide in humans: very little unchanged tartrate (<5%) has been detected in faeces. Although the concentration of radiolabelled carbon dioxide exhaled by humans 1 hour after intravenous dosing was small (18%), suggesting metabolism by tissue enzymes, up to 46% of the label was exhaled 4 hours after oral dosing (Chadwick et al., 1978).

Toxicological studies

Metatartaric acid

No acute toxicity studies were available.

Rats exposed to metatartaric acid in their drinking-water at concentrations up to 3.0% for 18 weeks had markedly reduced body weight due to a dose-related reduction in feed and water intake, owing to the poor palatability of metatartaric acid in water at all concentrations tested (Ingram et al., 1982). As a result, the Committee considered this study to be unsuitable for a safety assessment of metatartaric acid.

Metatartaric acid was not genotoxic in a reverse mutation assay.

No long-term toxicity and carcinogenicity, reproductive toxicity or developmental toxicity studies were available.

L(+)-Tartaric acid

The LD_{50} of sodium tartrate in mice was reported to be 4360 mg/kg bw; for disodium tartrate in male rabbits, it was greater than 3680 mg/kg bw (Locke et al., 1942).

The Committee noted that no new long-term toxicity studies had become available since the previous evaluation of L(+)-tartaric acid. However, the previously unpublished toxicity study that supports the ADI for tartaric acid had since been published. In that study, no treatment-related adverse effects were observed in rats with diets containing monosodium L(+)-tartrate at concentrations of 0, 25 600, 42 240, 60 160 or 76 800 mg/kg bw (reported to be equal to L(+)-tartaric acid doses of 0, 710, 1220, 1840 and 2460 mg/kg bw per day for males and 0, 930, 1600, 2360 and 3200 mg/kg bw per day for females, respectively) (Hunter et al., 1977). The Committee noted that the conversion reported in the publication used the molecular weight for disodium tartrate

rather than monosodium tartrate to calculate the doses of L(+)-tartaric acid. Using monosodium tartrate, the Committee calculated the doses to be 0, 770, 1400, 1900 and 2680 mg/kg bw per day for males and 0, 1030, 1780, 2630 and 3550 mg/kg bw per day for females, respectively. The Committee concluded that the NOAEL for L(+)-tartaric acid in the study was 2680 mg/kg bw per day, the highest tested dose.

In two in vitro assays including reverse mutation (*S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98) and chromosomal aberration (Chinese hamster fibroblast cell line), L(+)-tartaric acid showed no genotoxic potential at concentrations up to 1 mg/mL. However, although sodium L(+)-tartrate was negative in the reverse mutation assay, it was positive in a chromosomal aberration test at high concentrations of up to 15 mg/mL (Ishidate et al., 1984). The Committee noted that no testing of potential cytotoxicity was performed and that gaps had been counted in the chromosomal aberration test. The Committee concluded that these factors call into question the reliability of this study. In addition, the related compound, L(+)-tartaric acid, at 1 mg/mL was shown to be negative in the same assay. Sodium L(+)-tartrate was also negative using single intraperitoneal doses up to 3600 mg/kg bw in an in vivo micronucleus test in mice (Hayashi et al., 1988).

Assessment of dietary exposure

The sponsor requested the use of metatartaric acid as a food additive in wine at a maximum use level of 100 mg/L. The Committee conducted international dietary exposure assessments for metatartaric acid in wine using the GEMS/Food cluster diets database. The dietary exposure estimate for metatartaric acid ranged from 0.0004 (G14) to 0.2 mg/kg bw per day (G7) (per capita), assuming a 60 kg body weight and 100 mg/L of metatartaric acid as the maximum use level. The Committee also prepared international estimates of dietary exposure to metatartaric acid using wine (food category 14.2.3.1 "Still grape wine" and food category 14.2.3.3 "Fortified grape wine, grape liquor wine and sweet grape wine") consumption levels from the CIFOCOss database and 100 mg/L of metatartaric acid as the maximum use level. The estimates of mean dietary exposure to metatartaric acid for adult consumers of wine ranged up to 0.3 mg/kg bw per day, and the highest 95th percentile dietary exposures in adult consumers of wine reached 0.8 mg/kg bw per day. The Committee prepared dietary estimates to metatartaric acid in wine using consumption data from the 1995 Australian National Nutrition Survey, the 1997 New Zealand National Nutrition Survey and the USA National Health and Nutrition Examination Surveys, with the maximum use level of 100 mg/L. These estimates were 1.3, 1.3 and 0.3 mg/kg bw per day for the 95th percentile exposures for adult consumers of wine, respectively.

The Committee assumed that metatartaric acid hydrolyses to an approximately equivalent concentration of tartaric acid. The Committee noted that the dietary exposure to metatartaric acid for the highest 95th percentile adult consumers of wine (1.3 mg/kg bw per day, expressed as L(+)-tartaric acid) is appropriate for use in this safety assessment.

Evaluation

As metatartaric acid undergoes enzymatic hydrolysis to tartaric acid prior to systemic absorption, the biochemical and toxicological data on tartaric acid considered at previous meetings are relevant to the safety assessment of the metatartaric acid. Additional information to support the safety assessment of metatartaric acid includes the absence of any effects in a bacterial reverse mutation test. The present Committee evaluated a series of studies that had become available since L(+)-tartaric acid was last evaluated. The body of evidence suggests no change to the group ADI previously established for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid.

The Committee concluded that metatartaric acid (when used in winemaking) should be included in the group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid.

The Committee noted that the dietary exposure estimate for metatartaric acid for adult consumers of wine was 4% of the upper bound of the ADI and concluded that dietary exposure to metatartaric acid in wine at the maximum use level of 100 mg/L does not present a health concern.

A toxicological and dietary exposure monograph was prepared.

Tentative specifications and a Chemical and Technical Assessment were prepared.

The Committee received limited analytical data on metatartaric acid. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested:

- Characterization of the products (optical rotation, content of free tartaric acid, degree of esterification and molecular weight distribution) and the corresponding analytical methods;
- Infrared spectrum (in a suitable medium); and
- Analytical results including the above parameters from a minimum of five batches of products currently available in commerce, along with quality control data.

The Committee requests that this information be submitted by December 2018.

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3.1.7 Tamarind seed polysaccharide

Explanation

Tamarind seed polysaccharide (CAS No. 39386-78-2) is produced from the hulled seeds of *Tamarindus indica* Linne. Tamarind seed polysaccharide is a xyloglucan. Xyloglucans are a type of dietary fibre naturally present in the cell wall of plants, which are abundant in rice, vegetables and fruits (Shibuya & Iwasaki, 1978; Kato & Matsukura, 1994; Kato, 1995; Kato, Ito & Watanabe, 2001). Tamarind seed polysaccharide is permitted for use as a thickener, stabilizer, emulsifier and gelling agent in a variety of food products in China, Japan, the Republic of Korea and the USA.

Tamarind seed polysaccharide has not been previously evaluated by JECFA. The Committee evaluated tamarind seed polysaccharide at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016).

A toxicological dossier for tamarind seed polysaccharide was submitted. A comprehensive literature search was also conducted. None of the records retrieved added to the toxicological data submitted to the Committee for this meeting.

To address any data gaps, the Committee also considered safety data on other polysaccharide-based gums on the basis of their similar general structure, chemical and functional properties, technical uses, lack of absorption as intact substances and metabolism to normal dietary constituents (e.g. short-chain fatty acids) as a result of microbial fermentation in the large intestine.

Chemical and technical considerations

The tamarind tree is a large evergreen widely distributed in subtropical and tropical zones (Williams, 2006). *T. indica* L. is a monotypic genus and belongs to the subfamily Caesalpinioideae of the family Leguminosae (Fabaceae). The seeds of the tamarind fruit are smooth, glossy, flattened and oblong-shaped (Duke, 1981). Tamarind seed polysaccharide is also known as tamarind seed gum, tamarind gum, tamarind xyloglucan, tamarind seed xyloglucan and tamarind galactoxyloglucan.

Every part of the *T. indica* L. tree is used as food or in traditional medicine in most tropical countries (De Caluwé, Halamová & Van Damme,

2010). Traditional uses in food rely on the aroma and flavouring properties of the tamarind fruit, in its fresh or dried form. It is also used in herbal medicinal therapies (Williams, 2006).

Tamarind seed polysaccharide is produced from tamarind seeds that are sieved and toasted to remove the black testa (seed coat). The light brown tamarind kernel obtained is then pulverized and sieved to obtain tamarind kernel powder. The kernels contain 65–72% carbohydrate (polysaccharide and free sugars), 15–23% protein, 4–7% fat, 2–3% ash and 0.7–8% crude fibre, reported on a dry matter basis (Duke, 1981). The tamarind kernel powder is treated with methanol, and the pH is adjusted during treatment; this is followed by centrifugation to physically separate the insoluble tamarind seed polysaccharide from the supernatant, which contains the protein, fat and minerals. The polysaccharide is dried, pulverized, sieved and mixed with bulking agents to standardize the product. Depending on the pH treatment, downstream filtration, and acid or alkali treatment, products differing by viscosity can be manufactured.

Tamarind seed polysaccharide is composed of a linear chain of D -glucose units linked by β(1–4) glycosidic bonds. Single D-xylose units are attached to about 75% of these D-glucose units via α(1–6) bonds. Single D-galactose units are attached to some of the D-xylose units through β(1–2) bonds. The molar ratio of glucose:xylose:galactose is about 4:3:1 (Gidley et al., 1991). The tendency of xyloglucans to self-associate gives rise to a wide range of reported molecular weights (400–6000 kDa) (Nishinari et al., 2009).

Biochemical aspects

Absorption, distribution, metabolism or excretion data were not available on tamarind seed polysaccharide. Based on its size and chemical composition, tamarind seed polysaccharide, like other dietary fibres, is not expected to be absorbed intact or digested in the gastrointestinal tract (Cummings & Englyst, 1987). Based on its chemical composition, tamarind seed polysaccharide is expected to be enzymatically degraded and fermented by intestinal bacteria in the large intestine. The fermentation process would yield hydrogen gas, carbon dioxide and short-chain fatty acids, which could be absorbed and metabolized. It has been estimated that more than 75% of tamarind seed polysaccharide is fermented (Ministry of Health, Labour and Welfare, 2003). This extensive fermentation process is similar to that for other nondigestive polysaccharides, such as carob bean gum, cassia gum and tara gum.

Evidence supporting such a fermentation process includes the results of a 14-day dietary study in rats, which showed that oligosaccharides of tamarind seed polysaccharide generate short-chain fatty acids (specifically, lactic acid, propionic acid and butyric acid) in the caeca of test animals in greater amounts than in control rats fed a non-fibre diet (Ebihara & Nakamoto, 1998). In vitro

studies demonstrated that human microflora can also degrade and ferment tamarind seed polysaccharide (Hartemink et al., 1996). Specific bacteria that colonize the large intestine in humans are capable of enzymatic hydrolysis of the glucan backbone of xyloglucans, which would lead to fermentation (Hartemink et al., 1996; Larsbrink et al., 2014).

Toxicological studies

All toxicological tests were conducted using a commercial product in which the purity of the tamarind seed polysaccharide was between 80% and 85%. The remaining 15–20% included water, carbohydrates, protein and fat, which are normal dietary constituents that are not expected to pose a toxicological hazard. Tamarind seed polysaccharide is of low acute oral toxicity in mice and rats. The LD_{50} in each of these species was greater than 5000 mg/kg bw (4000 mg/kg bw when corrected for purity).

No toxicity was observed in a 13-week study in mice at concentrations of up to 50 000 mg/kg feed (equal to 8200 mg/kg bw per day, or 6642 mg/kg bw per day when corrected for purity) (Sano et al., 1996). There were no toxicologically relevant effects in a 4-week dietary study of tamarind seed polysaccharide in rats at concentrations up to 120 000 mg/kg feed (equal to 10 597 mg/kg bw per day, or 9113 mg/kg bw per day when corrected for purity) (Heimbach et al., 2013; Koetzner, 2013).

Similarly, no toxicologically relevant effects, including treatment-related tumours, were observed in a 78-week study in mice at concentrations of up to 50 000 mg/kg feed (equal to 6658 mg/kg bw per day, or 5380 mg/kg bw per day when corrected for purity) (Sano et al., 1996). No treatment-related toxicity, including tumours, was observed in a 104-week study in rats at concentrations of up to 120 000 mg/kg feed (equal to 5150 mg/kg bw per day, or 4161 mg/kg bw per day when corrected for purity) (Iida et al., 1978). The highest doses tested in these toxicity studies routinely equalled or exceeded the recommended dose limit of 5% of the diet for rodent toxicity studies.

The Committee concluded that the pivotal study was the 104-week study in rats (Iida et al., 1978). This was a well-conducted study performed before the implementation of GLP. The NOAEL was 5150 mg/kg bw per day (corrected to 4161 mg/kg bw per day for purity), the highest dose tested.

Tamarind seed polysaccharide tested negative in bacterial reverse mutation assays and in an in vitro chromosomal aberration assay. Despite the limitations of some of these assays (due to the poor solubility of the test substance at higher concentrations), based on the absence of chemical structural alerts and negative results, the Committee concluded that for tamarind seed polysaccharide, there was no concern with respect to genotoxicity.

No reproductive or developmental toxicity studies were conducted with tamarind seed polysaccharide. The Committee noted that histopathological analysis of reproductive organs from long-term feeding studies in mice and rats did not identify any effects on reproductive tissues. The Committee also noted that reproductive and developmental toxicity studies on other polysaccharide gums previously evaluated by the Committee did not raise concerns for reproductive or developmental effects. For example, when cassia gum was assessed in a two-generation reproductive toxicity study in rats, it was shown not to cause reproductive toxicity at 50 000 mg/kg feed (equal to 5280 mg/kg bw per day), the highest concentration tested. In a developmental toxicity study in rats, cassia gum did not cause embryotoxicity or teratogenicity at 1000 mg/kg bw per day, the highest dose tested. In a developmental toxicity study in rabbits, cassia gum did not cause any adverse effects on dams or numbers of implantations, postimplantation losses or fetal defects at 1000 mg/kg bw per day, the highest dose tested.

Based on the absence of histopathological effects on reproductive tissues in long-term rodent studies, the lack of absorption of intact tamarind seed polysaccharide, the degradation and fermentation of tamarind seed polysaccharide into normal dietary constituents, and the absence of reproductive or developmental toxicity observed with other polysaccharide gums, the Committee concluded that tamarind seed polysaccharide would be unlikely to pose a concern with respect to reproductive or developmental toxicity.

Observations in humans

No reports were found on food allergies or food intolerance to tamarind seed polysaccharide, despite its long-term use in several countries.

Assessment of dietary exposure

The Committee received an assessment of dietary exposure to tamarind seed polysaccharide from one sponsor in response to the call for data.

Two national estimates of dietary exposure to tamarind seed polysaccharide were included in the sponsor's submission and reviewed by the Committee: from Japan and the USA. These estimates of dietary exposure to tamarind seed polysaccharide were made by combining maximum use levels (assuming 85% polysaccharide in the commercial product) with 2014 food consumption data from the Japanese National Health and Nutrition Survey or with 2003–2006 food consumption data from USA National Health and Nutrition Examination Surveys. The estimated mean dietary exposure to tamarind seed polysaccharide ranged from 31 to 38 mg/kg bw per day, with the 90th percentile exposures up to 77 mg/kg bw per day. These estimates are conservative, in that it has been assumed that all products that might contain tamarind seed

polysaccharide would contain the substance at the indicated maximum use levels. Tamarind seed polysaccharide would be likely to substitute for other gums.

The Committee concluded that the estimated dietary exposure of 75 mg/kg bw per day was suitable for use in this safety assessment.

Evaluation

The Committee established an ADI "not specified" for tamarind seed polysaccharide. This ADI was based on the absence of toxicity in repeated-dose animal studies of tamarind seed polysaccharide. These included long-term rodent studies in which mice were fed up to 6658 mg/kg bw per day (corrected to 5380 mg/kg bw per day for purity) and rats up to 5150 mg/kg bw per day (corrected to 4161 mg/kg bw per day for purity). In addition, there was no concern regarding genotoxicity. Reproductive toxicity and developmental toxicity were not considered a concern based on the lack of absorption of intact polysaccharide, the degradation and fermentation of tamarind seed polysaccharide into normal dietary constituents and the absence of reproductive and developmental effects in other polysaccharide gums.

The estimated dietary exposure based on proposed uses and use levels was 75 mg/kg bw per day. The Committee concluded that this does not present a health concern.

A toxicological and dietary exposure monograph was prepared.

A new specifications monograph and a Chemical and Technical Assessment were prepared.

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3.1.8 Tannins

Explanation

Oenological tannins, which are derived from a variety of natural sources, including grape seeds and skins, stems and stalks, nutgalls and tannin-rich wood, are additives or processing aids used in wine. Tannins are also found in numerous other natural food items. Tannins are an extensive group of compounds that can be broadly divided into two main categories, condensed and hydrolysable tannins, with complex tannins being a mixture of the two. Condensed tannins are formed from polymerization reactions of leucocyanidins and flavan-3-ols and are largely derived commercially from grapes; the flavan-3-ol monomeric subunits include (+)-catechin and (−)-epicatechin and their gallates. Condensed tannins are divided into pro-anthocyanidins and profisetidin and are not susceptible to

hydrolytic cleavage. Hydrolysable tannins (gallotannins and ellagitannins) are largely derived from heartwood. They may undergo hydrolysis to yield gallic acid and ellagic acid and related saccharides.

Oenological tannins are used in winemaking to clarify musts and wines, prevent protein and metal–protein haze, stabilize red wine colour, prevent oxidization, inhibit the activity of the enzyme laccase, and improve the astringency and mouthfeel properties of wine.

The Committee evaluated tannic acid at its fifth, tenth, fourteenth, thirty-first and thirty-fifth meetings (Annex 1, references 5, 13, 22, *77* and *88*). The Committee revised metals and arsenic specifications for tannic acid at its sixty-third meeting (Annex 1, reference *173*). The Committee evaluated grape skin extract at its twenty-eighth meeting (Annex 1, reference *66*). The Committee revised metals and arsenic specifications for grape skin extract at its fifty-ninth meeting (Annex 1, reference *160*).

Anthocyanins were evaluated by the Committee at its twenty-sixth meeting, when an ADI of 0–2.5 mg/kg bw was established (Annex 1, reference *59*). This was based on a level of 7.5% grape skin extract in the diet, which caused no toxicological effect in a two-generation reproductive toxicity study in rats (Cox & Babish, 1978; Becci et al., 1983). Tannic acid (the major hydrolysable gallotannin) was evaluated by the Committee at its tenth, fourteenth, thirty-first and thirty-fifth meetings (Annex 1, references *13*, *22*, *77* and *88*). At its thirty-first meeting, the Committee established a temporary ADI "not specified" for tannic acid used as a filtering aid in food (Annex 1, reference *77*), and the temporary status was removed at the thirty-fifth meeting (Annex 1, reference *88*). At the fourteenth meeting, a temporary ADI of 0–0.6 mg/kg bw per day was established for tannins derived from Peruvian tara, and a temporary ADI of 0–0.3 mg/kg bw per day was established for tannins derived from Turkish Aleppo, Chinese tara and Sicilian sumac.

Tannins were evaluated based on a request from the Forty-eighth Session of the CCFA (FAO/WHO, 2016). The only submission in response to the JECFA call for data related to the use of tannins as a food additive in winemaking and consisted of a literature review rather than specifically commissioned studies. As there were already specifications for tannic acid, the submission largely covered the condensed tannins and the hydrolysable ellagitannins, although some data on tannic acid were also included. Additional studies related to the sources of tannins used in winemaking were identified in a literature search conducted by the Committee.

Chemical and technical considerations

Oenological tannins are manufactured from raw materials such as nutgalls, tara (*Caesalpinia spinosa*) pods, heartwoods (chestnut, oak, exotic wood such as

quebracho wood), other plants such as myrobolan fruits, or grape seeds and skins, stems and stalks. Most tannins available in commerce are extracted with water, steam, ethanol, ethyl acetate or acetone (or a mixture of these solvents), dried and milled. Different products may have undergone hydrolysis to varying degrees, pH and colour adjustment, sulfite addition and spray-drying. The composition of oenological tannins varies considerably depending on the botanical source of the raw material, extraction method, processing, purification and degree of polymerization (or the number of flavan-3-ol subunits).

Biochemical aspects

Tannins are high molecular weight carbohydrate polymers that are poorly absorbed; they may be broken down by gut microorganisms to smaller oligomers and monomers, which are more easily absorbed. Smaller oligomers are present in some of the tannin sources studied and may also be present in the oenological tannins, but the extent of this is unknown. Data on the absorption of proanthocyanidin polymers, procyanidin dimers and ellagitannins in laboratory species and/or humans were available. However, the relevance of these data to the oenological tannins used commercially is unclear, both because some of the tannin sources used are less well characterized (e.g. oak, chestnut, exotic woods) and because the composition of blends of oenological tannins that may be used is unknown. Both condensed and hydrolysable tannins are broken down by gut microflora into smaller units (monomers, dimers and oligomers), with further metabolism and some degree of conjugation then occurring. The available literature indicates that there is significant interindividual variation in metabolism of tannins in both humans and laboratory animal species. Data on tissue distribution and excretion for both groups of tannins are limited and indicate that distribution into specific tissues or organs may occur. Excretion of tannins and their metabolites occurs via the bile and/or the urine.

Toxicological studies

Data on acute, short-term and long-term toxicity, genotoxicity and reproductive toxicity were available for different tannins. For the condensed tannins, the majority of data relate to grape seed and grape skin extracts, whereas for the hydrolysable tannins, the majority of the data relate to tannic acid and pomegranate extract (a source of ellagitannins). However, the toxicological data available for both classes of tannins are limited, with information on long-term toxicity and reproductive and developmental toxicity particularly lacking. There are even fewer toxicological data specific to sources such as oak and chestnut tannins. A number of studies relate to proposed beneficial effects, where the tannins were used to prevent oxidative or genotoxic damage from toxicants; only limited information can be obtained from these studies. The rationale for

the inclusion of certain studies (notably on the hydrolysable ellagitannins, such as would be present in pomegranate extract) in the submission and how these compare to the oenological tannins as used are not clear.

The toxicity of condensed and hydrolysable tannins is low. Tannic acid is known to be hepatotoxic at high oral doses (1.7 g/kg bw in a 90-day rat study [Niho et al., 2001] and ≥2 g/kg bw in single-dose studies in rats [Boyd, Bereczky & Godi, 1965; Zhu, Filippich & Alsalam, 2001]), but there is no evidence of this occurring at lower doses. There are in vitro and in vivo genotoxicity studies available for both condensed and hydrolysable tannins; the results are largely negative, but some positive findings were reported. However, the significance of these findings relative to the oenological tannins used commercially is uncertain.

Observations in humans

Some of the tannins have been tested in human volunteer studies generally designed to assess their potential beneficial effects when used as food supplements. Some relevant information, particularly on the effects on haematology and serum biochemistry, can be obtained from these studies; there are no suggestions of adverse effects. However, how the tannins assessed in these studies compare to the oenological tannins used commercially is unclear.

Assessment of dietary exposure

The sponsor requested the use of (oenological) tannins in wine at use levels of 50–100 mg/L. To assess the potential exposure to tannins added to wine at these use levels, the Committee prepared international estimates of dietary exposure to tannins added to wine using wine consumption levels from the CIFOCOss database (food categories 14.2.3.1 "Still grape wine" and 14.2.3.3 "Fortified grape wine, grape liquor wine and sweet grape wine"). The mean dietary exposure to added tannins in wine at a use level of 75 mg/L ranged from 0.0005 to 0.12 mg/kg bw per day in adults. The highest exposure in adult consumers of wine (95th percentile combined with the maximum use level of 100 mg/L) was 0.77 mg/kg bw per day.

Tannins also occur naturally in many foods, including fruits, vegetables, cereals, beans, nuts, cocoa beans, tea and beer, as well as wine (Gu et al., 2004; USDA, 2004; Prior & Gu, 2005; Serrano et al., 2009). In several studies, the exposures to two types of tannins through the regular diet were reported: proanthocyanidins, the major tannins ingested in the western diet (Prior & Gu, 2005), and ellagitannins. Exposure estimates were reported for Finland (Ovaskainen et al., 2008), France (Pérez-Jiménez et al., 2011), Germany (Radtke, Linseisen & Wolfram, 1998), Spain (Saura-Calixtoa, Serrano & Goñi, 2007; Tresserra-Rimbau et al., 2013) and the USA (Gu et al., 2004). Mean exposures to proanthocyanidins in adults ranged from 71 mg/day in the USA to 450 mg/day

in Spain. The main contributors to the exposure to proanthocyanidins in these studies were red wine, fruit (berries, apples, oranges, peaches) and chocolate products. Only very limited exposure data on ellagitannins were available: from Germany (Radtke, Linseisen & Wolfram, 1998) and Finland (Ovaskainen et al., 2008). Reported mean exposures in adults were 5.2 and 12 mg/day, respectively. Assuming a body weight of 60 kg, the reported mean exposures to proanthocyanidins in adults would range from 1 to 8 mg/kg bw per day. These exposure levels from the diet are 1.3–10 times higher than the highest exposure in adults consuming wine (0.77 mg/kg bw per day) and 8–70 times higher than exposure in average consumers of wine. The Committee noted that because the exact composition of oenological tannins is unknown, these comparisons are to provide context only.

Overall, the exposure to tannins added to wine is expected to be lower than estimated by the Committee: tannins added to wine will be partially removed, as they precipitate with a proteinaceous matter that is subsequently removed by decantation or filtration. Furthermore, the addition of tannins to wine is technologically self-limiting, because the wine may become unacceptably astringent at high tannin levels.

Evaluation

The available data do not provide clear information on which tannin sources and individual tannin compounds are present in commercially used oenological tannins and thus how the oenological tannins would compare to the tannins used in the submitted studies. Therefore, it is not possible to establish which studies are relevant and consequently the extent of the data gaps. Some of the oenological tannins (e.g. grape seed and skin extracts) are better characterized than others (e.g. oak and chestnut).

Many of the available literature studies use a test substance that is derived from the same tannin source, such as grape skin extract, but this may have been an extract prepared in the laboratory or prepared commercially for use as a food additive or in food supplements: it is unclear how the compositions of these preparations compare to each other or to the same source when present in commercial oenological tannins.

The information on biochemical aspects is incomplete, with the implications of repeated dosing on absorption, tissue distribution and interindividual variation needing consideration. In general, there are also few data available on reproductive and developmental toxicity and/or long-term toxicity for some or all of the tannins.

In the absence of specifications and identification of the products in commerce, the Committee concluded that it is not possible to evaluate tannins used in winemaking.

A toxicological and dietary exposure monograph was not prepared.

No specifications monograph was prepared.

The Committee assessed the information received and concluded that there were insufficient data and information to prepare specifications for oenological tannins. The Committee requires data for the characterization of the products in commerce to be able to complete specifications for oenological tannins used as an antioxidant, colour retention agent and stabilizer in wine. The required information includes a detailed description of the manufacturing processes and thorough chemical characterization of the commercial products made from different botanical sources.

The following information is required:

- Composition of tannins derived from the full range of raw materials as well as the processes used in their manufacture;
- Validated analytical method(s) and relevant quality control data;
- Analytical data from five batches of each commercial product including information related to impurities such as gums, resinous substances, residual solvents, sulfur dioxide content and metallic impurities (arsenic, lead, iron, cadmium and mercury);
- Solubility of the products in commerce, according to JECFA terminology; and
- Use levels, natural occurrence and food products in which tannins are used.

Submitters are encouraged to offer a rationale for a single specifications monograph for oenological tannins covering all products or individual monographs.

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3.1.9 Yeast extracts containing mannoproteins

Explanation

Yeast extracts containing mannoproteins are used as food additives in winemaking. Yeast mannoproteins are extracted from purified yeast (*Saccharomyces cerevisiae*) cell walls by enzymatic treatment with β-glucosidase or by physicochemical extraction with thermal treatment. Yeast mannoproteins are galactomannans consisting almost exclusively of mannose units bound to proteins or peptides.

The name was changed from "yeast mannoproteins" to "yeast extracts containing mannoproteins" because the name "yeast mannoproteins" was not adequately descriptive. The products in commerce are extracts containing yeast components and mannoproteins, and not pure mannoproteins. Yeast extracts containing mannoproteins have not been previously evaluated by the Committee. The compounds were evaluated at the present meeting at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016). The JECFA call asked for data

on yeast mannoproteins in general; however, the only data that were submitted related to their use as a food additive in winemaking.

Wine contains significant concentrations of tartrates that can crystallize and precipitate during storage, resulting in unwanted sediment. Wine also contains small amounts of protein, which can produce a haze. Although yeast mannoproteins occur naturally in wine due to yeast fermentation, they are also added to inhibit the crystallization of tartrates and stabilize the proteins in the wine after bottling and during storage.

Yeast mannoproteins are approved for treatment of wine in Argentina, Australia, Canada, the European Union (Commission Regulation (EC) No. 2165/2005), New Zealand and the USA.

The sponsor submitted a dossier summarizing technological, toxicological and dietary exposure information relevant to the evaluation of yeast mannoproteins from *S. cerevisiae*. In addition, a literature search for toxicity data performed using multiple databases and search terms resulted in approximately 20 other potentially relevant papers. However, because few toxicological studies were available for yeast mannoproteins, relevant studies with *S. cerevisiae*, its constituents or substances derived from its fermentation were included in the assessment.

Chemical and technical considerations

Mannoproteins represent a large group of natural compounds from yeast (*S. cerevisiae*) in which polysaccharide chains are bound to proteins and peptides by covalent and non-covalent linkages (i.e. ionic interactions). The structures and molecular weights of mannoproteins vary, depending on the degree and type of glycosylation. The polysaccharide chains consist almost exclusively of mannose units linked by α-links forming a long α-1→6 linked backbone containing short α-1→2 and α-1→3 linked side-chains. Several of the side-chains may have phosphodiester linkages to other mannosyl residues. Yeast mannoproteins are extracted from purified yeast cell walls by enzymatic extraction using glucan 1,3-β-glucosidase (EC 3.2.1.58) or by thermal treatment. The enzyme hydrolyses the yeast cell wall, allowing the mannoproteins to be solubilized. The thermal treatment breaks the links with β-glucans in the cell wall to release the mannoproteins. The mannoproteins thus solubilized by either treatment are then separated from the insoluble cell wall material, concentrated and micro-filtered or ultra-filtered. The mannoproteins have molecular weights ranging from 20 kDa to more than 450 kDa.

There was limited information available to the Committee to fully characterize the yeast mannoprotein products in products of commerce. Information and data about the chemical composition of the range of commercial yeast mannoprotein products are required. There are also limited data available on the levels of yeast mannoproteins in wine. Wine contains yeast mannoproteins from the fermentation process as well as those added for the purpose of

precipitating tartrates. This results in potential levels higher than 400 mg/L of yeast mannoproteins in the wine.

Biochemical aspects

No relevant absorption, distribution, metabolism or excretion studies were available for yeast mannoproteins. The Committee assumed that mannoproteins extracted from *S. cerevisiae* in the test compound will behave similarly to those resulting from dietary exposure to the intact yeast or to other glucomannans consumed as part of a regular diet. Once mannoproteins have been hydrolysed by intestinal enzymes, the carbohydrate moiety can be fermented by intestinal microflora in the large intestine into, among others, organic acids or alcohols (den Besten et al., 2013 a,b; Bagenholm et al., 2017).

In a study using immortalized human hepatocytes (Fa2N-4 cells), a fermentation product of *S. cerevisiae* did not induce cytochrome P450 (CYP) CYP1A2 or CYP3A4 messenger ribonucleic acid or enzymatic activity and did not interfere with the induction of CYP1A2 or CYP3A4 by omeprazole or rifampin (also known as rifampicin), respectively (Schauss et al., 2012). The test article used in this study was described as the product of a proprietary fermentation process using *S. cerevisiae*, involving "both a unique substrate and a stress process". The test article, hereafter referred to as "yeast fermentate preparation", was also tested in several toxicology studies. The yeast fermentate preparation is reported to contain cell wall components, including mannoproteins, components from the medium, fermentative by-products and stress-induced metabolites. However, a more complete chemical characterization of the test article was not available.

Toxicological studies

In male rats given a daily dose of 10^8 viable cells or colony-forming units of *S. cerevisiae* RC016 by oral gavage for 60 days, no treatment-related effects were reported (González Pereyra et al., 2014).

In a 90-day study, groups of male and female rats were given 0, 30, 200 or 1500 mg/kg bw per day of a suspension of yeast fermentate preparation in water containing 1% methylcellulose. No deaths occurred, and no treatment-related changes in any of the parameters assessed at any dose were observed (Schauss et al., 2012).

Schauss et al. (2012) reported a chronic toxicity study in male and female rats administered 0, 20, 200 or 800 mg/kg bw per day of a suspension of yeast fermentate preparation in water containing 1% methylcellulose. No treatment-related or clinically relevant findings were reported in any of the parameters assessed at any dose.

A yeast fermentate preparation was negative in a bacterial reverse mutation assay and in a mouse lymphoma cell mutagenicity test (Schauss et al.,

2012). Bone marrow micronucleus and comet assays were negative in male rats given 10^8 viable cells or colony-forming units of *S. cerevisiae* RC016 daily for 60 days by oral gavage (González Pereyra et al., 2014).

No data were available regarding the carcinogenicity and reproductive or developmental toxicity of material relevant to yeast extracts containing mannoproteins.

The only study available with yeast extracts containing mannoproteins gave a negative result in a dermal sensitization study conducted on albino guinea-pigs (Richeux, 2002).

Owing to the high content of mannose in yeast, the Committee assumed that yeast mannoproteins, like other galactomannans, can interact with mannose receptors (Tizard et al., 1989). Binding of mannosylated proteins to mannose receptors is involved in various physiological mechanisms, including innate and specific immunity. The consequences of increased binding of mannoproteins to mannose receptors and the relevance of such data are still a matter of research.

Observations in humans

Yeast fermentate preparation from *S. cerevisiae* was not mitogenic in human peripheral lymphocytes (Schauss et al., 2012).

Bansal, Tadros & Bansal (2017) reported one case of allergy to beer, wine and cider resulting from immunoglobulin E reactivity to yeasts and moulds.

Assessment of dietary exposure

Yeast extracts containing mannoproteins are proposed for use at a recommended use level of 200 mg/L and at a maximum level of 400 mg/L in food category 14.2.3 "Grape wines" and its subcategories within the GSFA. Yeast mannoproteins also occur naturally in wine, as well as in other foods including bread, pastries, beer and yeast extracts, and in food supplements. The Committee evaluated the sponsor's submission and prepared international estimates of dietary exposure to yeast mannoproteins using the CIFOCOss database in combination with the recommended and maximum use levels in wine and the background occurrence of yeast mannoproteins in wine, bread, pastries and beer. No consumption data on yeast extracts and yeast-containing food supplements were available in CIFOCOss. The dietary exposure was calculated using datasets in the CIFOCOss that were related to food consumption data for adolescents (10–18 years), adults (18+ years) and the general population (ages not specified), assuming that 100% of the yeast extract was mannoproteins.

The mean background exposure to yeast mannoproteins ranged from 0.1 to 21 mg/kg bw per day. In consumers with high consumption of wine, the background exposure ranged from 2.5 to 21 mg/kg bw per day. The highest background exposures were calculated for adolescents. Addition of yeast extracts

containing mannoproteins to wine at the recommended level resulted in an increase in the mean dietary exposure to yeast mannoproteins in the datasets of less than 5% (<0.1–4.2%), resulting in a range of dietary exposure of 0.4–21 mg/kg bw per day. For consumers with high consumption of wine, the addition of yeast extracts containing mannoproteins to wine at the maximum level resulted in an increase of dietary exposure of, on average, 20%. The resulting high estimates of dietary exposure were 4.3–21 mg/kg bw per day. Dietary exposure to yeast mannoproteins was mainly (at least 90% in almost all datasets) determined by bread and pastries, due to both high consumption and a high concentration level. The additional dietary exposure to yeast mannoproteins via the consumption of yeast extract, based on FSANZ data (FSANZ, 2008), was estimated to be about 3 mg/kg bw per day.

Evaluation

The Committee noted that very few toxicity studies were available for the range of yeast extracts containing mannoproteins on the market. However, consumers are exposed to yeast mannoproteins from *S. cerevisiae* present in wine as well as in other fermented foods, including bread, pastries, beer and yeast extracts, and in food supplements. Therefore, the Committee considered that it was possible to use the available information relative to *S. cerevisiae* and its constituents for this evaluation. No indication for toxicity was identified from the available information, including the toxicological studies on one product that is poorly characterized (yeast fermentate preparation from *S. cerevisiae*). However, there were no data on reproductive and developmental toxicity or carcinogenicity for any relevant yeast preparation.

In addition to the natural presence of yeast mannoproteins in wine and the long history of consumption of yeast products in common foods, the Committee considered that the tentative product specifications for yeast extracts containing mannoproteins indicate that these do not contain chemical residues or microbiological contaminants of concern. In addition, the Committee estimated that the exposure to yeast mannoproteins due to the addition of yeast extracts containing mannoproteins to wine at the maximum level of 400 mg/L would result, on average, in a 20% increase in dietary exposure compared to the background exposure through the regular diet of 0.4–21 mg/kg bw per day, primarily driven by bread and pastries. These conservative dietary exposure estimates are based on the assumption that 100% of the yeast extracts containing mannoproteins is mannoproteins.

In considering the data and information regarding yeast and yeast-derived products, the Committee concluded that it is unlikely that there would be a health concern for the use of yeast extracts containing mannoproteins as a

food additive for oenological uses at maximum use levels up to 400 mg/L for the stabilization of wine.

The Committee noted that any change in the uses and/or use levels of yeast extracts containing mannoproteins as a food additive will require a new evaluation.

A toxicological and dietary exposure monograph was prepared.

A new tentative specifications monograph and a Chemical and Technical Assessment were prepared.

In order to remove the tentative designation of the specifications, the Committee requires chemical characterization of the product in commerce along with data to be able to complete specifications related to the use of yeast extracts containing mannoproteins in wine manufacture. The following information is required:

- Composition of yeast extracts containing mannoproteins as well as the processes used in their manufacture;
- Analytical data from five batches of each commercial product, including information related to impurities; and
- Data on concentrations of yeast mannoproteins in wine in which yeast extracts containing mannoproteins have been used.

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3.2 Revision of specifications and analytical methods

3.2.1 Microcrystalline cellulose

Microcrystalline cellulose was on the agenda of the present meeting for the revision of specifications related to its solubility in sodium hydroxide solution.

The Committee assessed the information submitted on the solubility of microcrystalline cellulose and redesignated its solubility as "Insoluble in water and ethanol. Practically insoluble or insoluble in sodium hydroxide solution (50 g/L)". The specifications were revised accordingly.

3.2.2 Silicon dioxide, amorphous

Silicon dioxide, amorphous was on the agenda at the present meeting for revisions related to pH, assay, loss on drying, loss on ignition and impurities. The Committee at its seventy-seventh meeting (Annex 1, reference *214*) evaluated silicon dioxide, amorphous as an anticaking agent. At its eightieth meeting (Annex 1, reference *223*), the Committee made the specifications tentative pending the receipt of further data and information.

The Committee at its present meeting received the requested information. The specifications were revised, and the tentative status was removed.

3.2.3 Sodium aluminium silicate

Sodium aluminium silicate was on the agenda at the present meeting for the revision of specifications. The Committee, at its eightieth meeting (Annex 1, reference *223*), made the specifications tentative and requested data on the solubility, the impurities soluble in 0.5 mol/L hydrochloric acid, and the suitability of the proposed assay method for the determination of aluminium, silicon and sodium. Information pertaining to functional uses other than anticaking agent was also requested.

At the current meeting, the Committee evaluated the data submitted for loss on ignition, impurities soluble in 0.5 mol/L hydrochloric acid and the assay.

Information received on functional uses confirmed that the substance is used only as an anticaking agent.

The specifications were revised, and the tentative status was removed.

3.2.4 Steviol glycosides

Steviol glycosides was on the agenda at the present meeting for the revision of the method of assay. The Committee at its eighty-second meeting (Annex 1, reference *230*) made the specifications for steviol glycosides tentative pending receipt of a validated method capable of assaying additional steviol glycosides, as well as supporting validation data and information from five sample batches of steviol glycosides using the proposed method. The Committee received a validated HPLC–ultraviolet (UV) method for the assay of steviol glycosides, for which reference standards are commercially available. The presence of steviol glycosides that exist in small quantities is confirmed using an HPLC–mass spectrometric method and quantified using HPLC–UV data. The Committee also received assay data for three batches of a commercial product using the proposed methods. The Committee, at its present meeting, assessed the information received and replaced the existing assay. Two additional saccharides (galactose and arabinose) have been identified in the extracts of *Stevia rebaudiana* Bertoni since the last evaluation of steviol glycosides (Annex 1, reference *230*). The Committee included the two saccharides in the definition of the specifications for steviol glycosides from *Stevia rebaudiana* Bertoni.

The Committee received additional information pertaining to enzymatically modified steviol glycosides and further comments on the ADI established by the Committee at the sixty-ninth meeting (Annex 1, reference *190*). However, the Committee noted that the data were outside the scope of the call for the current meeting and, in the interest of transparency, did not consider them at the current meeting.

The specifications were revised, and the tentative status was removed. The Chemical and Technical Assessment was also revised.

3.2.5 Sucrose esters of fatty acids

At the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016), sucrose esters of fatty acids was on the agenda of the present meeting for the revision of specifications related to solubility and to the chromatographic conditions in the assay method.

The Committee assessed the information submitted on the solubility of sucrose esters of fatty acids and revised the solubility criterion. In addition, the Committee reviewed the information submitted on the chromatographic conditions for the separation of the compounds and revised the UV integration instructions.

The specifications were revised.

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4. Future work and recommendations

General considerations

Information requirements for submissions on products derived from natural sources

The Committee emphasized that a full characterization of the products in commerce and a relevant set of biochemical and toxicological data on such products are essential in order to develop a specifications monograph and the related safety assessment.

Specific food additives

β-Carotenes

The Committee recommends that the group ADI for the sum of carotenoids, including β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters, be re-evaluated in light of evidence that shows very low absorption of β-carotene in rodents and rabbits in contrast to humans.

Jagua (Genipin–Glycine) Blue

In order to complete an evaluation of the safety of Jagua (Genipin–Glycine) Blue, the Committee recommends that additional biochemical and toxicological information, including using higher doses of the "blue polymer" and including the dimers be submitted with the following:

- Characterization of the low molecular weight components of the "blue polymer";
- A validated method for the determination of dimers; and
- Data on concentrations of dimers from five batches of the commercial product.

Metatartaric acid

In order to remove the tentative designation from the specifications, the Committee recommends that the following information on the products of commerce be submitted by December 2018:

- Characterization of the products (optical rotation, content of free tartaric acid, degree of esterification and molecular weight distribution) and the corresponding analytical methods;
- Infrared spectrum (in a suitable medium); and

- Analytical results including the above parameters from a minimum of five batches of products currently available in commerce, along with quality control data.

Tannins

In order to complete specifications for oenological tannins used as an antioxidant, colour retention agent and stabilizer in wine, the Committee recommends that the following information be submitted for evaluation:

- Composition of tannins derived from the full range of raw materials as well as the processes used in their manufacture;
- Validated analytical method(s) and relevant quality control data;
- Analytical data from five batches of each commercial product including information related to impurities such as gums, resinous substances, residual solvents, sulfur dioxide content and metallic impurities (arsenic, lead, iron, cadmium and mercury);
- Solubility of the products in commerce, according to JECFA terminology; and
- Use levels, natural occurrence and food products in which tannins are used.

Submitters are encouraged to offer a rationale for a single specifications monograph for oenological tannins covering all products or individual monographs.

Yeast extracts containing mannoproteins

In order to remove the tentative designation of the specifications, the Committee recommends that the following information be submitted for evaluation:

- Composition of yeast extracts containing mannoproteins as well as the processes used in their manufacture;
- Analytical data from five batches of each commercial product, including information related to impurities; and
- Data on concentrations of yeast mannoproteins in wine in which yeast extracts containing mannoproteins have been used.

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Annex 1

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179. Safety evaluation of certain food additives. WHO Food Additives Series, No. 56, 2006.

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181. Evaluation of certain veterinary drug residues in food (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006.

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183. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 57, 2006.

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192. Compendium of food additive specifications. FAO JECFA Monographs 5, 2009.

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196. Evaluation of certain food additives (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.

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216. Compendium of food additive specifications. FAO JECFA Monographs 14, 2013.

217. Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 988, 2014.

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219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.

220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.

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222. Compendium of food additive specifications. FAO JECFA Monographs 16, 2014.

223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.

224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.

225. Compendium of food additive specifications. FAO JECFA Monographs 17, 2015.

226. Evaluation of certain veterinary drug residues in food (Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 997, 2016.

227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.

228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 18, 2016.

229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.

230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.

231. Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.

232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.

233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives) WHO Technical Report Series, No.1002, 2017.

ANNEX 2

Toxicological information, dietary exposures and information on specifications

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Brilliant Blue FCF	R[a]	The Committee concluded that the available data support the revision of the ADI for Brilliant Blue FCF. In a long-term toxicity study in rats, a no-observed-adverse-effect level (NOAEL) of 631 mg/kg body weight (bw) per day was identified, based on a 15% decrease in mean terminal body weight and decreased survival of females at 1318 mg/kg bw per day. **The Committee established an ADI of 0–6 mg/kg bw** based on this NOAEL by applying an uncertainty factor of 100 for interspecies and intraspecies differences. The Committee noted that the conservative dietary exposure estimate of 5 mg/kg bw per day (95th percentile for children) is less than the upper limit of the ADI of 0–6 mg/kg bw established for Brilliant Blue FCF and **concluded that dietary exposure to Brilliant Blue FCF for children and all other age groups does not present a health concern.** **The previous ADI of 0–12.5 mg/kg bw was withdrawn.**
β-Carotene-rich extract from *Dunaliella salina*	N	The Committee noted that data have become available since the previous evaluation that show large differences in absorption of β-carotene between rodents and humans. The Committee considered that rodents are inappropriate animal models for establishing an ADI for β-carotene. The Committee noted that the toxicity of the other components of the β-carotene-rich d-limonene extract of *D. salina* (hereafter referred to as *D. salina* d-limonene extract) can be evaluated using the results of rodent studies. A short-term toxicity study in rats gave a NOAEL of 3180 mg/kg bw per day, the highest dose tested. No long-term toxicity or reproductive studies have been conducted. The *D. salina* d-limonene extract did not show genotoxicity or developmental toxicity. Correction of the NOAEL of 3180 mg/kg bw per day for the percentage of the algal component (20–35%) gives an adjusted NOAEL of 636–1113 mg/kg bw per day for the algal lipid component of the *D. salina* d-limonene extract. The margin of exposure for this algal lipid component is 2120–3710 using a dietary exposure of 18 mg/day (0.3 mg/kg bw per day). The Committee concluded that exposure to the algal component of the extract does not pose a health concern. The Committee noted that the total dietary exposure to β-carotene is not expected to increase when *D. salina* d-limonene extract is used as a food colour.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		The Committee concluded that there was no health concern for the use of β-carotene-rich extract from *D. salina* when used as a food colour in accordance with the specifications established at this meeting. The Committee emphasized that this conclusion applies to the use of this extract as a food colour, not as a food supplement.
Fast Green FCF	R[a]	The ADI of 0–25 mg/kg bw established previously by the Committee was based on a long-term rat dietary that identified a NOAEL of 5% Fast Green FCF (equivalent to 2500 mg/kg bw per day), the highest concentration tested. The Committee concluded that the new data that had become available since the previous evaluation gave no reason to revise the ADI and **confirmed the ADI of 0–25 mg/kg bw.** The Committee noted that the conservative dietary exposure estimate for Fast Green FCF of 12 mg/kg bw per day (95th percentile for adolescents) was below the upper bound of the ADI. **The Committee concluded that dietary exposures to Fast Green FCF for adolescents and all other age groups do not present a health concern.**
Gum ghatti	R[b]	The Committee took into account the lack of systemic exposure to gum ghatti because of its high molecular weight and polysaccharide structure, its lack of toxicity in short-term studies, the lack of concern for genotoxicity and the absence of treatment-related adverse effects in studies of gum arabic and other polysaccharide gums with a similar profile. The Committee concluded that gum ghatti is unlikely to be of health concern and **established an ADI "not specified"[c] for gum ghatti that complies with the specifications.** **The Committee concluded that the estimated dietary exposure to gum ghatti of 12 mg/kg bw per day does not present a health concern.**
Jagua (Genipin–Glycine) Blue	N,T	The Committee noted that the highest doses tested in two 90-day toxicity studies in rats and dogs were only 330 and 338 mg/kg bw per day (expressed on a "blue polymer" basis[d]), respectively. The Committee was concerned that the possible effects of the low molecular weight component of the "blue polymer" that could be absorbed were not adequately investigated. A comparison of the dietary exposure estimate (11 mg/kg bw per day) with the NOAEL from the 90-day studies of oral toxicity in rats and dogs (approximately 330 mg/kg bw per day) gives a margin of exposure of about 30. Because of the limited biochemical and toxicological database and the low margin of exposure, **the Committee was unable to complete the evaluation for Jagua (Genipin–Glycine) Blue.**

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Metatartaric acid	T	As metatartaric acid undergoes enzymatic hydrolysis to tartaric acid prior to systemic absorption, the biochemical and toxicological data on tartaric acid considered at previous meetings are relevant to the safety assessment of metatartaric acid. Previously evaluated and new studies suggest no change to the group ADI previously established for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid. **The Committee concluded that metatartaric acid (when used in winemaking) should be included in the group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid.** The Committee noted that the dietary exposure estimate for metatartaric acid for adult consumers of wine was 4% of the upper bound of the ADI **and concluded that dietary exposure to metatartaric acid in wine at the maximum use level of 100 mg/L does not present a health concern.**
Tamarind seed polysaccharide	N	The Committee noted the absence of toxicity in long-term rodent studies and lack of concern regarding genotoxicity, reproductive toxicity and developmental toxicity, and **established an ADI "not specified"[c] for tamarind seed polysaccharide.** **The Committee concluded that the estimated dietary exposure of 75 mg/kg bw per day based on proposed uses and use levels does not present a health concern.**
Tannins (oenological tannins)	–	The Committee noted that the available data do not provide clear information on which tannin sources and individual tannin compounds are present in commercially used oenological tannins and, thus, how the oenological tannins would compare to the tannins used in the submitted studies. Therefore, it is not possible to establish which studies are relevant and, consequently, the extent of the data gaps. The information on biochemical aspects is incomplete, with the implications of repeated dosing on absorption, tissue distribution and interindividual variation needing consideration. In general, there are also few data available on reproductive and developmental toxicity and/or long-term toxicity for some or all of the tannins. **In the absence of specifications and identification of the products in commerce, the Committee concluded that it was not possible to evaluate tannins used in winemaking.**
Yeast extracts containing mannoproteins	N,T	In addition to the natural presence of yeast mannoproteins in wine and the long history of consumption of yeast products in common foods, the Committee considered that the tentative product specifications for yeast extracts containing mannoproteins indicate that these do not contain chemical residues or microbiological contaminants of concern. In addition, the Committee estimated that dietary exposure to yeast mannoproteins due to the addition of yeast extracts containing mannoproteins to wine at the maximum level of 400 mg/L would result, on average, in a **20% increase in dietary exposure compared to the background exposure through the regular diet of 0.4–21 mg/kg bw per**

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		day, primarily driven by bread and pastries. These conservative dietary exposure estimates are based on the assumption that 100% of the yeast extracts containing mannoproteins is mannoproteins. In considering the data and information regarding yeast and yeast-derived products, **the Committee concluded that it is unlikely that there would be a health concern for the use of yeast extracts containing mannoproteins as a food additive for oenological uses at maximum use levels up to 400 mg/L for the stabilization of wine.** The Committee noted that any change in the uses and/or use levels of yeast extracts containing mannoproteins as a food additive will require a new evaluation.

–: no specifications prepared; N: new specifications; R: existing specifications revised; T: tentative specifications

[a] A maximum limit for manganese was added. High-performance liquid chromatography (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water or aqueous ammonium acetate.

[b] An HPLC method for the identification of the gum constituents was added to replace the thin-layer chromatography (TLC) method. One identity method, using a mercury-containing reagent, was removed. L-Rhamnose was added as one of the constituents of gum ghatti, based on current literature reports.

[c] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect; it should not conceal food of inferior quality or adulterated food; and it should not create a nutritional imbalance.

[d] "Blue polymer" refers to the blue-coloured genipin–glycine polymer and dimer content of Jagua (Genipin–Glycine) Blue.

Food additives considered for specifications only

Food additive	Specifications
Microcrystalline cellulose	R[a]
Silicon dioxide, amorphous	R[b]
Sodium aluminium silicate	R[c]
Steviol glycosides	R[d]
Sucrose esters of fatty acids	R[e]

R: existing specifications revised

[a] The Committee assessed the information submitted on the solubility of microcrystalline cellulose and redesignated its solubility as "Insoluble in water and ethanol. Practically insoluble or insoluble in sodium hydroxide solution (50 g/L)".

[b] Silicon dioxide, amorphous was on the agenda at the present meeting for revisions related to pH, assay, loss on drying, loss on ignition and impurities. The Committee at its present meeting received the requested information. The tentative status was removed.

[c] At the current meeting, the Committee evaluated the data submitted for loss on ignition, impurities soluble in 0.5 mol/L hydrochloric acid and the suitability of the proposed assay method for the determination of aluminium, silicon and sodium. Information received on functional uses confirmed that the substance is used only as an anticaking agent. The tentative status was removed.

[d] The Committee received a validated HPLC–ultraviolet (UV) method for the assay of steviol glycosides, for which reference standards are commercially available. The presence of steviol glycosides that exist in small quantities is confirmed using an HPLC–mass spectrometric method and quantified using HPLC–UV data. The Committee also received assay data for three batches of a commercial product using the proposed methods. The Committee, at its present meeting, assessed the information received and replaced the existing assay. Two additional saccharides (galactose and arabinose) have been identified in the extracts of *Stevia rebaudiana* Bertoni since the last evaluation of steviol glycosides. The Committee included the two saccharides in the definition of the specifications for steviol glycosides from *S. rebaudiana* Bertoni. The tentative status was removed.

[e] The Committee assessed the information submitted on the solubility of sucrose esters of fatty acids and revised the solubility criterion. In addition, the Committee reviewed the information submitted on the chromatographic conditions for the separation of the compounds and revised the UV integration instructions.

Annex 3

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

84th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
FAO Headquarters, Rome, 6—15 June 2017

Opening:
Philippine Room (C277) 6 June at 9.30 h

Draft Agenda

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 Agenda

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

6. C6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full Committee)

7. Evaluations

    7.1 Brilliant Blue FCF
    7.2 Carotenes from *Dunaliella salina*
    7.3 Fast Green FCF
    7.4 Gum ghatti
    7.5 Jagua (*Genipa americana*) extract
    7.6 Metatartaric acid
    7.7 Tamarind seed polysaccharide
    7.8 Tannins (oenological tannins)
    7.9 Yeast mannoproteins

8. Specifications

   8.1 Microcrystalline cellulose
   8.2 Silicon dioxide, amorphous
   8.3 Sodium aluminium silicate
   8.4 Steviol glycosides
   8.5 Sucrose esters of fatty acids

9. Other matters to be considered (general considerations)
   - Update from IPCS on risk assessment work: chemical-specific adjustment factors (CSAF), mixtures
   - Update of EHC240: (for information)
   - Development on guidance on the evaluation of genotoxicity studies
   - Updated guidance on dose–response modelling for the use in risk assessment
   - Exposure assessments

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

11. Adoption of the report

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of Certain Contaminants in Food
Eighty-third Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No.1002, 2017 (166 pages)

Evaluation of Certain Food Additives
Eighty-second Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1000, 2016 (162 pages)

Evaluation of Certain Veterinary Drug Residues in Food
Eighty-first Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 997, 2016 (110 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Eighty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 72, 2016 (162 pages)

Evaluation of Certain Food Additives and Contaminants
Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 995, 2016 (114 pages)

Safety Evaluation of Certain Food Additives and Contaminants
Eightieth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 71, 2015 (132 pages)

Evaluation of Certain Food Additives
Seventy-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 990, 2015 (124 pages)

Safety Evaluation of Certain Food Additives
Seventy-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 70, 2015 (369 pages)

Evaluation of Certain Veterinary Drug Residues in Food
Seventy-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 988, 2014 (127 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Seventy-eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 69, 2014 (241 pages)

Further information on these and other WHO publications can be obtained from
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Evaluation of certain food additives

This report represents the conclusions of a Joint FAO/WHO Expert Committee (JECFA) convened to evaluate the safety of various food additives and to prepare specifications for the identity and purity of the food additives.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of compounds on the agenda and includes information requirements for submissions on products derived from natural sources. Also described are updates on activities relevant to JECFA from the Forty-ninth Session of the Codex Committee on Food Additives (CCFA), the International Programme on Chemical Safety (IPCS) and JECFA publications. Next is a summary of the Committee's evaluations of technical, toxicological and dietary exposure data for nine food additives: Brilliant Blue FCF; β-carotene-rich extract from *Dunaliella salina*; Fast Green FCF; gum ghatti; Jagua (Genipin–Glycine) Blue; metatartaric acid; tamarind seed polysaccharide; tannins; and yeast extracts containing mannoproteins.

Specifications for the following food additives were revised: microcrystalline cellulose; silicon dioxide, amorphous; sodium aluminium silicate; steviol glycosides; and sucrose esters of fatty acids.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to all of the food additives as well as toxicological information, dietary exposures and information on specifications.

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