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1036

Evaluation of certain food additives and contaminants

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

Food and Agriculture Organization of the United Nations

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Virtual meeting, 1–12 February 2021

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List of abbreviations and acronyms

24HDR	24-hour dietary recall
ADI	acceptable daily intake
ARfD	acute reference dose
BMD	benchmark dose
bw	body weight
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CIFOCOss	Chronic Individual Food Consumption Database – Summary Statistics
CONTAM	European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain
EA	ergot alkaloid
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EPA	(United States) Environmental Protection Agency
ESI	electrospray ionization
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCID	Food Commodity Intake Database (US Environmental Protection Agency)
GC-FID	gas chromatography with flame ionization detection
GC-MS	gas chromatography–mass spectrometry
GEMS/Food	Global Environment Monitoring System, Food Contamination Monitoring and Assessment Programme
GIFT	(FAO/WHO) global individual food consumption data tool
HBGV	health-based guidance value
HPLC	high-performance liquid chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LB	lower bound
LD_{50}	median lethal dose
LC-GC-FID	on-line coupled liquid chromatography-gas chromatography-flame ionization detection
LC-HRMS	liquid chromatography-high resolution mass spectrometry
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LOD	limit of detection
LOEL	lowest observed effect level
LOQ	limit of quantification

MCV	mean corpuscular volume
ML	maximum limit
MOE	margin of exposure
MS	mass spectrometry
MTBE	methyl tertiary butyl ether
MTDI	maximum tolerable daily intake
NOAEL	no-observed-adverse-effect level
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
P97.5	97.5th percentile
PAH	polycyclic aromatic hydrocarbon
PTMI	provisional tolerable monthly intake
PTWI	provisional tolerable weekly intake
RP	reference point
SCF	EU Scientific Committee on Food
SIDS	Screening Information Dataset
SPE	solid phase extraction
TBA	tertiary butyl alcohol
TDI	tolerable daily intake
TEF	toxic equivalency factors
TLC	thin-layer chromatography
TRS	Technical Report Series
UB	upper bound
UF	uncertainty factor
UHPLC	ultra-high-performance liquid chromatography
UL	upper intake level
USA	United States of America
WHO	World Health Organization

1. Introduction

The ninety-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was convened by video-conference from 1 to 12 February 2021. The meeting was opened on behalf of the Director-General of the Food and Agriculture Organization of the United Nations (FAO) by Mr Jamie Morrison (Director of the Food Systems and Food Safety Division) and on behalf of the Director-General of the World Health Organization (WHO) by Mr Kim Petersen (Programme Manager, Department of Nutrition and Food Safety). Mr Morrison in his opening remarks welcomed all meeting participants, and stressed that, despite the challenges of the ongoing COVID-19 pandemic, the work of JECFA had progressed and continued to provide sound scientific advice to Codex and the Member States, largely thanks to the efforts and work of the JECFA experts. He reminded the participants about their responsibility to impart the most unbiased and best scientific advice possible, and that they had been invited to serve solely in their capacity as scientific experts to provide sound scientific advice and not as representatives of their employer or country. He closed by reiterating his sincere gratitude to all participants for providing their time and expertise to this JECFA meeting.

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

1.1 Procedural matters

Owing to the travel restrictions and lockdowns due to the COVID-19 pandemic in many countries, it was not possible to convene a physical meeting and it was instead decided to hold it online by video-conferencing. In view of the time differences in the countries of origin of the invited experts, the only possible time for a video-conference was restricted to a 4-hour time slot (12:00–16:00 CET) each day. This allowed only 40% of the usual daily length (8–10 hours) of a typical JECFA meeting. Although the experts participated fully, they noted that online meetings do not permit the necessary in-depth, robust scientific discussions that are characteristic of JECFA meetings and are therefore not a suitable substitute for face-to-face JECFA meetings. In particular, the experts felt that the online format did not foster the atmosphere of trust, inclusiveness and openness that has marked all physical JECFA meetings. The experts considered that the success of the ninety-first meeting was mainly due to the cohesion between them, which

stemmed from the trust built on the relationships they had formed during previous face-to-face meetings. The experts also decried the significant difficulty of holding any informal meetings outside the scheduled meeting times because of the widely differing time zones. They noted that such informal interactions during the physical meetings were instrumental in solving problems and discussing issues in depth, bilaterally or in small groups, and added that such informal settings often gave rise to equitable solutions to stubborn problems.

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

In recognition of the difficulties and the tremendous efforts made, the Joint FAO/WHO Secretariat expressed its deep gratitude to all the experts for their commitment and flexibility, not least as the scheduled meeting times were exceedingly inconvenient for many.

The meeting report was adopted on 25 February 2021.

1.2 Declarations of interests

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

1.3 Adoption of the agenda

The meeting agenda was adopted without any modification.

2. Contaminants

The Committee re-evaluated cadmium and evaluated the ergot alkaloids for the first time.

2.1 Cadmium (exposure assessment from all food sources)

2.1.1 Explanation

Cadmium was evaluated by the Committee at its sixteenth, thirty-third, forty-first, fifty-fifth, sixty-first, sixty-fourth, seventy-third and seventy-seventh meetings (Annex 1, references *30*, *83*, *107*, *149*, *166*, *176* and *202*). At the sixty-first and sixty-fourth meetings, the Committee noted that the estimated total mean dietary exposure to cadmium from all foods, derived from per capita data from the five GEMS/Food regional diets, ranged from 40% to 60% of the provisional tolerable weekly intake (PTWI) applicable at that time of 7 µg/kg bw. The seven commodity groups that contributed significantly to total mean dietary exposure to cadmium were rice, wheat, root vegetables, tuber vegetables, leafy vegetables, other vegetables and molluscs (40–85% of the total mean dietary exposure to cadmium across the five regional diets).

At its seventy-third meeting in 2011, the Committee re-evaluated cadmium and established a provisional tolerable monthly intake (PTMI) of 25 µg/kg bw, reflecting the long half-life of cadmium in humans. Reported national estimates of mean dietary exposure to cadmium from all foods for adults ranged from 2.2 to 12 µg/kg bw per month, or 9–48% of the PTMI. For European children up to 12 years of age, this estimate was 11.9 µg/kg bw per month or 47% of the PTMI. High percentile dietary exposures to cadmium for adults from Europe, Lebanon and the United States of America (USA) were reported to range from 6.9 to 12.1 µg/kg bw per month (28–48% of the PTMI), and from 20.4 to 22.0 µg/kg bw per month (82–88% of the PTMI) for children aged 0.5–12 years from Australia and the USA. Data on cadmium occurrence and consumption of foods containing cocoa and its derivatives were included in all 2011 estimates. Although not all estimates of dietary cadmium exposure evaluated at the seventy-third meeting reported the major contributing foods, for those estimates that did report this information, cereals and cereal products and vegetables were consistently reported as major contributors, with seafood and meat, including offal, also reported in some studies. None of the studies reported cocoa products as major contributors to dietary cadmium exposure.

At its seventy-seventh meeting in 2013, the Committee assessed dietary exposure to cadmium from cocoa and cocoa products at the request of the sixth session of the Codex Committee on Contaminants in Foods (CCCF). The

Committee considered the exposure to cadmium from foods containing cocoa and its derivatives in the context of overall dietary exposure. The estimates of mean dietary exposure to cadmium from foods containing cocoa and its derivatives ranged from 0.005 to 0.39 μg/kg bw per month or 0.2–1.6% of the PTMI across the 17 GEMS/Food cluster diets, assuming a body weight of 60 kg. Mean dietary exposure estimates for individual cocoa products based on national food consumption data ranged from 0.001 to 0.46 μg/kg bw per month or 0.004–1.8% of the PTMI. The cocoa products included were cocoa beverages, cocoa powder and other cocoa products. The highest high exposure (97.5th percentile, P97.5) was estimated at 12 μg/kg bw per month for European children 7–11 years of age solely due to the consumption of cocoa powder. Combining the highest P97.5 dietary exposure estimate for adults and children out of the three cocoa products with the mean dietary exposure estimates for both age groups from the whole diet, the Committee estimated a total dietary exposure of 7.4–17.2 μg/kg bw per month or 30–69% of the PTMI for adults and 23.9 μg/kg bw per month or 96% of the PTMI for children aged 0.5–12 years. The Committee noted that these estimates of total dietary cadmium exposure very likely overestimated the exposure, because the estimates from the whole diet also included a contribution from cocoa and cocoa products.

At the request of the thirteenth session of CCCF for more comprehensive occurrence data for cadmium in food, the JECFA Secretariat issued a call for data on cadmium in chocolate and cocoa-derived products in 2019. The submissions included a wider geographical range of occurrence data for cadmium in cocoa products than considered at the seventy-seventh meeting of the Committee. The occurrence data also showed a higher mean concentration for cadmium in cocoa products than previously noted by the Committee. As a result, the JECFA Secretariat considered it appropriate to revise the dietary exposure assessment of cadmium to include not only chocolate and cocoa products but also the contribution from all food sources. At the present meeting the Committee reassessed cadmium exposure to include the contribution of all food sources, particularly cocoa products.

2.1.2 Data submitted or available to the Committee

The GEMS/Food contaminants database was queried for records relating to cadmium in any food. The database query was restricted to records submitted since the previous assessment of dietary cadmium exposure from the whole diet by the Committee in 2011. Data submitted since 1 January 2011 originated from 27 countries or country groups (WHO European Region, WHO African Region), representing 10 of the 17 GEMS/Food cluster diets. It should be noted that for several of the countries or clusters the available data were limited in quantity or

restricted to a narrow range of foods. For example, the sole country providing data from cluster G09 (Indonesia) submitted analytical results for 30 samples of cocoa products only. Five clusters (G07, G08, G10, G11 and G15) cover the countries of Europe; however, most of the contaminant concentration data available for these countries were only identified at the level of the WHO European Region and it was not possible to examine differences in contamination profile between these clusters using these data.

The final data set contained 277 292 records, of which 216 373 (78%) were from the WHO European Region. A considerable body of non-European data was available for cluster G10, submitted by Canada (n = 21 501), Japan (n = 5332) and the USA (n = 5887). Records were widely spread across different food types, with the most commonly analysed food types being edible pig offal (7.3%), marine fish (6.9%) and cattle meat (3.7%).

Given the focus of the current assessment on cadmium in cocoa and cocoa products, an overview of these data as included in the dietary exposure assessment was prepared. In total, 6957 records for cocoa and cocoa products were available, representing 2.5% of all records in the final data set. These records related to five groups of cocoa products: cocoa beans (n = 108), cocoa beverage (n = 20), cocoa butter (n = 20), cocoa mass (n = 218), cocoa powder (n = 2583) and chocolate (n = 4008). As for the whole database, the main single source of records for cocoa products was the WHO European Region, accounting for 2293 records (33%).

The Committee additionally evaluated published data on dietary exposure to cadmium at a national level. Since the evaluation of cadmium at the seventy-third meeting of the Committee in 2011, several national evaluations of chronic dietary exposure have been published. The Committee evaluated 44 national studies conducted worldwide in 32 countries and a country grouping, as reported in the literature. Studies evaluated were from Australia, Bangladesh, Benin, Brazil, Cameroon, Canada, Chile, China, Denmark, Europe, France, French Polynesia, Germany, Hong Kong Special Administrative Region (SAR), Ireland, Islamic Republic of Iran, Italy, Japan, Republic of Korea, Mali, the Netherlands, New Zealand, Nigeria, Poland, Serbia, Spain, Sri Lanka, Sweden, Thailand, the United Kingdom, the USA and Viet Nam. Evaluation was restricted to studies that included most of the foods commonly eaten in the country.

Given the large number of national estimates of dietary cadmium exposure available from the literature, their coverage of countries across the world, and their consistency, the Committee considered that deriving less refined international and national estimates of dietary exposure was inappropriate. The GEMS/Food cluster diets were used only to examine the contribution of cocoa products to dietary cadmium exposure.

2.1.3 National estimates of dietary exposure

The mean dietary exposure to cadmium from the total diet at a national level ranged from 0.6 μg/kg bw per month for adults in the Sikasso region of Mali (2.4% of the PTMI) up to 24 μg/kg bw per month in children aged 4–11 years in China (96% of the PTMI). The maximum reported high percentile estimate of dietary cadmium exposure was 66 μg/kg bw per month in boys aged 8 years from Australia (260% of the PTMI). However, this estimate was based on a 1-day 24-hour dietary recall (24HDR), which may have inflated the high percentile estimate. The highest high percentile estimate of dietary cadmium exposure based on multiple-day dietary records was for children aged 4–11 years in China (48.2 μg/kg bw per month; 190% of the PTMI). High percentile estimates of adult dietary cadmium exposure were only occasionally above the PTMI and were typically 20–60% of the PTMI. The main sources of cadmium exposure were grain and grain-based products, vegetables, and fish and seafood.

2.1.4 Temporal trends in dietary cadmium exposure

Due to differences in study design and study location, it is not possible to identify any trends in dietary exposure to cadmium across the Committee evaluations (sixty-first, sixty-fourth, seventy-third and current). Most studies continue to report estimated mean dietary exposure to cadmium approximately in the range of 10–40% of the health-based guidance value (HBGV), and sometimes higher. Similarly, the major foods contributing to dietary cadmium exposure have not changed, with cereals, vegetables and seafood, especially molluscs, being consistent major contributors across evaluations. None of the Committee's evaluations have identified cocoa products as major contributors to dietary cadmium exposure.

2.1.5 Contribution of cocoa products to dietary exposure

Where included in the published national estimates of dietary exposure, the contribution of cocoa products to the total mean dietary exposure to cadmium ranged from 0.2 to 9%.

Further estimates of the contribution of cocoa products to dietary cadmium exposure were derived using the GEMS/Food cluster diets and global estimates of mean concentrations of cadmium derived from all extracted data in the GEMS/Food contaminants database (277 292 records). Across cluster diets, cocoa products contributed 0.1–5.9% of dietary cadmium exposure. Clusters with the highest contributions to dietary cadmium exposure from cocoa products were the "westernized" clusters (G07, G08, G10 and G15), including predominantly European and North American countries. Contributions for these clusters ranged from 3.4–5.9%, with the greatest contribution for G07. These contributions reflect the higher consumption of chocolate and, more particularly, cocoa powder in the

countries within these clusters, as the cadmium concentrations in foods were assumed not to differ between clusters.

The major producers of cocoa are African countries (Cameroon, Côte d'Ivoire, Ghana and Nigeria), Indonesia and South and Central American countries (Brazil, Colombia, Dominican Republic, Ecuador and Peru). These countries are represented by the clusters G03, G05, G09 and G13. Interestingly, cocoa products were generally very low contributors to dietary cadmium exposure (<1%) in these regions.

The potential impact on the contribution of cocoa products to dietary cadmium exposure of consuming products sourced from a single geographical region (GEMS/Food cluster) was explored for the cluster diet (G07) with the greatest contribution from cocoa products to cadmium exposure. In addition, sufficient information for such an analysis was also available from the European dietary exposure assessment, carried out by the European Food Safety Authority (EFSA) *(1)*. Based on these data, the Committee conducted a more detailed analysis of the impact of consumption of cocoa products from a single geographical region on dietary cadmium exposure for different age groups in Europe. The results of these analyses are summarized in Table 1. This analysis suggests that there are potential scenarios under which cocoa products would be the main contributor to dietary cadmium exposure.

2.1.6 Impact of established and proposed maximum limits for cadmium on cocoa product rejection rates and dietary cadmium exposure

The Codex Alimentarius *General Standard for Contaminants and Toxins in Food and Feed* includes maximum limits (MLs) for cadmium in:

- chocolate containing or declaring ≥ 50% to < 70% total cocoa solids on a dry matter basis of 800 µg/kg; and
- chocolate containing or declaring ≥ 70% total cocoa solids on a dry matter basis of 900 µg/kg.

At the thirteenth meeting of CCCF in 2019, further MLs were discussed and it was proposed to derive MLs proportional to the cocoa solids content of the cocoa products:

- ML of 300 µg/kg for chocolates containing or declaring <30% total cocoa solids on a dry matter basis;
- ML of 500 µg/kg for chocolates containing or declaring ≥30% to <50% total cocoa solids on a dry matter basis; and
- ML of 1500 µg/kg for cocoa powder (100% total cocoa solids on a dry matter basis, sold for final consumption).

Table 1
Impact of the source of cocoa products consumed on the contribution of cocoa products to dietary cadmium exposure, GEMS/Food cluster G07 and European countries

	Contribution of cocoa products to dietary cadmium exposure (%) dependent on the source of cocoa products consumed			
Population[a]	**All[b]**	**G03[c]**	**G05[c]**	**G09[c]**
Literature national estimate	0.1–9.4			
Cluster G07[c]	5.9	0.9	9.8	3.8
European countries				
Infants	0.2	0.1	1.3	0.4
Toddlers	4.2	1.2	19.7	7.0
Other children	9.4	3.9	39.4	17.6
Adolescents	9.4	4.2	39.5	17.9
Adults	4.6	1.4	21.1	7.6
Elderly	2.6	0.7	12.6	4.2
Very elderly	2.8	0.8	13.7	4.7

[a] Infants: 12 weeks–11 months; toddlers: 12–35 months; other children: 3–9 years; adolescents: 10–17 years; adults: 18–64 years, elderly: 65–74 years; very elderly: ≥ 75 years.
[b] For the GEMS/Food cluster G07, "all" refers to the total data set on cadmium concentrations in cocoa products submitted to the GEMS/Food contaminants database. For literature and European estimates *(1)*, "all" refers to the cadmium concentration data used in the original analyses.
[c] Cluster G03 includes African countries, G05 includes mainly South and Central American countries, G09 includes mainly South-East Asian countries, and G07 includes mainly European countries, Australia, Bermuda and Uruguay.

Of the 4008 records in the GEMS/Food contaminants database related to chocolate, it was only possible to establish the percentage of cocoa solids for 638 (15.9%). These records were virtually all from countries in cluster G05 (South/Central America). The proportion of samples that exceeded the established or proposed ML ranged from 2.1% for chocolate with a ≥30 to <50% cocoa solids content to 16.3% for cocoa powder. Virtually all cocoa powder samples with cadmium concentrations above the ML were from countries in cluster G05 (South/Central America), resulting in a substantially higher potential rejection rate for cocoa powder samples from this cluster (405 of 1345 samples, 30.1%).

A summary of potential rejection rates for chocolate and cocoa powder from application of established and proposed MLs and the impact of applying the MLs on mean cadmium concentrations is provided in Table 2.

Using the data across all clusters with sufficient information to allow application of the MLs, the mean contribution of cocoa products to dietary cadmium exposure was 2.2% without application of the MLs and 1.5% with application of MLs (see Table 3). Application of the MLs resulted in a mean reduction in dietary cadmium exposure of 0.7% across all clusters with reductions ranging from 0.0% (cluster G16) to 2.4% (cluster G07).

Application of the MLs had the greatest impact on dietary cadmium exposure when it was assumed that cocoa powder was sourced entirely from

Table 2
Proportion of chocolate samples in different cocoa solids content classes and cocoa powder from different sources exceeding the established or proposed Codex maximum limit (ML) and the impact on mean cadmium concentration (medium bound)

	Chocolate, classified by cocoa solids content (%)[a]				Cocoa powder			
	<30	≥30 to <50	≥50 to <70	≥70	All	G03	G05	G09
ML (µg/kg)	300	500	800	900	1500	1500	1500	1500
Number of samples	114	187	251	86	2583	74	1345	9
Number of samples with cadmium concentration > ML (%)	3 (2.6)	4 (2.1)	27 (10.7)	4 (4.7)	420 (16.3)	0 (0.0)	405 (30.1)	0 (0.0)
MB mean, all samples	121	180	474	318	971	141	1600	609
MB mean, sample ≤ ML only (µg/kg)	110	172	418	255	502	141	814	609

G03: mainly African countries; G05: mainly South/Central American countries; G09: mainly South-East Asian countries; LOD: limit of detection; MB: medium bound, analytical results below the limit of detection (LOD) are substituted by a value equal to LOD/2.
[a] Samples for which the cocoa solids content was available were almost all from countries in cluster G05.

countries in cluster G05. This is not surprising as, for clusters G03, G05 and G09, only cocoa powder samples from cluster G05 had cadmium concentrations above the ML (30.1%, see Table 2). For cocoa products sourced from countries in clusters G03 and G09, application of the MLs had a negligible impact on dietary cadmium exposure, as the changes in exposure were only due to changes in the mean cadmium concentration for chocolate. The results of these analyses are summarized in Table 3.

2.1.7 Evaluation

The Committee assessed information related to exposure to cadmium from all food sources, with a particular focus on cocoa products. Information assessed was restricted to the period since the previous assessment of dietary exposure to cadmium in 2011. The Committee summarized dietary cadmium exposure estimates from 44 national studies conducted worldwide in 32 countries and a country grouping as reported in the literature. The mean dietary exposure to cadmium from the whole diet ranged from 0.6 µg/kg bw per month (2.4% of the PTMI) for adults in the Sikasso region of Mali up to 24 µg/kg bw per month (96% of the PTMI) in children aged 4–11 years in China. These children from China also had the highest high percentile estimate of dietary cadmium of 48.2 µg/kg bw per month (190% of the PTMI). High percentile estimates of adult dietary cadmium exposure were only occasionally above the PTMI and were typically 20–60% of the PTMI. Consistent with the previous evaluations of the Committee, the present evaluation identified the main sources of dietary cadmium exposure

Table 3

Impact of maximum limits (MLs) for cadmium in chocolate and cocoa powder and source of cocoa products on potential rejection rates and the contribution of cocoa products to dietary cadmium exposure for GEMS/Food cluster diets

Source of cocoa products[a]	Potential rejection rate (%) for cocoa powder samples from application of ML[b]	Mean contribution (range) of cocoa products to dietary cadmium exposure, GEMS/Food cluster diets (%)		Mean reduction (range) in dietary cadmium exposure due to application of MLs, GEMS/Food cluster diets[c] (%)
		Without MLs applied	With MLs applied	
All[d]	16.3	2.2 (0.1–6.6)	1.5 (0.1–4.3)	0.7 (0.0–2.4)
Cluster G03	0.0	1.1 (0.0–2.9)	1.1 (0.0–2.6)	0.1 (0.0–0.3)
Cluster G05	30.1	2.9 (0.2–9.3)	1.9 (0.1–5.7)	1.1 (0.0–3.8)
Cluster G09	0.0	1.7 (0.1–5.0)	1.6 (0.1–4.8)	0.1 (0.0–0.3)

ML: maximum limit, both proposed and established MLs were applied in this analysis; G03: mainly African countries; G05: mainly South/Central American countries; G09: mainly South-East Asian countries.

[a] Cocoa products included in the GEMS/Food cluster diets are cocoa beans, cocoa butter, cocoa mass, cocoa powder and chocolate.

[b] Potential rejection rates for chocolate are not given, as submitted data with sufficient information to allow application of MLs were only received from countries in cluster G05. The total rejection rate for chocolate samples was 4.9%.

[c] The percentages in this column are the percentage decreases in the estimated dietary cadmium exposure due to application of the MLs, rather than the difference in the contribution from cocoa products.

[d] "All" refers to the total data set on cadmium concentrations in cocoa products submitted to the GEMS/Food contaminants database with sufficient information to apply the MLs.

in these national studies as cereals and cereal-based products, vegetables, and fish and seafood. Of the 44 studies reviewed, only nine reported the contribution of cocoa products to the total mean dietary exposure to cadmium, which ranged from 0.2 to 9%.

Given the large number of national estimates of dietary cadmium exposure available from the literature, their coverage of countries across the world, and their consistency, the Committee considered that deriving less refined international and national estimates of dietary exposure was unnecessary.

Based on data on the concentration of cadmium in foods submitted to the GEMS/Food contaminants database since 1 January 2011, the Committee examined the contribution of cocoa products to the mean dietary exposure to cadmium using the GEMS/Food clusters diets. Analyses using these data showed that the contribution of cocoa products to the dietary exposure to cadmium was consistent with the estimates based on national dietary exposure studies, ranging from 0.1% to 5.9%. The highest contributions were calculated for European and North American countries, reflecting the higher consumption of chocolate and cocoa powder in these countries.

The potential impact of consumption of cocoa products from a single geographical region, as represented by GEMS/Food clusters, was examined. For the cluster with the greatest contribution to dietary cadmium exposure from cocoa products (G07, mainly European countries, 5.9%) this contribution would

decrease to 0.9% or increase to almost 10% if cocoa products were sourced only from countries in cluster G03 (Africa) or G05 (South/Central America), respectively. The Committee carried out a similar analysis using data (mean concentrations of cadmium in cocoa products, dietary cadmium exposure estimates and contributions of cocoa products to dietary exposure) for European countries reported by EFSA *(1)*. In the EFSA study, the age group with the greatest contribution to dietary cadmium exposure from cocoa products was children aged 3–9 years (contribution 9.4%). From the Committee's analysis, if this age group were to consume cocoa products sourced solely from cluster G03 (Africa), dietary cadmium exposure would decrease modestly (16.8 to 15.8 μg/kg bw per month), while the contribution from cocoa products would decrease to 3.9%. If this group were to consume cocoa products sourced solely from cluster G05 (South/Central America), dietary cadmium exposure would increase to 25.1 μg/kg bw per month, with cocoa products contributing 39% of dietary cadmium exposure.

CCCF has proposed MLs for chocolate with proportions of total cocoa solids of <30% and ≥30% to <50% on a dry matter basis and for cocoa powder with 100% total cocoa solids on a dry matter basis. These MLs are proposed in addition to existing MLs for chocolate with ≥50% to <70% and ≥70% total cocoa solids on a dry matter basis. Cocoa solids content information was available for a limited subset (15.9%) of the chocolate records in the GEMS/Food contaminants database. Comparing the cadmium concentrations in chocolate and cocoa powder in the GEMS/Food contaminants database to the existing and proposed MLs showed that 2.1–10.7% of the chocolate samples and 16.3% of the cocoa powder samples had concentrations higher than the MLs and could potentially be rejected by importing countries through application of the MLs. Applying these MLs compared to not applying them resulted in an average decrease in the contribution of cocoa products (including also cocoa beans, cocoa butter and cocoa mass) to the dietary exposure to cadmium of 0.7% across all clusters.

At its seventy-third meeting in 2011, the Committee established a PTMI of 25 μg/kg bw, reflecting the long half-life of cadmium in humans. The PTMI was not reviewed at the current meeting. The national exposure estimates were predominantly below this PTMI, with some exceptions for young children or adults living in China. The Committee noted that the current JECFA PTMI for cadmium is based on long-term bioaccumulation in the kidney, with steady-state not achieved until after 45–60 years of exposure. The Committee concluded that dietary exposure above the PTMI for limited periods may be of lesser concern in younger age groups. However, there may be a health concern in areas where the cadmium exposure during adulthood exceeds the PTMI.

The Committee concluded that major contributors to dietary cadmium exposure were cereals and cereal products, vegetables and seafood. The

contribution of cocoa products to dietary cadmium exposure was minor in comparison (0.1–9.4% for national studies and estimates based on GEMS/Food cluster diets), even in countries in which the consumption of cocoa products is relatively high.

Application of both established and proposed MLs for chocolate and cocoa powder may result in substantial rejection rates (up to 30%) for products from some regions, but has only a minor impact (mean decrease across clusters of 0.7%, range 0.0–2.4%) on total dietary cadmium exposure.

A dietary exposure monograph was prepared.

Reference

1. European Food Safety Authority. Scientific report of EFSA: Cadmium dietary exposure in the European population. EFSA J. 2012;10:2551.

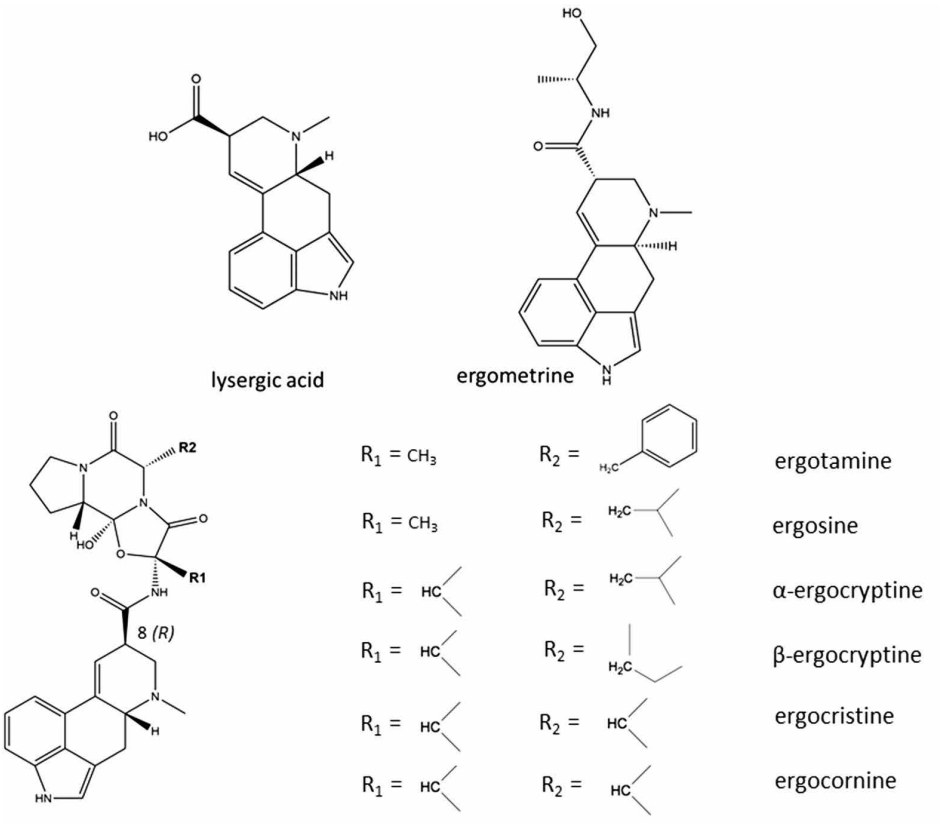
2.2 Ergot alkaloids

2.2.1 Explanation

Ergot is a disease of plants caused by fungi of the genus *Claviceps*. Ergot also refers to the typically elongated fungal structure, technically known as a sclerotium (plural sclerotia), which replaces kernels on grain ears or seeds on grass heads of diseased plants. There are 40 known species of *Claviceps*; the species infecting hosts relevant for the food chain are primarily *C. purpurea* (ubiquitous, infects grasses and cereals such as rye, wheat and triticale), *C. africana* (infects sorghum) and *C. fusiformis* (infects only pearl millet).

Ergot alkaloids (EAs) are a group of toxic fungal metabolites (mycotoxins) produced by *Claviceps* spp., in sclerotia. Structurally, EAs are closely related to biogenic amines such as norepinephrine, dopamine and serotonin. The EAs are characterized by the ergoline ring system consisting of four fused rings in which position N6 carries a methyl group, and there is a double bond at either C8,9 or at C9,10. Substitution at C8 gives rise to the naturally occurring range of alkaloids. Of those considered in this evaluation, ergometrine (ergonovine) is a simple lysergic acid derivative and the others are peptide alkaloids (known as ergopeptines or ergopeptides), in which the substituent at C8 is a cyclized tripeptide (Fig. 1). Based on the EAs identified in sclerotia of *Claviceps* spp. and occurrence data, the Committee concluded that the assessment should focus on the (*R*)-epimers: ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (a mixture of α- and β-isomers), ergocornine, and the corresponding (*S*)-inine

Fig. 1
Chemical structures of lysergic acid, ergometrine and selected peptide ergot alkaloids



epimers. Ergotamine and ergometrine have been used in human medicine for the treatment of migraine headache as well as management of the third stage of labour and postpartum blood loss.

EAs have not previously been reviewed by JECFA. The Committee evaluated EAs at the present meeting in response to a request from the CCCF in 2016.

The search for biological data followed JECFA guidance on conducting a comprehensive literature review (2017). For animal data, the search was performed in PubMed and Scopus (first search in May 2020, updated in October 2020 and January 2021). For human data, PubMed was searched for records from 2010 to December 2020 to identify studies of the adverse effects in humans associated with the main EAs used in medicine. For analytical methods, sampling, processing and decontamination, the search was performed on Web of Science,

PubMed and Scopus as well as official sources and Google Scholar. The literature search on occurrence and dietary exposure to EAs was performed using PubMed and Scopus from 2000 to December 2020.

2.2.2 Biochemical aspects

No reports of kinetic studies are available for most of the naturally occurring EAs (e.g. ergosine, ergocristine, ergocryptine and ergocornine). However, for ergotamine and ergometrine some human and limited animal data were available.

Absorption following oral administration of radiolabelled ergotamine in rats and Rhesus monkeys ranged from 40% to 10%, respectively *(1, 2)*. Following absorption in rats and Rhesus monkeys, ergotamine was excreted mostly in bile (33% and 24%, respectively) whereas only a small amount (9% and 7%, respectively) was present in urine *(1, 2)*. In humans, excretion of various radiolabelled EAs including some dihydro-derivatives in urine, was low (range 1 to 5%) *(3–7)*. No EAs were detected in cow's milk following consumption of ergot-contaminated feed (equal to 4.1–16.3 µg total alkaloids/kg bw per day) *(8)*.

Apart from its likely presence in liver and kidneys, little is known about the distribution of EAs in other tissues and organs. No information is available on EAs administered by the oral route in laboratory animals. In mice, after intraperitoneal administration, ergotamine is found mainly in the kidneys with relatively low levels detected in liver and brainstem. Following intravenous administration in pregnant rats, radiolabelled ergotamine could be detected in the uterus, placenta and yolk-sac with minor amounts in amniotic fluid and fetal tissues. The transplacental passage of ergotamine was estimated to be around 2.8% *(9, 10)*.

In humans, most data on the pharmacokinetics come from studies involving a range of radiolabelled synthetic or semi-synthetic EAs administered to healthy volunteers. Wide variability in the pharmacokinetic parameters was observed for different alkaloids and between individuals. Following oral administration of 0.2 mg of ergometrine maleate, the elimination half-life from plasma was calculated to be 1.9 hours *(11)*. Peak plasma ergotamine levels following oral administration (1 mg/kg bw) are generally achieved 2 hours later but have been reported to occur as early as 30 minutes after administration in some studies. Absorption of ergotamine is up to 62% but bioavailability of parent compound is approximately 1% or less due to extensive metabolism during its passage through the intestinal wall and liver *(4, 5, 12, 13)*.

There is no information on the likely presence of ergometrine in the milk of lactating women *(14)*. However, for the analogue methylergometrine, up to 1.3 µg/L was present in the milk of lactating women after oral administration of 0.25 mg/day *(15, 16)*.

Only limited data are available on the metabolic pathway of EAs either in humans or in laboratory animals. Ergotamine metabolism occurs largely through undefined pathways but is likely to involve cytochrome P450 3A4 (CYP3A4), an important phase I drug-metabolizing enzyme in humans. The evidence comes from co-administration of therapeutic compounds known to be potent CYP3A4 inhibitors, such as clarithromycin *(17)* and ritonavir *(18)*. Both are reported to be associated with ergotism following co-administration with ergotamine. The most likely biotransformation of the ergopeptine alkaloids involves opening the tricyclic amino acid ring structure at the proline moiety *(2)*. Maurer & Frick *(6)* have proposed that in humans dihydroergotamine undergoes oxidation of the peptide moiety. After a single oral administration of dihydroergotamine to healthy volunteers, the plasma levels of 8'-OH-dihydroergotamine were several times greater than those of the parent compound *(19, 20)*. This metabolite, 8'-OH-dihydroergotamine, has been shown to have approximately the same potency as dihydroergotamine for vasoconstrictive activity in human volunteers *(21)*.

2.2.3 Toxicological studies

Oral median lethal dose (LD_{50}) values are available for some of the naturally occurring EAs, namely ergometrine, ergotamine, ergocornine, ergocryptine, ergostine and ergonine *(22)*. Oral LD_{50} values range from 150–3200 mg/kg bw for mice, rats and rabbits, with the exception of ergometrine in rabbits (27.8 mg/kg bw). Based on available oral LD_{50} values, ergometrine is the most toxic of the naturally occurring EAs. The clinical signs of acute sublethal poisoning relate to neurotoxicity, including restlessness, miosis or mydriasis, muscular weakness, tremors and rigidity. Tail gangrene was observed in rats 5–7 days after a single intraperitoneal exposure to a mixture of EAs (ergocornine, α- and β-ergocryptine and ergocristine) at 25 mg/kg bw. More recent studies of the effects of a single intraperitoneal administration to rats and mice of naturally occurring alkaloids *(10, 23)* also reported signs of neurotoxicity (head and whole-body shakes, reciprocal forepaw treading, lateral head weaving, flat body posture and hind limb abduction) and cardiotoxicity (bradycardia and elevated systolic and diastolic blood pressure).

Short-term toxicity (4-week) studies have been conducted in rats treated with ergotamine tartrate *(24)*, ergometrine maleate *(25)* and α-ergocryptine *(26–28)* and in pigs given feed contaminated with ergot sclerotia *(29, 30)*. A further study was conducted in pigs exposed for 50 days to feed contaminated with ergot sclerotia *(31)*.

Ergotamine tartrate at concentrations of 0, 4, 20, 100 or 500 mg/kg diet was given to five groups of six rats per group per sex for 4 weeks *(24)*. At the highest concentration (500 mg/kg diet), redness of the tail tip was seen in all

animals tested, which in some cases progressed to necrosis of the tail tip (two of the six males and three of the six females). A significant decrease in body weight and feed intake was observed in both sexes at 100 and 500 mg/kg. Slight changes in some haematological parameters were seen in the groups that received the 100 and 500 mg/kg concentrations. Increases in relative weights of some organs (heart, brain and liver) were observed in the females fed ergotamine tartrate at 20, 100 and 500 mg/kg.

Ergometrine maleate at concentrations of 0, 2, 10, 50 or 250 mg/kg diet was administered to six groups of six rats per group per sex for 4 weeks *(25)*. Two control groups were included: one received the control diet ad libitum and the other was pair-fed with the highest concentration group to determine any effects secondary to a decreased feed intake. No treatment-related clinical signs were observed during the experiment. Tail tips were not affected. Body weight was not influenced by ergometrine maleate treatment, except in females fed 10 mg/kg diet after 4 weeks of exposure, which showed a significant weight increase. Plasma glucose levels were significantly decreased in females fed 50 and 250 mg/kg diet, but not in males. T4 levels were significantly decreased in males fed 250 mg/kg diet. Prolactin was determined in serum samples taken from a limited number of animals and showed a wide interindividual variation in all groups. The authors reported that the levels were markedly decreased in the 50 and 250 mg/kg diet groups for both sexes (without statistical analysis). In females given the highest concentration, relative organ weights of heart, liver and ovaries were increased. Microscopic examination showed moderate reactive hyperplasia in enlarged mediastinal lymph nodes. Treatment-related histopathological changes were observed in the liver of males and females fed 250 mg/kg diet, with significant evidence of increased glycogen storage *(25)*.

Alpha-ergocryptine at concentrations of 0, 4, 20, 100 or 500 mg/kg diet was given to six groups of six rats per group per sex for 28–32 days *(26–28)*. Two control groups were included: one received the control diet ad libitum and the other was pair-fed with the highest concentration group. Mean body weight, body weight gain, feed intake and feed efficiency decreased in both sexes in a non-monotonic manner. In animals receiving concentrations higher than 4 mg/kg diet, significant changes were observed in some haematological parameters (decreased mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH)), serum enzyme activities (slightly increased or decreased alanine aminotransferase (ALAT), serum aspartate aminotransferase (ASAT) and gamma-glutamyltransferase (GGT)), serum urea concentrations (increased), glomerular filtration (decreased creatinine and urea clearances). Prolactin was decreased in animals of both sexes in the 20, 100 and 500 mg/kg diet groups. U-shaped changes were observed for some parameters, which might be caused by the U-shaped concentration–response relationship for feed intake, owing

to the dopaminergic properties of α-ergocryptine. This could be related to inhibition of feed intake at an intermediate concentration due to activation of satiety mechanisms in the lateral hypothalamic area and/or the activation in the forebrain of behaviours incompatible with feeding *(26)*. Microscopic examination at autopsy revealed treatment-related findings in the kidneys (nephrosis), liver (atrophy, glycogen storage), thymus (atrophy), tail (muscular degeneration), ovaries (atrophy) and uterus (atrophy). The muscular degeneration in the tail was assumed to be due to the vasoconstrictive properties of ergocryptine. Ergocryptine influenced carbohydrate metabolism and affected thyroid and pituitary function *(27)*.

From the available studies in rats, the Committee concluded that there is no major potency difference in the subacute toxicity of ergotamine, ergometrine and α-ergocryptine *(24, 25, 27, 28)*. The most prominent effect of ergotamine and ergocryptine is vasoconstriction, whereas the most prominent effect of ergometrine is smooth muscle contraction.

Feed contaminated with 0, 1.2 or 2.5 g of sclerotia/kg was given to three groups of 24 weaned piglets for 28 days *(30)*. Based on the mean feed intake and body weight, the doses of total EAs were 159 and 83 μg/kg bw per day after 2 and 4 weeks, respectively for the animals given the low dose. The most abundant alkaloid was ergotamine, followed by ergosine, ergocristine and their corresponding -inine epimers. During the experimental period, the daily feed intake of animals exposed to the higher dose of ergot was reduced by about 18% in comparison with that in the control group. This reduction in feed ingestion was associated with a decrease in animal weight gain. Exposure to ergot led to mild to moderate lesions of the liver and the jejunum of the pigs. In the liver, tissue disorganization of hepatic cords, inflammation and vacuolation of hepatocytes, megalocytosis and necrosis were the main morphological alterations contributing to a significant increase in the liver lesion score. The main histological changes observed in the jejunum were villi atrophy, oedema of lamina propria and cytoplasmic vacuolation of enterocytes. Animals exposed to the higher dose of ergot displayed a significant increase of the lesion score in the jejunum compared to control animals.

Wheat ergot sclerotia were added to a basal diet at concentrations of 0, 1.04, 2.07, 5.21, 10.41 and 20.82 mg/kg for total alkaloids and fed to 32 weaned pigs per concentration for 28 days. The most abundant alkaloid was ergocristine, followed by ergotamine, ergosine, ergocryptine and ergocornine. Pigs fed the highest concentration gained 82% and 38% less weight than the control animals in weeks 1 and 2 respectively, and body weight on day 28 was significantly reduced. EAs decreased average daily feed intake and feed efficiency over the entire period, but average daily feed intake was not affected during the initial 14 days. EAs significantly decreased serum prolactin in all treated groups as

measured in samples collected on day 28 *(29)*. The Committee noted that there was no dose–response relationship in the prolactin reduction *(29)*.

Barley ergot sclerotia were added to a basal diet (0.0, 0.225, 0.45, 0.9 or 1.8 g ergot sclerotia/kg diet) and fed for 50 days to 10 weaned pigs, paired one male to one female *(31)*. Ergot sclerotia contained 2.27 g alkaloid/kg, mainly ergocristine, followed by ergotamine, ergocristinine, ergocryptine, ergometrine, ergosine and ergocornine. Pigs fed the highest concentration of ergot were less efficient at conversion of feed to weight gain, and animals receiving the higher levels of ergot were observed to be much more excitable and difficult to restrain than control animals after 3 weeks of feeding with ergot sclerotia. No other clinical signs were observed. Histopathological changes (for example, cellular vacuolation and cytoplasmic disruption) were observed in the liver, kidney and spleen. The severity of the changes was associated with the concentration of ergot in the diet.

Four groups of 10 rats per group per sex fed ergotamine tartrate at concentrations of 0, 5, 20 or 80 mg/kg diet were observed for 13 weeks *(32)*. Both body weight gain and feed intake were significantly decreased in females (but not in males) fed the high concentration (80 mg/kg diet) compared to the controls. The only treatment-related histopathological finding was muscular atrophy in the caudal longitudinal muscles of the tail of animals in groups fed the higher concentration (see Table 8). The atrophy consisted of partial or total disappearance of fibres, tinctorial changes and fibrosis. The low incidence in the control and lower concentration groups was considered by the authors to be a background level. In addition to the atrophy in the high-concentration group, degenerative changes of nerve fibres in that region were also apparent. No vascular abnormalities could be detected that might be responsible for these putative ischaemic changes.

No long-term toxicity studies of specific naturally occurring EAs (i.e. ergometrine, ergotamine, ergosine, ergocristine, ergocryptine or ergocornine) were available. In an early study *(33)*, three series of experiments were conducted with rats (3 weeks of age) fed powdered crude ergot (composition not known) in a high protein diet. In a first experiment, groups of 20 females received a diet containing 0, 1, 2 or 5% crude ergot for 6 months. In a second experiment, groups of nine males and nine females received a low protein diet with 0, 1, 2 or 5% crude ergot for 6 months. In a third experiment, groups of eight males and eight females received a low protein diet with 0 or 5% crude ergot, 5% defatted ergot, 5% ergot oil, or ergotoxine ethanesulfonate at a concentration equivalent to 5% ergot for 1 to 2 years. The body weight gain was significantly reduced at week 15 in the 5% groups compared to the controls. The pathological changes observed only in the treated animals included: neurofibromas of the ears, necrosis and calcification of the lower ends of the renal pyramids, and enlargement of the

ovaries from marked corpus luteum hyperplasia. These lesions were noted in 46, 45 and 41 of the 218 treated animals, respectively. The earliest tumour was noted after 9 months of exposure. The tumours regressed when the feeding of ergot was stopped and resumed growth when it was begun again.

In vitro and in vivo genotoxicity studies are available only for a limited number of naturally occurring EAs or their salts. Ergometrine tartrate showed no mutagenic potential in vitro in *Salmonella* Typhimurium strains *(34)*. Alpha-ergocryptine did not induce sister chromatid exchange in Chinese hamster ovary cells *(35)*. Agroclavine (a precursor compound) showed a weak mutagenic response when activated with rat liver S9 in *S.* Typhimurium strains *(36)*. However, ergometrine maleate and ergotamine tartrate induced chromosomal damage in human leukocytes in vitro *(35, 37, 38)*. Ergotamine tartrate and ergometrine maleate induced sister chromatid exchange in Chinese hamster ovary cells *(35)*. Semi-synthetic dihydrogenated derivatives (dihydroergocristine and α-dihydroergocryptine) also gave negative results in tests with *S.* Typhimurium strains *(39, 40)*.

In vivo, ergotamine tartrate showed no genotoxic potential in the micronucleus test after intraperitoneal injection to mice and Chinese hamster *(41)* and gave negative results in the dominant lethal test after intraperitoneal injection in mice *(42, 43)*. Semi-synthetic dihydrogenated derivatives (dihydroergocristine and α-dihydroergocryptine) also gave negative results in the in vivo mouse micronucleus assay after oral administration *(39, 40)*. However, ergotamine tartrate was reported to induce a significant number of chromosomal aberrations in bone marrow cells after intraperitoneal injection in mice *(44)*.

Taking all the available information into account, the Committee concluded that naturally occurring EAs do not raise concerns for genotoxicity.

In animals, EAs induce effects on ovulation, implantation, early pregnancy, and on embryonic and fetal development, resulting in abortion, high neonatal mortality, fetal malformations and growth retardation *(22, 45–52)*. The effects vary according to the EA *(22)*. The Committee noted that the effects on implantation and pregnancy maintenance in rodents are due to reduced secretion of prolactin and this is not relevant for humans at that stage of pregnancy *(53)*. EAs inhibit prolactin secretion and impair lactation in rodents and humans *(22, 54–58)*.

The pharmacological mechanisms associated with ergot toxicity are complex and have not been fully delineated *(52)*. They include peripheral vasoconstriction, peripheral adrenergic blockade, reduced secretion of prolactin and stimulation of uterine smooth muscle *(25)*.

EAs are structurally related to biogenic amines such as norepinephrine, dopamine and serotonin. This structural similarity allows EAs to interact with G-protein coupled receptors (GPCR) (e.g. dopaminergic, noradrenergic and

serotoninergic ones), as agonists and/or antagonists. The receptor affinity and selectivity, as well as the intrinsic activity (efficacy), of these compounds are highly dependent upon the substituents present at positions 1, 6, 8 and 10 of the lysergic acid moiety. In addition, the specific interaction between EAs and monoaminergic receptors appears to be organ-specific *(59)*. Ergotamine displayed high affinity for adrenergic (a1, a2), dopaminergic (D1, D2) and serotoninergic ((5-hydroxytryptamine) 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT5A, 5-HT5B and 5-HT6) receptors. Ergotamine behaves as an antagonist at adrenergic receptors, partially inhibits the 5-HT1 receptor, modulates neurotransmitter release presynaptically, and excites the 5-HT2, 5-HT3, 5-HT4, 5-HT6 and 5-HT7 receptors. Because of their structural differences from the physiological monoamine neurotransmitters, EAs are generally characterized by a low specificity and selectivity with respect to the above-mentioned neuroreceptors and, depending on the individual structure, they can display a complex behaviour as receptor agonists, partial agonists or antagonists *(60)*.

The vascular effects of EAs have been known for centuries. The occurrence of gangrene in various animal species correlates closely with the vasoconstrictor potential of the EAs *(22)*. Repeated-dose toxicity experiments with ergotamine in rats showed necrosis and fibrosis in the tail tips of animals in the high-concentration groups, explained by the vasoconstrictive properties of ergotamine *(24, 32)*.

Cherewyk et al. *(61)* showed in vitro that four (*S*)-epimers (ergocryptinine, ergocristinine, ergocorninine and ergotaminine) were vasoactive and produced a concentration-dependent arterial contractile response similar to that reported for the (*R*)-epimers. The arterial contractile response to ergotaminine was the strongest, followed by ergocorninine, ergocristinine and ergocryptinine.

Several hydroxylated metabolites were found to retain the biochemical activity and receptor-binding potential of the parent compound in vitro *(62)*.

EAs have an agonist effect on the α-adrenergic receptors. Activation of α1-adrenergic receptors produces anorexia. Reduced appetite was observed in rat studies *(24, 27, 28, 32)* and in rabbit studies *(63, 64)* and, consequently, decreased body weight.

In the uterus, EAs can play an agonist role on α-adrenergic receptors leading to oxytocic effects (promotion of uterine contractions). The activation of receptors is characterized by an increase in the three parameters of uterine contraction: frequency, amplitude and basic tone.

2.2.4 Observations in domestic animals/veterinary toxicology

EAs have negative impacts on growth (decreased feed intake and weight gain) and reproductive performance (decreased prolactin levels, lower conception

rates and birth weights (and, in males, reduced fertilization potential) of domestic animals, such as cattle, horses, pigs and sheep (reviewed by Klotz *(65)*). Gangrenous ergotism (i.e. fescue foot or fescue lameness in livestock) is one of the most acute and obvious visible effects of exposure to EAs. EAs induce vasoconstrictive responses in arteries and veins *(66–70)*. The effects of EAs on lactation vary with the livestock species. Consumption of EAs reduces milk yield in cattle, horses and sheep *(71)*. Poole & Poole *(71)* reviewed the effects of EAs on female reproduction in grazing livestock species. The effects reported included altered cyclicity, suppressed hormone secretion, reduced pregnancy rates, early embryonic loss, agalactia and reduced offspring birth weights. In cattle, reproductive failure following exposure to EAs can be attributed to altered ovarian follicle development, luteal dysfunction and reduced concentrations of circulating steroid hormones, leading to reduced pregnancy rates.

2.2.5 Observations in humans

2.2.5.1 Biomarkers

A small number of studies of EA exposure biomarkers were identified. One study measured EAs in serum and urine and collected 24HDR data that were used to estimate dietary exposure for 600 men and women participating in the European Food Consumption Validation Project *(72)*. The six EAs relevant to this report and their -inine epimers were included in the study. Overall, EAs were detected in 116 of 268 serum samples and 106 of 188 urine samples. Across all mycotoxins evaluated, only slight agreement was observed between exposure estimates and concentrations in serum or urine, suggesting that biological measurements were generally not sufficient for describing chronic dietary exposure, although they might provide useful information following single exposures. No studies of biomarkers of effect were identified.

2.2.5.2 Clinical observations

Ergotamine tartrate is used to treat migraine and cluster headaches *(73, 74)*. The usual oral dose is 2 mg (1–2 mg, and can be repeated at 30-minute intervals up to a maximum of 6 mg per day, 8 mg per attack, 12 mg per week and two courses per month) *(75)*.

The adverse effects of ergotamine are related to its vasoconstrictive properties and its effects on the central nervous system. Common side-effects at therapeutic doses, including nausea and vomiting, are well characterized. Side-effects typically occur as a result of prolonged use for migraine headaches rather than after acute single doses. In cases of prolonged use, effects consistent with ergotism have been observed (i.e. cardiovascular effects, gangrene, confusion and convulsions) *(75)*. In rare cases, pleural and peritoneal fibrosis and fibrosis

of the cardiac valves have been reported *(75)*. Recent case studies report severe cardiovascular effects (i.e. acute coronary syndrome, electrocardiogram changes compatible with myocardial infarction, valvulopathy and decompensated heart failure) and drug interactions in HIV-infected individuals on antiretroviral therapy and individuals taking macrolide antibiotics. These case reports involved the oral administration of ergotamine tartrate at therapeutic doses for several days in the case of drug interactions (e.g. 1 mg ergotamine plus 100 mg caffeine daily for 5 days *(76)*), and up to several decades in patients treated for migraine (e.g. 1–3 mg ergotamine plus caffeine for 30 years *(77)*).

Ergometrine maleate is used to induce uterine contractions and prevent postpartum haemorrhage in the third stage of labour and to treat excessive haemorrhage postpartum. Although all EAs have uterotonic effects, ergometrine (or methylergometrine) has been used clinically because it is more active as a uterine-stimulating agent than ergotamine *(78)*. The usual oral dose is 0.2 mg. This dosage can be increased to 0.4 mg, 2–4 times daily for up to 2–7 days. Uterotonic effects can be observed in women postpartum within 10 minutes after oral administration of 0.2 mg of ergometrine; however, a larger initial dose may be required given the wide variation in patient response *(78)*. Ergometrine is contraindicated in patients with cardiovascular disease, sepsis, hepatic or renal impairment, during the first stage of labour, in women with pre-eclampsia or eclampsia, and those who are at risk of preterm birth. Side-effects of ergometrine maleate, including vomiting and nausea, are well characterized at the normal therapeutic doses *(75)*. Overdosages may cause seizures and gangrene as well as more severe gastrointestinal symptoms, vascular effects, dizziness or loss of consciousness and cardiovascular effects *(75)*.

2.2.5.3 Epidemiology

Epidemiological studies report associations of overuse of ergotamine or dihydroergotamine (i.e. daily doses for 60–90 days in a period of 6 months to 1 year) with serious cardiovascular effects (i.e. ischaemic complications including angina, myocardial infarction, stroke, cerebral ischaemia and peripheral vascular disease) *(79, 80)*. Analyses of poison control data indicate that most overdoses involving oral ergotamine (including 1 mg ergotamine/100 mg caffeine tablets) have not resulted in major effects or deaths *(81)*, and that most of the symptoms reported after overdose in another study were due to interactions with CYP3A4 inhibitors *(82)*. Similarly, of 56 accidental exposures to ergotamine in children less than 7 years of age reported to the California Poison Control System, none were characterized as serious (median dose in children with mild clinical symptoms: 1 mg (range: 0.2–11 mg)) *(83)*.

One epidemiological study found an association of ergotamine (0.3 mg (tablets) to 1.5 mg (drops) for 1 day to 7 months) with low birth weight and preterm birth, but maternal smoking, a known cause of low birth weight, was not controlled for in the analysis *(84)*. No increase in the overall incidence of congenital abnormalities was found in a study of 924 children (*n*=31 abnormalities) of women who had migraine headaches, 71% of whom were said to have taken ergotamine at some time during pregnancy (month/trimester not stated) *(85)*. Two analyses, which were based on either three or six cases, from the Hungarian Case-Control Surveillance of Congenital Abnormalities dataset reported associations of ergotamine use during pregnancy with neural tube deficits *(86, 87)*.

Ergometrine may be administered orally or via the intravenous or intramuscular routes to control postpartum haemorrhage. One study was identified that examined the effects of oral ergometrine administered for this purpose *(88)*. Women in the ergometrine group received two 0.2 mg tablets or 0.4 mg total; no significant elevation of blood pressure was observed in women for whom these data were available. Poisonings in neonates accidentally administered ergometrine are described, including a fatality involving a 0.2 mg oral dose of ergometrine maleate *(89, 90)*. Of 37 cases reported to the California Poison Control System of oral ergometrine exposure in children less than 7 years of age, five resulted in symptoms, all of which were characterized as minor (median dose: 0.4 mg (range: 0.2–2)) *(83)*.

Oral preparations of sclerotia of *C. purpurea* were previously used to accelerate labour (single dose indications ranged from 0.2–3 mg with daily doses from 6–7.5 mg) *(89)*. This practice has been discontinued due to an increased risk of stillbirth.

The two most recent outbreaks of gangrenous ergotism occurred in Ethiopia. The first of these involved 2–3 months of exposure to grain with a 0.75% ergot content *(89, 91)* and the second was associated with exposure to concentrations ranging from 2.1–26.6 mg ergotamine/kg and 0.9–12.1 mg ergometrine/kg *(89, 92, 93)*. Outbreaks associated with *C. fusiformis* from contaminated pearl millet have occurred in India. The concentrations reported in unaffected villages ranged from 1–38 g ergot/kg (15–26 mg total ergot alkaloids/kg), whereas concentrations in affected villages ranged from 15–175 g ergot/kg (15–199 mg total ergot alkaloids/kg) *(89, 94, 95)*.

2.2.6 Analytical methods

The Committee reviewed the analytical methods for the determination of the 12 EAs most commonly associated with contaminated cereals, namely the lysergic acid derivative, ergometrine, and the ergopeptines, ergocornine, ergocristine,

ergotamine, ergocryptine and ergosine, as well as their -inine epimers. Although ergocryptine and ergocryptinine can occur as both α- and β-analogues, these are seldom determined separately.

EAs are generally soluble in organic solvents, charged at acid pH and uncharged at neutral or alkaline pH. Those with a C9,10 double bond have natural fluorescence properties. They are also light sensitive, and prone to epimerization at C8 during long storage and chemical analysis, requiring determination of epimers.

EAs may be extracted with non-polar organic solvents under alkaline conditions or with polar solvents under acidic conditions. They are isolated by either liquid/liquid partitioning reversed-phase solid phase extraction (SPE), strong cation exchange SPE, mixed cation/reversed-phase SPE, silica gel columns or immunoaffinity columns.

Separation of individual EAs by one-dimensional or two-dimensional thin-layer chromatography (TLC) has been only partially successful, whereas total EA content can be resolved as a single spot by high-performance TLC. Quantification is either by fluorodensitometry or by using selected spray reagents. Commercial enzyme-linked immunosorbent assays (ELISAs) are available for rapid screening of cereals for total EAs; however, questions have been raised as to whether cross-reactivity is the same for all 12 forms.

Gas chromatography may be used to determine structurally simpler clavine alkaloids and lysergic acid derivatives, but ergopeptines tend to decompose in hot injector ports and only fragments can be determined, usually by mass spectrometry (MS).

Quantitative determination of the main EAs associated with contaminated cereals is generally achieved by high-performance liquid chromatography (HPLC) with either UV/fluorescence detection or MS detection. Earlier HPLC methods were only able to identify a limited number of alkaloids, possibly owing to the lack of reference standards. Detection by natural fluorescence provides better sensitivity and selectivity with reported detection limits typically in the low µg/kg range. Recently, lysergic acid diethylamide (LSD) has been used as an internal standard for determination of the 12 EAs.

HPLC-MS has been used to obtain both quantitative results and confirmatory mass spectra. It is the instrumental technique of choice for most mycotoxin analyses and provides a platform for the development of multi-mycotoxin methods incorporating toxins of very different chemistries. For HPLC-MS/MS of EAs, reversed-phase HPLC is frequently performed with mobile phases containing volatile weak acids to provide efficient positive electrospray ionization (ESI) at the MS interface.

Matrix effects (signal enhancement or suppression) are common in HPLC-MS/MS analysis. The level of signal suppression can vary widely between

matrices and the individual EAs, even among varieties of the same cereal. Signal suppression is strongly influenced by extract purification technique and can be improved with the use of ultra-high-performance liquid chromatography (UHPLC). The use of atmospheric pressure chemical ionization rather than ESI produced strong signal enhancement and an over-estimation of EA levels. Given the lack of labelled standards, some analysts use EA calibrants prepared in an extract of toxin-free sample to account for these effects.

EAs have been included in multi-mycotoxin HPLC-MS/MS methods. The development of multi-mycotoxin analytical methods has resulted in the use of so-called "dilute-and-shoot" techniques. Another popular method is termed QuEChERS (quick, easy, cheap, effective, rugged and safe), which removes interfering substances such as lipids and pigments and is potentially followed by dispersive SPE.

2.2.7 Sampling protocols

The Committee reviewed the available information regarding sampling protocols. It noted that, in general, designing statistically-based sampling plans for mycotoxins is a complex task because of the heterogeneity of contamination. Sampling was discussed at the fifty-sixth meeting of the Committee (Annex 1, reference *152*). In the absence of sampling protocols designed specifically for EAs, sampling protocols for aflatoxin are used for EA analysis. Further investigation of their distribution in different foods is needed to develop specific sampling protocols for EAs.

2.2.8 Effects of processing

Information on the effects of various processing procedures on the levels of EAs in food comes largely from Canada, Europe and the USA where EAs frequently occur in cereal crops, particularly in rye and wheat. Contamination of cereal grains with EAs is mostly associated with fungal sclerotia and shrivelled, discoloured grains. Conventional grain-cleaning equipment (e.g. scalpers, shaker decks, gravitational separators and electronic sensor-based sorters) can reduce EA contamination. Methods using either high-velocity air cleaning of grains or electronic sensor-based handling have been shown to reduce EA levels.

In common with other mycotoxins, the milling of cereals does not destroy EAs, but merely distributes them among the milling fractions. In general, fractions intended for human food have lower levels of EAs than those intended for animal feed.

Heat treatment can reduce EA levels in final processed products; this reduction is strongly dependent on processing temperature and duration. Temperatures above 100 °C used for frying, roasting, toasting and extrusion

cooking reduce EA levels; however, thermal treatment leads to epimerization and a shift in the ratio of the -ine to the -inine forms *(96)*.

In beer brewing, the steeping and kilning steps lead to a reduction of the final EA content.

2.2.9 Prevention and control

Factors that affect the concentration of EAs in plants are poorly documented. Among the cereals, rye and triticale are the most susceptible to infection by *Claviceps* species, followed by wheat, barley and oats. Cultivar differences also play a role in susceptibility. Wild grasses growing within or outside fields are the primary source of ergot inoculum due to the wide range of hosts for *C. purpurea*. Crop rotation can be used to control EA contamination; however, this strategy is only effective if grasses are simultaneously controlled. Tillage practices can also contribute to the control of plant contamination, as deep ploughing buries sclerotia in the soil. Lastly, the use of ergot-free or certified seed improves control by preventing the introduction of a primary inoculum into fields.

There are very few studies on post-harvest control of EAs. Cleaning grain during and after harvest by removing sclerotia will reduce EA contamination.

Effective decontamination measures must be irreversible, products must be non-toxic and grain should keep its nutritional value while maintaining storability and palatability (Codex Alimentarius, 2015). Removal of sclerotia by sieving, opto-electric sorting or winnowing prior to sorting are effective physical procedures. While treatment with chemicals such as chlorine, sulfur dioxide and hydrogen chloride, or flotation in saline solution has some effect, these measures would need to be allowed for use in foods. The Committee did not find any authorized procedures in national food legislation.

2.2.10 Levels and patterns of contamination in food commodities

The GEMS/Food contaminants database contains 178 184 records for EAs submitted between 2004 and 2019 originating from four WHO regions: African Region (Benin, Cameroon, Mali and Nigeria), European Region, Region of the Americas (Canada) and Western Pacific Region (China, Hong Kong SAR, New Zealand and Singapore). No concentration data for EAs have been submitted from countries in the WHO South-East Asian and Eastern Mediterranean regions. Most of the analytical records were supplied by the European Region (83.8%), followed by the Region of the Americas (13.6%), African Region (1.4%) and Western Pacific Region (1.2%). These data represented 13 of the 17 GEMS/Food cluster diets.

All samples from Benin, Cameroon, Mali, Nigeria and New Zealand, as well as most samples from the European Region were analysed for 12 EAs (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and

the corresponding -inine epimers). Most samples from Canada were analysed for three EAs (ergocristine, ergocryptine and ergosine), whereas the samples from Hong Kong SAR (China), and Singapore were analysed for total EAs with no indication of the specific EAs included.

For 11 EAs, the highest levels were observed in food samples from the European Region: ergocristine (9279 µg/kg), ergocristinine (3538 µg/kg), ergocornine (619 µg/kg), ergocorninine (396 µg/kg), ergocryptine (661 µg/kg), ergocryptinine (1007 µg/kg), ergosine (1287 µg/kg), ergosinine (1066 µg/kg), ergometrine (760 µg/kg), ergometrinine (234 µg/kg) and ergotaminine (339 µg/kg). For ergotamine the highest level (3343 µg/kg) was observed in food samples from the Region of the Americas.

As the relative potency of individual EAs is uncertain, the Committee calculated the total EA concentration as the simple sum for all food samples for which concentration data were available for individual EAs. The UB estimate of concentration for the sum of EAs was only calculated for food commodities for which at least one quantified sample was available. The UB estimates per food sample were derived by adding quantified results to the mean of the LODs or LOQs of the individual EAs reported as not detected or not quantified. For samples with only EAs reported as not detected or not quantified, UB estimates were derived by averaging the LODs or LOQs.

The Committee did not consider food commodities for which results were reported only as "none detected" or "none quantified". Despite large differences in the number of analyses reported, the only foodstuffs that tested positive for EAs were cereals and cereal-based products and, to a lesser extent, legumes and pulses. In all regions, contamination (10.2 to 32.7% positive samples) was observed in cereals and cereal-based products, but the level of contamination was higher in the European Region (mean UB level 89.8 µg/kg) and in the Region of the Americas (mean UB level 40.4 µg/kg) than in the Western Pacific Region (mean UB level 9.8 µg/kg) or in the African Region (mean UB level 7.2 µg/kg). In the European Region and the Western Pacific Region, contamination (8.6 to 10% positive samples) was also observed in legumes and pulses at lower levels than in cereals and cereal-based products (mean UB level 11.7 µg/kg and 2.8 µg/kg, respectively). Table 4 provides a summary of data from the GEMS/Food contaminants database on the sum of concentrations of EAs by WHO region.

The highest levels of total EAs were observed in rye (13 783 µg/kg) and wheat-based-products (5649 µg/kg) from the European Region.

2.2.11 Food consumption and dietary exposure estimates

National and international assessments of chronic and acute dietary exposure to EAs reported from the scientific or grey literature or derived by the Committee

Table 4

Summary of data from the GEMS/Food contaminants database on concentrations of ergot alkaloids (EAs) by WHO region according to the food commodities used in the GEMS/Food cluster diets[a,b]

	WHO African Region				WHO European Region				WHO Western Pacific Region				WHO/PAHO Region of the Americas			
Food commodities	**No. of samples**	**% <LOD or LOQ**	**Mean LB (µg/kg)**	**Mean UB (µg/kg)**	**No. of samples**	**% <LOD or LOQ**	**Mean LB (µg/kg)**	**Mean UB (µg/kg)**	**No. of samples**	**% <LOD or LOQ**	**Mean LB (µg/kg)**	**Mean UB (µg/kg)**	**No. of samples**	**% <LOD or LOQ**	**Mean LB (µg/kg)**	**Mean UB (µg/kg)**
Cereals and cereal-based products	**59**	**89.8**	**6.2**	**7.0**	**9381**	**66.9**	**63.5**	**89.8**	**496**	**67.3**	**9.1**	**9.8**	**5593**	**72.2**	**33.0**	**40.4**
Barley	NA				84	99	1.6	11.6	11	91	49.1	49.4	264	66.3	45.9	54.8
Bran, unprocessed of cereal grain (except buckwheat, canihua, quinoa)	NA				1	100	0.0	2.0	NA				186	90.3	3.2	11.9
Bread and other cooked cereal products	9	33	40.9	43.0	2334	62.9	29.8	61.0	149	45	7.7	8.2	1425	69.1	16.9	23.9
Buckwheat	NA				110	89.1	3.7	11.5	5	100	0.0	0.5	140	95	2.3	7.8
Cereal grains NES	NA				519	95	9.5	28.7	15	100	0.0	0.5	NA			
Cereals and cereal-based products NES	NA				4261	61.4	99.4	126.1	176	81.3	3.7	4.6	612	98	0.8	6.0
Maize	16	100	0.0	0.5	123	100	0.0	16.2	9	100	0.0	0.5	449	8.4	0.4	5.7
Oats	NA				183	90.7	7.8	20.0	26	92.3	1.7	3.3	434	90.8	7.2	14.9
Rice	16	100	0.0	0.5	155	100	0.0	15.4	23	95.7	0.4	1.9	420	100	0.0	6.8
Rye	NA				910	54.1	93.4	124.6	29	34.5	15.8	16.1	132	41.7	195.0	200.5
Wheat	NA				549	83.6	15.0	33.7	18	83	3.5	3.9	1404	38.5	83.9	93.5
Wheat flour	NA				NA				17	29.4	4.6	5.2	NA			
Wheatgerm	NA				NA				3	0	464.0	464.0	NA			
White bread	NA				72	87.5	4.1	23.5	6	16.7	15.0	15.1	NA			
Wholemeal bread	NA				38	39.5	68.5	114.3	3	66.7	14.0	14.3	NA			

Food commodities	WHO African Region				WHO European Region				WHO Western Pacific Region				WHO/PAHO Region of the Americas			
	No. of samples	% <LOD or LOQ	Mean LB (µg/kg)	Mean UB (µg/kg)	No. of samples	% <LOD or LOQ	Mean LB (µg/kg)	Mean UB (µg/kg)	No. of samples	% <LOD or LOQ	Mean LB (µg/kg)	Mean UB (µg/kg)	No. of samples	% <LOD or LOQ	Mean LB (µg/kg)	Mean UB (µg/kg)
Legumes and pulses	**18**	**100**	**0.0**	**0.5**	**197**	**91.4**	**3.5**	**11.7**	**10**	**90**	**0.9**	**2.8**	**4**	**100**	**0.0**	**2.9**
Beans, shelled (immature seeds)	NA				67	91	6.5	14.2	NA				NA			
Peas (dry)	2	100	0.0	0.5	1	100	0.0	20.0	7	85.7	1.3	3.3	NA			
Soybean (dry)	NA				109	89.9	2.3	9.1	NA				4.0	100	0.0	2.9

LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; NA: not analysed; UB: upper bound.

[a] The total content of EAs in each sample was estimated by summing the reported concentrations for each of the individual alkaloids. Numbers of individual alkaloids tested were: 12 for the African Region (Benin, Cameroon, Mali and Nigeria: ergometrine, ergocornine, ergocristine, ergocryptine, ergosine, ergotamine and their corresponding -inine (*S*)-epimers); between 3 (ergosine, ergocristine, ergocryptine) and 12 (ergometrine, ergosine, ergocornine, ergotamine, ergocristine and their corresponding -inine (*S*)-epimers) for the Region of the Americas and the European Region depending on the sampling year and the food tested; either 12 for the Western Pacific Region (New Zealand; ergometrine, ergocornine, ergocristine, ergocryptine, ergosine, ergotamine and their corresponding -inine (*S*)-epimers) or expressed as EAs for China, Hong Kong Special Administrative Region and Singapore).

[b] The mean LB estimates were derived by substituting zero for analytical results below the LOD or LOQ. The mean UB estimates were derived by averaging the sum of individual EAs reported as not detected or not quantified.

Table 5
Summary of national and international LB–UB estimates of chronic dietary exposure to ergot alkaloids (EAs) from the literature or derived by the Committee

WHO region	Population group (age in months or years)	Estimated dietary exposure, mean (P90 or P95) in µg/kg bw per day [b]	Reference
National estimates			
African Region (Benin, Cameroon, Mali and Nigeria)	Adult (18 to <65 years)	<0.01–0.09 (<0.01–0.18)	Derived by this Committee
European Region (22 countries)[a]	Infants (<12 months)	0.01–0.03 (0.05–0.76)	(EFSA, 2017) *(97)*
	Toddlers (12 to <36 months)	0.03–0.47 (0.07–0.86)	
	Other children (36 months to <10 years)	0.02–0.46 (0.05–0.79)	
	Adolescents (10 to <18 years)	0.01–0.29 (0.03–0.56)	
	Adults (18 to <65 years)	0.01–0.18 (0.02–0.37)	
	Elderly (65 to <75 years)	0.01–0.14 (0.02–0.28)	
	Very elderly (over 75 years)	0.01–0.16 (0.02–0.28)	
Western Pacific Region (New Zealand)	Children (5–15 years)	0.01–0.03 (0.03–0.06)	(NZFS, 2020) *(98)*
	Adults (>15 years)	<0.01–0.01 (0.01–0.02)	
International estimates [c]			
African Region (clusters G03, G13, G16)	Adult (>15 years)	0.01–0.04 (0.03–0.09)	Derived by this Committee
Region of the Americas (clusters G05, G12)	Adult (>15 years)	0.11–0.15 (0.23–0.31)	Derived by this Committee
European Region (clusters G02, G07, G08, G10, G11, G15)	Adult (>15 years)	0.05–0.18 (0.1–0.37)	Derived by this Committee
Western Pacific Region (clusters G14, G17)	Adult (>15 years)	0.01–0.02 (0.02–0.04)	Derived by this Committee

[a] Austria, Belgium, Bulgaria, Croatia, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Lithuania, Luxembourg, Malta, Poland, Slovenia, Sweden, Switzerland, the Netherlands and the United Kingdom. Range from minimum LB to maximum UB estimates across studies.
[b] The range of dietary exposure estimates refers to LB and UB estimates of mean dietary exposure. National and international dietary exposures to EAs were reported from scientific or grey literature or derived by the Committee. The LB mean estimates were derived by substituting zero for analytical results below the LOD or LOQ. The UB mean estimates were derived by averaging the sum of individual EAs reported as not detected or not quantified. All international exposure estimates are rounded and based on a 60 kg body weight.
[c] High percentiles are an approximation of the 90th percentile dietary exposure, calculated as twice the mean dietary exposure *(99)*.

were all based on total EA concentration calculated as the simple sum of individual EAs. Table 5 provides a summary of the national and international estimates of chronic dietary exposure to EAs from the literature or derived by the Committee. The Committee evaluated published studies on chronic dietary exposure to EAs in sub-Saharan African countries (Benin, Cameroon, Mali and Nigeria), European countries and New Zealand.

Across national estimates of dietary exposure, LB–UB estimates of mean dietary exposure to the sum of EAs were in the range of 0.010–0.47 µg/kg bw per day for children and <0.01–0.18 µg/kg bw per day for adults. High percentile LB–UB estimates of dietary exposure (P95) were in the range of 0.03–0.86 µg/

kg bw per day for children and <0.01–0.37 µg/kg bw per day for adults. For all national estimates, the main foods contributing to dietary exposure were wheat and wheat-based products.

In addition to national estimates of chronic dietary exposure, the Committee derived international estimates of chronic dietary exposure to EAs using the 13 GEMS/Food cluster diets for the African Region, the Region of the Americas, the European Region and the Western Pacific Region, with the concentration data for EAs in foods from the same WHO regions and food commodities described in Table 4. Even though some clusters defined in 2013 do not map exactly to a single WHO region, the Committee considered that differences noted in clustering were limited to a small number of countries and are not likely to affect the chronic dietary exposure estimates. No international estimates were derived for clusters within WHO regions from which no data had been submitted to the GEMS/Food contaminants database. Therefore, no dietary exposure was estimated for clusters G01, G04 and G06 associated with the Eastern Mediterranean Region and cluster G09 associated with the South-East Asia Region.

LB–UB mean international estimates of chronic dietary exposure to EAs ranged from 0.01 (G14, Western Pacific Region) to 0.18 µg/kg bw per day (G02, European Region). LB–UB high estimates of dietary exposure (90th percentile, P90) to EAs ranged from 0.02 (G14, Western Pacific Region) to 0.37 µg/kg bw per day (G02, European Region). Wheat and wheat-based products were the main foods contributing to dietary exposure to EAs in these clusters (ranging from 67 to 100%). Rye and rye products also contributed to dietary exposure in these clusters, but to a lesser extent (ranging from 2 to 33%).

Except for European countries, no acute dietary exposure estimates for EAs were reported in the scientific literature. Mean acute exposure ranged from 0.02 µg/kg bw per day for "infants" up to 0.32 µg/kg bw per day for children aged 3–9 years. High estimates of acute dietary exposure (P95) to EAs ranged from 0.1 to 0.49 µg/kg bw per day for adults and from 0.13 to 0.98 µg/kg bw per day for children aged 3–9 years. The food types contributing most to the acute dietary exposure to EAs were "mixed wheat and rye bread and rolls" and "rye bread and rolls".

Since there was no match between the countries in the FAO/WHO global individual food consumption database (GIFT) and the countries that submitted data on concentrations of EAs in foods, the Committee did not derive additional national estimates of acute dietary exposure.

The Committee noted that for Europe no major differences were observed between estimates of chronic and acute dietary exposure to EAs, indicating that the main contributors to the exposure are foods consumed on a regular basis within particular populations. Considering that wheat and wheat-based products

have been identified as the main foods contributing to both chronic and acute dietary exposure to EAs in European estimates, it is likely that, for other regions in the world where wheat-based products are staple foods, the chronic exposure estimates will be comparable to acute exposure estimates.

2.2.11.1 Transfer from feed to food

The very limited data on tissue distribution and residual concentrations in edible tissues, milk and eggs provide no evidence of accumulation of EAs in edible tissues.

2.2.12 Dose–response analysis

2.2.12.1 Dose–response data in humans

Data on the use of drugs containing EAs in humans provide relevant information for assessing the acute effects of EAs, both with respect to doses with a pharmacological (therapeutic) effect and those that may have a toxic effect (Table 6). The therapeutic use of oral ergometrine maleate in management of the third stage of labour or to prevent or treat postpartum haemorrhage has largely been superseded by other drugs, sometimes in combination with ergometrine, administered by the intramuscular or intravenous routes. But from its use in the past, it is known that an oral dose of 0.2 mg ergometrine maleate has pharmacological activity. This is the lowest single dose of an EA used therapeutically; oral doses of ergotamine tartrate used for treatment of migraine are 1–2 mg at onset (up to 4–6 mg per day).

2.2.12.2 Dose–response data in animals

The key studies in experimental animals identified by the Committee are summarized in Table 7.

The Committee identified the tail muscular atrophy effect as the critical effect suitable for the hazard characterization. This tail muscular atrophy can be explained by vasoconstriction. Dose–response analyses were performed on the available information on experimental animals from the two 4-week studies on ergotamine tartrate *(24)* and on α-ergocryptine *(26)*, and from the 13-week study on ergotamine tartrate *(32)* (Table 8).

Dose–response modelling on data from experimental animals was performed by means of the benchmark dose (BMD) analysis using the US EPA software BMDS 3.2[1] (Table 8). Models available in BMDS 3.2 for dichotomous (quantal) response were used as the default set of models and Bayesian model averaging (Bayes MA) was used for the calculation of BMD confidence intervals

[1] https://www.epa.gov/bmds

Table 6

Daily oral doses of ergot alkaloids used therapeutically and doses causing adverse effects in humans

Ergot alkaloid (EA) (Effect)	Dose (as mg EA salt/person)	Dose (as mg EA/ person)	Dose (as mg EA/kg bw)	Reference
Ergometrine maleate				
Therapeutic dose, adult (Uterine contraction, vasoconstriction)	0.2	0.15	0.0025[a]	Martindale, 2010 *(75)*
Adverse effects, neonate (Vasoconstriction, CNS, respiratory, renal)[c]	0.2	0.15	0.04–0.05[b]	EFSA, 2012 *(89)*
Adverse effects, children <7 years old (Gastrointestinal symptoms)[e]	0.4 (median dose) 0.2–2 (range)	0.9–1.8	0.015–0.03[d]	Martindale, 2010 *(75)*
Ergotamine tartrate				
Therapeutic dose, adult[f] (Vasoconstriction cerebral arteries)	1–2	0.9–1.8	0.015–0.03[a]	Martindale, 2010 *(75)*
Adverse effects, children <7 years old (Gastrointestinal, CNS, respiratory symptoms)[g]	1 (median dose) 0.2–11 (range)	0.9	0.045–0.06[d]	Armenian & Kearney, 2014 *(83)*

CNS, central nervous system.
[a] Calculated for a 60 kg adult.
[b] Calculated on the basis of a body weight range at birth of 3.0–3.5 kg.
[c] Accidental administration of adult dose of ergometrine instead of vitamin K, including one death.
[d] Calculated on the basis of a body weight range at 3–7 years of age of 15–20 kg.
[e] Four cases of gastrointestinal symptoms, none serious, in 37 reports of exposure to ergometrine.
[f] Up to 6 mg/person per day.
[g] Fifteen cases of symptoms, none serious, in 56 reports of exposure.

Table 7

Summary of all critical studies of ergot alkaloids in experimental animals and their NOAELs/ LOAELs

Substances	Species	NOAELs	LOAELs	Effect at the LOAEL	Duration	Reference
Ergotamine tartrate Ergotamine[a]	Rat	4 mg/kg diet 0.34 mg/kg bw per day (♀ and ♂)	20 mg/kg diet 1.7 (♀), 1.6 (♂) mg/kg bw per day	Increased relative organ weight in ♀	4 weeks	Speijers et al., 1992 *(24)*
Ergometrine maleate Ergometrine[b]	Rat	10 mg/kg diet 0.72 (♀), 0.70 (♂) mg/kg bw per day	50 mg/kg diet 3.3 (♀), 3.4 (♂) mg/kg bw per day	Decreased plasma glucose level in ♀	4 weeks	Peters-Volleberg et al., 1996 *(25)*
α-ergocryptine	Rat	4 mg/kg diet 0.37 (♀), 0.34 (♂) mg/kg bw per day	20 mg/kg diet 1.7 (♀), 1.4 (♂) mg/kg bw per day	Increased relative organ weight in ♀	28–32 days	Janssen et al., 1998, 2000[a,b] *(26–28)*
Ergot sclerotia	Pig	Not determined	0.1 mg EAs/kg bw per day[c]	Jejunum lesions	4 weeks	Maruo et al., 2018 *(30)*
Ergotamine tartrate Ergotamine[a]	Rat	5 mg/kg diet 0.48 (♀), 0.42 (♂) mg/kg bw per day	20 mg/kg diet 1.99 (♀), 1.65 (♂) mg/ kg bw per day	Tail muscular atrophy in ♀	13 weeks	Speijers et al., 1993 *(32)*

EAs, ergot alkaloids; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.
[a] For the conversion of concentrations in diet to doses in mg/kg bw, the Committee used the molecular weights of ergotamine tartrate (1313.4 g/mol) and ergotamine (1163.4 g/mol for two molecules) and the data on body weights and feed intake available in the report.
[b] For the conversion of concentrations in diet to doses in mg/kg bw, the Committee used the molecular weights of ergometrine maleate (441.5 g/mol) and ergometrine (325.4 g/mol) and the data on body weights and feed intake available in the report.

Table 7 (continued)

[c] LOAEL of 0.1 mg/kg bw identified by the authors based on body weights and feed intake. The exposure was 0.159 and 0.083 mg/kg bw per day after 2 and 4 weeks of exposure, respectively.

Table 8
Dose–response analysis using the BMD approach (BMR = 10%) in rats (BMDS 3.2 Bayesian Model Averaging)

Concentration of ergot alkaloids in the diet	Sex	Dose mg/kg bw per day	BMD mg/kg bw per day	BMD confidence interval mg/kg bw per day	Tail effect and incidence	Reference
4-week studies						
Ergotamine tartrate		Ergotamine			Abnormality	Speijers et al.,
0, 100, 500 mg/kg diet	Females	0, 7.3, 41	16.6	5.25–64.7	0/4, 0/6, 3/6	1992 (24)
	Males	0, 7.5, 37	6.37	1.68–28.4	0/3, 2/6, 4/5	
α-Ergocryptine		α-Ergocryptine			Muscle degeneration	Janssen et al., 2000a (27)
0, 4, 20, 100, 500 mg/kg diet	Females	0, 0.37, 1.7, 8.9, 60	18.1	5.36–37.4	0/6, 0/6, 0/6, 0/5, 4/6	
	Males	0, 0.34, 1.4, 6.6, 44	3.88	1.29–9.59	0/6, 0/6, 0/6, 2/6, 6/6	
13-week studies						
Ergotamine tartrate		Ergotamine			Muscular atrophy	Speijers et al.,
	Females	0, 0.48,1.99, 7.63	1.55	0.61–3.20	0/10, 0/10, 2/10, 7/10	1993 (32)
0, 5, 20, 80 mg/kg diet	Males	0, 0.42, 1.65, 6.18	1.69	0.63–3.53	1/10, 1/10, 1/10, 7/10	

BMD, benchmark dose; BMDS 3.2, US EPA software (https://www.epa.gov/bmds); BMR, benchmark response.

(BMD-CI) following recent JECFA guidance (EHC 240 Chapter 5 in *(100)*). The lower confidence bound ($BMDL_{10}$) corresponding to a BMR_{10} for extra risk of tail muscular atrophy, was selected as the reference point for the hazard characterization.

2.2.13 Evaluation

The Committee identified the pharmacological effect of ergometrine maleate on the uterus – causing uterine contractions in humans during late pregnancy and postpartum – as the critical effect for the evaluation of EAs in the diet.

The Committee established an acute reference dose (ARfD), based on the following considerations:

1) The lowest oral therapeutic dose of 0.2 mg ergometrine maleate (equivalent to 2.5 µg/kg bw, expressed as ergometrine) is considered a pharmacological effect level in the most sensitive individuals, i.e. those with high absorption.

2) Of the EAs that have been used as drugs, ergometrine is known to have the highest potency for uterine contractions and its uterotonic effect increases towards the end of pregnancy.

In selecting an uncertainty factor (UF) for extrapolation from the pharmacological effect level at the therapeutic dose (LOEL) to a NOEL, the Committee took into consideration that the data relate to a short-lived, reversible, pharmacological effect, seen within a very sensitive subpopulation (women in late pregnancy or postpartum). A UF of 2 was considered appropriate for extrapolating from a pharmacological LOEL to a NOEL.

To derive an ARfD from a NOEL based on human data, in the absence of additional information, the default UF would normally be 10. However, for a substance that reversibly interacts with specific receptors, as is the case here, with a pharmacological effect that is predominantly dependent on its maximum plasma concentration (i.e. C_{max}), a UF for toxicokinetic differences is considered unnecessary. The Committee therefore applied the UF of 3.16 to cover possible interindividual toxicodynamic differences.

Applying a composite UF of 6.3 (2×3.16) results in an ARfD of 0.4 µg ergometrine/kg bw ($2.5 \div 6.3 = 0.4$). The Committee noted that it is appropriate to establish a group ARfD for EAs but concluded that the available data are not sufficient to establish toxic equivalency factors (TEFs) for different EAs. Therefore, the ARfD is established as a group ARfD for the simple sum of total EAs in the diet.

This ARfD would also be protective for other potentially sensitive subgroups in the population, such as children, based on similar calculations in relation to adverse effects (gastrointestinal symptoms) in that group following unintentional exposure to ergometrine maleate.

Limited data from two 4-week studies on ergotamine tartrate and α-ergocryptine in rats allowed the determination of a reference point ($BMDL_{10}$) of 1.3 mg/kg bw for EAs, based on muscular degeneration in the tail, secondary to vasoconstriction. The Committee noted that the human pharmacological effect level of 2.5 µg/kg bw and its derived NOEL provided a much more sensitive reference point for derivation of an ARfD than the $BMDL_{10}$ value from a downstream toxic effect in animals.

As a first approach to establishing a TDI, the Committee considered the data from repeated-dose animal studies and selected the lowest $BMDL_{10}$ value of 0.6 mg/kg bw per day calculated for ergotamine, based on tail muscular atrophy, secondary to vasoconstriction, observed in the 13-week study in rats *(32)* as reference point. Applying a default UF of 100 for intra- and inter-species differences, a UF of 2 for extrapolation from a 13-week study to chronic exposure

and an additional UF of 3 to take into account the limitations of the available toxicity data would indicate derivation of a TDI of 1 μg/kg bw per day.

The Committee considered that a TDI should not be higher than the ARfD and decided to establish a group TDI for the sum of total EAs in the diet at the same value as the group ARfD of 0.4 μg/kg bw per day.

The Committee noted that some estimates of the mean (0.46–0.47 μg/kg bw per day) and high percentile (0.56–0.86 μg/kg bw per day) chronic dietary exposure in children and some estimates of the high percentile acute dietary exposure in children (0.65–0.98 μg/kg bw per day) and in adults (0.49 μg/kg bw per day) exceeded the EAs group health-based guidance value (HBGV), and that this may indicate a human health concern.

Recommendations

The Committee recommended the following:

- additional data on the EAs to allow for the derivation of toxic equivalency factors (TEFs);
- additional data on the occurrence of EAs (at least for the 12 considered at this meeting) in wheat and wheat-based products and in rye and rye products from WHO regions and clusters for which no data were submitted for this evaluation;
- the establishment of sampling plans for EAs.

A toxicological monograph and an exposure assessment were prepared.

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3. Previous cargoes – solvents and reactants

3.1 Introduction

Fats and oils destined to be used as food are transported and stored in large volumes. Transportation in large volumes by sea is exempted from many land-based regulations as it is not practical to have fleets of ships solely dedicated to the transportation of food in large tanks, since the trade is generally unidirectional from producer to consumer. Furthermore, the construction and dependency on the availability of a limited number of single-use carriers would make the transport of fats and oils extremely expensive. To address the economic realities, certain types of ships are permitted to carry different classes of cargo in their tanks on their outbound and onward journeys. A non-food item may be carried in a tank in one direction and a single type of fat or oil on the further voyage. Since ships are constructed to have several individual tanks, each may contain a cargo destined for a different location and may be used to carry either a food or non-food item depending on the contract.

A number of organizations have been involved in the development of codes of practice, transportation contracts, ship construction, cargo segregation, environmental issues and food safety. The Codex Alimentarius Commission (CAC) adopted and published a code of practice for the storage and transport of edible fats and oils in bulk, which was developed by Codex Committee on Fats and Oils (CCFO) in 1987 *(1)*. At that time, CCFO recognized the need to assess the acceptability of previous cargoes transported in a tank subsequently used for the transportation of an edible fat or oil. Commercial trade contracts recognized the need to specify that certain chemicals should never be acceptable previous cargoes for subsequent cargoes of edible fats or oils. These substances formed the basis of the "banned lists" of previous cargoes. In 2001, a combined list of chemicals banned as previous cargoes was developed by CCFO and adopted by CAC *(2)*; it was added to the Codex code of practice as Appendix 1. Other substances carried in bulk were considered to pose a low risk to public health as a contaminant in edible fats or oils; these formed the basis of "acceptable lists" of previous cargoes. The development of a CCFO acceptable list of previous cargoes was also based on trade experience. A preliminary list was reviewed by the Scientific Committee on Food and their findings were reported to CCFO in 1999; 14 substances were identified for which there were insufficient data to make a safety determination. After further discussion at subsequent CCFO meetings, a list of 23 potentially safe previous cargoes that require evaluation was developed. CCFO asked for scientific advice from FAO/WHO on these 23 substances that lacked safety evaluations. Groups 2 to 5 (18 of the 23 substances) were evaluated

at the ninetieth meeting. The present evaluation by JECFA addresses the Group 1 substances (5 of the 23 substances) on the current list of chemicals acceptable as previous cargoes by CCFO.

3.2 Background

3.2.1 Global production and consumption of fats and oils

The global trade in edible fats and oils is more than 200 million metric tonnes annually and valued at approximately US$ 120 billion *(3)*. By far the largest contributors are palm (36%) and soybean oil (28%), followed by rapeseed/canola (14%), sunflower seed (10%), palm kernel (4%), peanut (3%), cottonseed (3%), coconut (2%) and olive oils (2%).

Many vegetable oils are produced in regions (for example: soybean – Argentina, Brazil, USA; rapeseed – Australia, Canada; sunflower seed – Ukraine; palm – Indonesia and Malaysia; and coconut – equatorial latitudes) far from the major sites of consumption. Olive oil is produced in regions with a Mediterranean climate in both the northern and southern hemispheres. International trade in fats and oils uses the most economical method of ocean transportation since global trade in edible fats and oils is primarily unidirectional. Soybean oil from Argentina and Brazil, for example, is shipped to both Asian and European markets, but there is unlikely to be a complementary cargo of fat or oil available for transportation in the reverse direction. Similarly, oils from tropical regions are traded globally, often without reciprocal trade in fats and oils.

3.2.2 Regulations affecting fats and oils

Shipment of fats and oils is described in numerous national and international regulations and agreements. Land-based transportation is regulated by local and national guidelines and/or legislation, whereas international trade is subject to commercial agreements, international shipping regulations and various codes of practice. The development of banned lists and acceptable lists of previous cargoes is founded on existing trade contracts.

About 85% of the fats and oils are traded globally using FOSFA (The Federation of Oils, Seeds and Fats Associations, London) contracts. The balance is traded under contracts issued by NIOP (National Institute of Oilseed Products) or other organizations. A contract under "banned list terms" requires that fats and oils are not shipped in tanks that have contained a substance on the banned list as the immediate previous cargo. For certain chemicals, this requirement is extended to the three previous cargoes. Alternatively, a contract may state that "the immediate previous cargo shall be a product on the FOSFA List of Acceptable Previous Cargoes". In this case, the receiver will only accept the cargo if the

previous cargo is on FOSFA's acceptable list. These two lists only cover a small proportion of the chemicals transported by sea; thus many substances appear on neither list and their acceptability as a previous cargo is subject to agreement by the contracting parties.

3.2.3 Global transport of fats and oils

Transportation by sea is regulated by the International Maritime Organization. The International Convention for the Prevention of Pollution from Ships (MARPOL) aims to prevent operational and accidental pollution from ships. MARPOL limits the carriage of different classes of liquid cargoes to specific tanker vessels based on ship construction and the class of chemical. Under this convention, fats and oils may not be transported in vessels designated to carry cargoes of crude oil, fuel oil, heavy diesel oil or lubricating oil. The International Code for the Construction and Equipment of Ships Carrying Dangerous Chemicals in Bulk (IBC Code) lists chemicals carried as bulk liquids, their pollution category, the type of ship design and any relevant restrictions or derogations. The previous cargoes under consideration (see Table 9) are in the medium- or low-risk categories for marine pollutants. The single exception is propylene tetramer, which is considered a high-risk marine pollutant. MARPOL also deals with tank washing and material discharge. Pentane falls into an additional category of oil-like substances requiring additional attention between cargoes.

3.2.4 The interrelationship of national, regional and trade interests

The practice of acceptable list trading was discussed in line with regional initiatives to protect consumer health. The adoption of the hazard analysis and critical control point (HACCP) principles and their inclusion in the Codex Alimentarius approach to the safe trade of food and food products can be applied to the transport of oils and fats by sea. The CAC adopted the *Code of Practice for the Storage and Transport of Fats and Oils in Bulk* developed by CCFO in 1987 (CAC-RCP 36-1987). The Code has been revised periodically and a banned list of substances was added in 2001. The list of acceptable previous cargoes adopted by the European Union (EU) and based on existing trade lists, was evaluated by EFSA.

3.2.5 Development of the Codex Code of Practice for Storage and Transport of Edible Fats and Oils in Bulk

CCFO discussions highlighted the need for lists of banned and acceptable previous cargoes. The topic of contamination by previous cargoes led to the incorporation of the FOSFA and NIOP trade lists into the Code by reference

Table 9
List of substances submitted by CCFO for evaluation by JECFA for addition to the list of acceptable previous cargoes

Substance (synonyms)	CAS number	Assessment group[a]
Acetic anhydride (ethanoic anhydride)	108-24-7	1
1,4-Butanediol (1,4-butylene glycol)	110-63-4	2
Butyl acetate, *sec*-	105-46-4	1
Butyl acetate, *tert*-	540-88-5	1
Calcium ammonium nitrate solution	15245-12-2	4
Calcium lignosulfonate liquid (lignin liquor; sulphite lye)	8061-52-7	4
Calcium nitrate (CN-9) solution	35054-52-5	4
Cyclohexane	110-82-7	1
Fatty alcohols		
iso Decyl alcohol (isodecanol)	25339-17-7	2
Myristyl alcohol (1-tetradecanol, tetradecanol)	112-72-1	2
iso Nonyl alcohol (isononanol)	27458-94-2	2
iso Octyl alcohol (isooctanol)	26952-21-6	2
Tridecyl alcohol (1-tridecanol)	112-70-9	2
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[b]		3
Methyl tertiary butyl ether (MTBE)	1634-04-4	5
Mineral oil, medium and low viscosity, class II		3
Mineral oil, medium and low viscosity, class III		3
Montan wax	8002-53-7	3
Pentane	109-66-0	1
1,3-Propanediol (1,3-propylene glycol)	504-63-2	2
Propylene tetramer (tetrapropylene, dodecene)	6842-15-5	3
Soybean oil epoxidized	8013-07-08	3
Ethyl tertiary butyl ether (ETBE)	637-92-3	5

[a] Only Group 1 was not considered at this meeting.
[b] Discussed with Group 2 – alcohols.

only. In 2001, CAC adopted the "Banned List" and it appears in the current code of practice as Appendix 3.

The development of a List of Acceptable Previous Cargoes by CCFO began with attempts to harmonize the FOSFA and NIOP trade lists with an EU list. The Acceptable List was further refined in 1999 when CCFO considered a list of substances proposed by the EU that had been reviewed by the Scientific Committee on Food (SCF). Having developed a list of acceptable previous cargoes, it was determined that there were 14 substances on it that required further evaluation; these 14 substances formed the basis of the CCFO Proposed Draft List of Acceptable Previous Cargoes, which was adopted by CAC 34 in 2011. For consideration at this meeting a list of 23 substances was proposed to FAO/

WHO (Table 9) by CCFO for scientific advice on their suitability as previous cargoes for the carriage of fats and oils by sea-going vessels upon its evaluation against the four criteria. Each substance on the list has been assigned to Groups 1–5 (1 – solvents/reactants; 2 – alcohols; 3 – oils and waxes; 4 – solutions; 5 – butyl ethers). Substances in Group 1 were evaluated at the present meeting.

3.3 Development of criteria

As a result of the CCFO request to FAO/WHO for scientific advice on the development of criteria for the assessment of the safety of residues of previous cargoes in the tanks of sea-going vessels carrying edible fats and oils, a technical meeting was convened (in November 2006) at the Dutch National Institute of Public Health and the Environment (RIVM). RIVM prepared a technical background document (Appendix II *(4)*) and drafted the meeting report with FAO/WHO *(5)*.

Discussions were limited to the assessment of previous cargoes in the transport of edible fats and oils in bulk by sea and the consideration of safety implications in terms of human health. The experts accepted that the quality of the fats and oils cargo could change as a result of hydrolysis and oxidation, but they acknowledged that these changes were already taken into account in trade contracts.

The experts considered a list of parameters originating from discussions at CCFO meetings, noting that previous cargoes are generally liquid chemical substances, slurries of solid particles or aqueous solutions. To further frame the deliberations, the experts decided to consider only a generic worst-case scenario since developing criteria to cover every possible combination of previous cargo, type of tank, cleaning regime and possible further processing of the subsequent cargo of fat or oil would not be a realistic approach.

The experts developed the following worst-case scenario: the smallest commercially viable tank size (200 m^3), coated with a polymer that absorbs the previous cargo, is filled to 60% capacity (as required by contract), and the cargo of fat or oil is not to be further processed or refined. The model also assumed that the tank and associated pipework has been cleaned according to defined standards, inspected and considered clean and dry. Under these circumstances, the maximum level of contamination in the subsequent fat or oil cargo by the previous cargo was calculated to be 100 mg/kg. This value was used to determine a single estimate of worst-case human exposure of 0.1 mg/kg bw per day. Based on this generic exposure value, the experts considered that for the evaluation of previous cargoes, the ADI (or TDI) should be greater than or equal to 0.1 mg/kg bw in order to provide sufficient protection for children and high-intake

Table 10
Criteria adopted by CAC 34 and included in RCP-36-1987

1.	The substance is transported/stored in an appropriately designed system with adequate cleaning routines, including the verification of the efficacy of cleaning between cargoes, followed by effective inspection and recording procedures.
2.	Residues of the substance in the subsequent cargo of fat or oil should not result in adverse human health effects. The ADI (or TDI) of the substance should be greater than or equal to 0.1 mg/kg bw per day. Substances for which there is no numerical ADI (or TDI) should be evaluated on a case-by-case basis.
3.	The substance should not be or contain a known food allergen, unless the identified food allergen can be adequately removed by subsequent processing of the fat or oil for its intended use.
4.	Most substances do not react with edible fats and oils under normal shipping and storage conditions. However, if the substance does react with edible fats and oils, any known reaction products must comply with criteria 2 and 3.

consumers. Negligent or fraudulent practices were not considered to be part of the criteria. The experts identified four criteria necessary to determine the acceptability of a previous cargo (see *(4)*).

The criteria as adopted by CAC 34 (2011) are listed in Table 10.

3.4 Basis of evaluation

3.4.1 Chemistry/reactivity

Edible fats and oils are normally chemically stable; however, there may be potential for reactions with residues of previous cargoes that could give rise to products that are hazardous to human health. Consideration should be given to chemical substances that can react with edible fats and oils under normal transportation conditions. Minor oxidation and hydrolysis are normally anticipated in trade contracts and are not considered a consequence of contact with a previous cargo, unless accelerated degradation occurs. Although many possible reactions require the presence of specific catalysts or temperatures well in excess of those anticipated during transportation, potential reactions of the previous cargo with triglycerides and free fatty acids or other minor components present in the fat or oil should still be considered.

3.4.2 Methods of analysis

In a few cases where contamination is considered critical there has been an international effort to develop specific analytical methods. Cases of contamination with diesel fuel (alkanes) and mineral oils (mineral oil saturated hydrocarbons, MOSH; mineral oil aromatic hydrocarbons, MOAH) led to the development of relevant international standards. Although many of the substances under review at the present meeting can be analysed by gas or liquid chromatography using appropriate detector systems, little progress has been made in the application

of these technologies to their contamination of oils and fats. It is assumed that available methods with suitable modifications will be capable of determining the maximum anticipated level of 100 mg/kg of previous cargo in the subsequent cargo of fats or oils.

3.4.3 Dietary exposure assessment for previous cargo chemical substances

As a consequence of considering a range of previous cargo chemical substances at its ninetieth meeting, the Committee concluded that it was appropriate to review the approach to estimating dietary exposure set out in the 2006 document *Development of criteria for acceptable previous cargoes for fats and oils* (criteria document) *(4)*.

The Committee noted that since the 2006 criteria document was drafted, newer and better-quality data on the consumption of fats and oils by adults, infants and young children have become available.

The Committee also noted that some of the previous cargo chemical substances assessed have additional sources of dietary exposure and expressed the view that it may be necessary to consider this in the exposure assessment.

The considerations used at the present meeting are identical to those developed and used by the Committee at its ninetieth meeting.

3.4.3.1 Exposure estimates in the 2006 criteria document

Based on the best available data at that time, the 2006 criteria document set out the following approach to dietary exposure assessment of previous cargo chemical substances present in fats and oils:

- Estimated mean per capita consumption of 0.025 kg/day of a single type of fat or oil. The value was rounded up from the maximum per capita consumption of refined soybean oil of 22 g/person per day from the GEMS/Food cluster diets.
- A factor of 2.5 to cover children and high consumers was derived from a rounded ratio between the mean and 97.5th percentile consumption of total vegetable oil from a food consumption survey in the United Kingdom (20 and 52 g/person per day for the population aged > 18 years). The criteria document also noted that dietary exposure of children to contaminants is frequently 2.5 times that of adults.
- A worst-case concentration of 100 mg/kg for a previous cargo contaminant in fats or oils.
- A body weight of 60 kg.

These data were used to define a worst-case dietary exposure estimate:

$$\frac{\text{Consumption of oil (0.025 kg/day)} \times 2.5 \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

= 0.1 mg/kg bw per day

Based on the **mean per capita consumption of fats and oils, and a factor of 2.5**, there would be no health concern to the general population from exposure to previous cargoes if the acceptable daily intake (ADI) or tolerable daily intake (TDI) is sufficiently protective, for example, the ADI or TDI is greater than, or equal to **0.1 mg/kg bw per day**.

3.4.3.2 Exposure estimates based on up-to-date consumption data for adults

Since 2006, the GEMS/Food cluster diets have been revised, and the FAO/WHO Chronic Individual Food Consumption – summary statistics database (CIFOCOss) has become available *(6)*. The 2006 criteria document noted that food consumption information from dedicated surveys would be more appropriate than the food consumption estimates from the GEMS/Food cluster diets *(7)*. However, it used the cluster diets, as food consumption survey data were only available from a very limited number of countries at that time. CIFOCOss currently contains food consumption data from 37 countries.

From the current version of CIFOCOss, the maximum mean consumption for a single fat or oil type is 35 g/person per day for consumption of virgin or extra-virgin olive oil by elderly Italians. The maximum 95th percentile (p95) consumption of a single fat or oil is 138 g/person per day for edible cottonseed oil by women (age 15–49 years) from Burkina Faso. This group also has the highest 97.5th percentile consumption of 189 g/person per day.

Based on the protocols currently used by JECFA for veterinary drugs, the number of consumers of cottonseed oil in the Burkina Faso survey (n = 116) would suggest that the 95th percentile is the highest reliable percentile *(8, 9)*.

These data suggest that for adults, a mean fat or oil consumption of 35 g/person per day and a high consumption of fat or oil of 140 g/person per day would be a conservative estimate consistent with available data.

The use of updated food consumption data will result in a revised estimated worst-case dietary exposure for adults:

$$\frac{\text{p95 consumption of oil (0.140 kg/day)} \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

= 0.2 mg/kg bw per day

3.4.3.3 Exposure estimates for infants and young children

Potentially vulnerable population groups, like infants and young children, were not specifically considered in the 2006 criteria document. Since then, individual consumption data for several population groups, including infants and young children, have become available through CIFOCOss and other sources. Infants and young children should be considered in the risk assessment because they could potentially experience high exposure to previous cargo chemical substances per kg body weight while they are undergoing growth and development.

Information on consumption of food oils by infants and young children was also available from the US Environmental Protection Agency's Food Commodity Intake Database (FCID) *(10)*, which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumption for infants and young children based on FCID is comparable to those in the CIFOCOss database; however, oil consumption information based on FCID takes into account individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and p95 consumption by infants and young children were 7.6 and 19 g/day, respectively. Estimated mean and p95 consumption on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively.

These data were used to define a worst-case dietary exposure estimate for infants and young children:

p95 consumption of oil (0.003 kg/kg bw/day) × concentration (100 mg/kg fat or oil)

= 0.3 mg/kg bw per day

3.4.3.4 Exposure from other dietary sources

For some previous cargo chemical substances potentially present in food oils, there are additional sources of dietary exposure, such as contamination (for example, contaminated drinking-water) or food additive uses (Table 11). Dietary exposures from these different sources should be considered in exposure assessment.

3.4.3.5 Conclusion

The Committee concluded that, based on up-to-date data on consumption of single fats and oils in the general population, which have become available since 2006, the generic human exposure value of 0.1 mg/kg bw per day used in the 2006 Criterion no. 2 to determine the acceptability of a previous cargo should be revised. Consequently, the updated, more conservative generic human exposure

Table 11
List of substances for evaluation by JECFA arising from the development of a list of acceptable previous cargoes by the Codex Committee on Fats and Oils: Other sources of exposure

Substance (synonyms)	Other sources of exposure
Acetic anhydride	Acetylation agent; used in food contact material
sec-Butyl acetate	Naturally present in vinegar; flavouring agent
tert-Butyl acetate	None
n-Pentane	Used in manufacture of food contact material
Cyclohexane	Extraction solvent in preparation of flavourings; diluent in colour additive mixtures

value of 0.3 mg/kg bw per day should be used in the evaluation of these substances.

The Committee noted that these estimates of dietary exposure were derived from a more conservative approach to using data on consumption of single fats and oils and a worst-case concentration of previous cargo chemicals in a single fat or oil of 100 mg/kg.

The Committee also concluded that additional sources of dietary exposure need to be considered in exposure assessment of previous cargo chemical substances.

3.4.4 Approach to toxicological evaluation

The Committee received no submitted data and, therefore, reviewed monographs from previous evaluations of individual substances conducted by JECFA, WHO, International Agency for Research on Cancer, and national and regional governmental authorities to retrieve additional relevant references for completing the present assessment. The Committee also conducted literature searches. The details are included in the consideration of individual substances.

At its present meeting, the Committee revised the generic value for assumed worst-case human dietary exposure from 0.1 to 0.3 mg/kg bw per day and used this revised generic exposure value for the evaluation of previous cargoes. The Committee also considered data on exposure to the substances from sources other than previous cargoes. Thus, the ADI (or TDI) should be greater than or equal to the estimated dietary exposure (0.3 mg/kg bw per day plus exposure from other possible dietary sources) in order to provide sufficient protection for infants, children and high-intake consumers. In situations where no appropriate numerical ADI (or TDI) was available from JECFA, the Committee considered other previously established health-based guidance values or calculated a MOE based on a reference point characterizing the toxicological hazard (such as a no-observed-adverse-effect level (NOAEL), etc.) identified from the available

data divided by the estimated dietary exposure. Interpretation of this MOE is a matter of expert judgement that takes into account limitations in the available toxicological database.

3.4.5 Recommendations

The Committee reiterated the recommendations made at the ninetieth meeting that the CCFO consider revising Criterion no. 2 in RCP-36-1987 as adopted by CAC 34 (2011).

1) Based on the consumption of fats and oils by infants and young children, there is no health concern for the general population from dietary exposure to previous cargo chemical substances if the ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to 0.3 mg/kg bw per day. Substances for which there is no numerical ADI or TDI should be evaluated on a case-by-case basis (e.g. margin of exposure (MOE) approach).
2) Where there are additional sources of dietary exposure to the previous cargo chemical substances, they should be considered in the exposure assessment.

References

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7. GEMS/Food consumption database [Online]. Geneva: World Health Organization; 2012 (https://www.who.int/nutrition/landscape_analysis/nlis_gem_food/en/, accessed 4 November 2020).

8. Boobis A, Cerniglia C, Chicoine A, Fattori V, Lipp M, Reuss R, et al. Characterizing chronic and acute health risks of residues of veterinary drugs in food: latest methodological developments by the Joint FAO/WHO Expert Committee on Food Additives. Crit Rev Toxicol. 2017;47:889–903. doi:10.1080/10408444.2017.1340259.

9. Arcella D, Boobis A, Cressey P, Erdely H, Fattori V, Leblanc J-C, et al. Harmonized methodology to assess chronic dietary exposure to residues from compounds used as pesticide and veterinary drug. Crit Rev Toxicol. 2019;49:1–10. DOI: 10.1080/10408444.2019.1578729.

10. What we eat in America – food commodity consumption database 2005–10 [Online]. Washington (DC): US Environmental Protection Agency – Office of Pesticide Programs; 2020 (https://fcid.foodrisk.org/, accessed 22 July 2020).

11. WHO/FAO. Food Safety Collaborative Platform [Online]. Geneva/Rome: World Health Organization/Food and Agriculture Organization of the United Nations; 2019 (https://apps.who.int/foscollab/Download/DownloadConso, accessed 14 May 2020).

3.5 Evaluation of substances – solvents and reactants (Group 1)

3.5.1 Acetic anhydride

Explanation

Acetic anhydride is rapidly hydrolysed to acetic acid in the presence of water. Consequently, the toxicological information on acetic acid and its previous evaluations are considered relevant to the assessment of acetic anhydride.

Previous evaluations of acetic anhydride

Acetic anhydride has not been previously considered by JECFA. In 1997 the Scientific Committee on Food (SCF) and EFSA (2012) *(1, 2)* concluded that since acetic anhydride will be converted to acetic acid during tank washing, use of acetic anhydride as a previous cargo for fats and oils is considered acceptable.

Previous evaluations of acetic acid

Acetic acid was first reviewed by the Committee at its ninth and tenth meetings in 1967 (Annex 1, references *11* and *13*). At those two meetings, the Committee concluded that for the purposes of evaluation, all sources of acetate used as food additives should be considered together. Since acetic acid has a sufficiently acid taste to limit the amount used in foods, it was not considered necessary to specify an ADI. However, at its seventeenth meeting, the Committee allocated a group ADI "not specified",[1,2] to acetic acid and its potassium and sodium salts. The Committee

[1] Originally the term "not limited" was used; however, this term was replaced by "not specified" at the Committee's eighteenth meeting (Annex 1, reference *35*).

[2] The current term "not specified" is applicable to a food substance of very low toxicity that, on the basis of the available chemical, biochemical and toxicological data as well as the total dietary intake of the

noted acetic acid's established metabolic pathways and its consumption as a normal constituent of the diet (Annex 1, reference *32*). At its forty-ninth meeting in 1998, the Committee re-evaluated the safety of acetic acid as a flavouring agent and concluded that there were no safety concerns based on the negative genotoxicity profile, the absence of any adverse effects at 350 mg/kg bw per day in a short-term toxicity test (63 days) in rats and a relatively small contribution from use as a flavouring agent (Annex 1, reference *131*). The Committee also noted that acetic acid can be predicted to undergo complete metabolism to endogenous products via the fatty acid and tricarboxylic acid pathways. In the opinion of the Committee the endogenous levels of metabolites from these substances would not give rise to perturbations outside the physiological range.

Present evaluation

For the present evaluation, previous assessments (monographs) completed by JECFA, SCF or EFSA, and national and regional governmental authorities were identified by searching their respective websites. This was followed by a comprehensive search to identify any critical new data for the assessment of human health risk on PubMed and PubChem. The search terms used were acetic anhydride and synonyms (e.g. acetyl acetate and acetanhydride), CAS number (108-24-7), toxicity and toxicokinetics. Given the paucity of information relevant to the oral toxicity of acetic anhydride, secondary searches for relevant information on its hydrolysis product (acetic acid) were also conducted to supplement this assessment. The cut-off date for inclusion in this report was 29 December 2020.

Chemical and technical considerations

The chemical and technical considerations for acetic anhydride are summarized in Table 12.

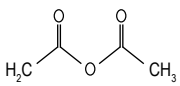
Assessment

Biochemical aspects

Acetic anhydride readily hydrolyses to acetic acid in the presence of water. The washing of containers after transporting a cargo, and moisture within the edible

substance (from its use at the levels necessary to achieve the desired effect and from its acceptable background in food), does not, in the opinion of the Joint FAO/WHO Expert Committee on Food Additives, represent a hazard to health. For that reason, and for reasons stated in 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: that is, it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance. (https://apps.who.int/iris/bitstream/handle/10665/44065/WHO_EHC_240_13_eng_Annex1.pdf;jsessionid=BDA8CB8E9D2770D3A11D7C8AA1FF2AA0?sequence=13).

Table 12
Chemical and technical considerations for acetic anhydride

Name: Acetic anhydride (ethanoic anhydride)	
CAS number	**Alternative CAS numbers**
108-24-7	None
Chemical details	Acetic anhydride; acetyl acetate; acetic acid, 1,1′-anhydride; ethanoyl ethanoate; acetic acid anhydride; acetyl acetate; acetyl oxide; acetic oxide Colourless liquid with a strong, pungent, vinegar-like odour  Molar mass: 102.09 g/mol Melting point: −73.1 °C Boiling point: 139.5 °C Soluble in water. Hydrolyses to acetic acid in aqueous solution
Route(s) of synthesis	Manufactured by different processes: 1) acetic acid process (ketene process) from acetic acid to form a ketene and the further reaction of the ketene with acetic acid; 2) acetaldehyde oxidation process; and 3) carbonylation of methyl acetate process.
Composition	Technical quality acetic anhydride (max. 97%) often contains colour bodies, heavy metals, phosphorus and sulfur compounds. Acetic anhydride manufactured by the ketene process sometimes contains ketene polymers, e.g. acetylacetone, diketene, dehydroacetic acid, and particulate carbon or soot. Polymers of allene, or its equilibrium mixture, methylacetylene-allene, are reactive impurities which slowly autoxidize to peroxides if exposed to air.
Uses	The primary industrial application of acetic anhydride is for acetylation reactions in the production of cellulose acetates. Acetic anhydride is used in the manufacture of fibres, plastics, pharmaceuticals, dyes and explosives. In food applications it is used in the starch industry as an acetylation compound in production of modified starches.
Analytical methods	None reported for previous cargoes. Residues of acetic acid can be detected in cleaning water and wipe samples with LC-MS/MS, IC or UHPLC.
Potential reaction(s) with a subsequent cargo of fat or oil	On contact with water (e.g. during tank washing) acetic anhydride hydrolyses to acetic acid. Acetic anhydride acetylates free hydroxyl groups without a catalyst, but esterification is more complete in the presence of acids; acetic anhydride and acetic acid could react with alcohols (for example mono- and diglycerides) forming acetates.

LC-MS/MS, liquid chromatography–tandem mass spectrometry; IC, ion chromatography; UHPLC, ultra-high-performance liquid chromatography.

oil is likely to transform almost all residual acetic anhydride to acetic acid. Any traces of acetic anhydride remaining are expected to be readily hydrolysed to acetic acid when ingested. Acetic acid is a product of normal metabolism in humans. It is expected to be readily absorbed, metabolized and rapidly excreted *(3, 4)*.

Toxicological studies

Since ingested acetic anhydride is highly corrosive to the mucous membrane of the gastrointestinal tract, the available toxicity database for oral toxicity in laboratory

animals is restricted. The reported oral median lethal dose (LD_{50}) values in rats range from 630 mg/kg bw *(5)* up to 1800 mg/kg bw *(6)*. Acetic acid is highly irritating to the skin and mucous membranes and following ingestion, early signs of toxicity can be attributed to its irritating properties at high concentrations. JECFA (1998) previously noted no effects at a dose of 350 mg/kg bw per day acetic acid from a study in male rats administered sodium acetate via oral gavage for 63 days (Annex 1, reference *132; (7)*). In a developmental toxicity study in mice, a maternal NOAEL of 74.3 mg acetic acid/kg bw per day (based on decreased body weight gain at 345 mg/kg bw per day) and a developmental toxicity NOAEL of 345 mg acetic acid/kg bw per day were identified (based on increases in the number of litters containing dead fetuses, and incomplete ossification at 1600 mg/kg bw per day). No evidence of developmental toxicity was observed in rats up to doses of 1600 mg acetic acid/kg bw per day *(8)*.

The Committee concluded that the available information on acetic anhydride and acetic acid in vitro and in vivo does not raise concerns for genotoxicity. Although acetic acid has been associated with tumour promotion *(9, 10)*, the Committee considered that the tumour-promoting and tumour progression effects reported are likely to be the result of the site-of-contact irritating/cytotoxic potential of acetic acid at high concentrations and would not be of concern at the low concentrations that occur in fats or oils following its transport as a previous cargo.

Allergenicity

The Committee concluded that, considering the widespread presence of acetic acid in the diet, it is unlikely that exposure to acetic acid resulting from the breakdown of acetic anhydride present in low concentrations such as when transported as a previous cargo will produce an allergic response.

Impurities

The Committee noted that fats and oils for human consumption have to conform to the MLs for heavy metals set out in the Codex *General Standard for Contaminants and Toxins in Food and Feed*, CXS-193-1995 *(11)*, and it is therefore not necessary to estimate exposures to heavy metals in food oils due to carryover from acetic anhydride previous cargoes. Additional impurities in acetic anhydride, depending on the method of manufacture, include ketene polymers (e.g. acetylacetone, diketene and dehydroacetic acid), polymers of allene or its equilibrium mixture, methylacetylene-allene and particulate carbon or soot. The quantities of these impurities in acetic anhydride are unknown; exposure to ketene polymers, polymers of allene (or its equilibrium mixture) and particulate

carbon or soot, therefore, cannot be estimated. The Committee noted that some of these impurities may be genotoxic, e.g. diketene.

Assessment of dietary exposure

Acetic anhydride is approved in some countries as an acetylation agent for use in the preparation of modified food starches and acetylated monoglycerides. Acetic anhydride is rapidly hydrolysed to acetic acid, a constituent present naturally in vinegar (at up to 15%) and other foods *(12, 13)*. Environment and Climate Change Canada and Health Canada *(13)* determined that the dietary exposure to acetic anhydride from food additive uses, if any, is likely to be negligible.

Acetic anhydride may also be used in the manufacture of paper food packaging or paper trays. However, Environment and Climate Change Canada and Health Canada *(13)* determined that exposure to acetic anhydride from packaging uses is not expected due to negligible residual levels in the finished packaging materials.

No data were found on concentrations of acetic anhydride in food oils due to carryover from previous cargoes. A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances. Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day, based on a worst-case concentration of 100 mg/kg and an oil intake of 3 g/kg bw per day by infants and young children who are high consumers (see section 3.4.3, Dietary exposure assessment for previous cargo chemical substances). It is not expected that exposure to acetic acid present due to hydrolysis of acetic anhydride in carryover from previous cargoes would add significantly to total acetic acid exposures, estimated at 2.1 g/day (equivalent to 35 mg/kg bw per day for adults) based on Life Sciences Research Office data *(14)*.

Evaluation

No information regarding the short-term and long-term toxicity of acetic anhydride was identified. However, upon evaluation of the available information, the Committee noted that it had previously allocated a group ADI "not specified" to acetic anhydride's immediate hydrolysis product, i.e. acetic acid and its potassium and sodium salts (Annex 1, reference *32*). Since acetic anhydride is anticipated to be rapidly hydrolysed to acetic acid during tank washing, within the edible oil cargo and after ingestion, the group ADI "not specified" for acetic acid and its potassium and sodium salts is considered directly relevant for this assessment of acetic anhydride. The US National Research Council estimated that mean exposure to acetic acid from all food sources is 2.1 g/day for persons above 2 years of age *(14)*, which is equivalent to 35 mg/kg bw per day for adults based

on a body weight of 60 kg. It is not expected that exposure to acetic acid present due to hydrolysis of acetic anhydride in carryover from previous cargoes would add significantly to total exposures to acetic acid. Therefore, acetic anhydride at the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day would only contribute marginally to the overall dietary exposure to acetic acid and is not expected to result in adverse effects on human health.

The Committee concluded that, considering the widespread presence of acetic acid in the diet, it is unlikely that exposure to acetic acid resulting from the breakdown of acetic anhydride present in low concentrations such as when transported as a previous cargo will produce an allergic response.

Acetic anhydride acetylates free hydroxyl groups without a catalyst, but esterification is more complete in the presence of acids, so acetic anhydride and acetic acid could react with alcohols (for example mono- and diglycerides) forming acetates *(15)*. Reaction rates are likely to be slow at ambient temperature.

Although exposure to acetic anhydride and acetic acid as a result of transporting acetic anhydride as a previous cargo does not appear to be a health concern, there is uncertainty concerning the purity or "grade" of acetic anhydride that is transported as a previous cargo. Since acetic anhydride may contain impurities (e.g. diketene), which are potentially genotoxic, the Committee could not reach a conclusion on the safety of transporting acetic anhydride as a previous cargo for edible fats and oils until the nature and quantities of these impurities have been clarified.

3.5.2 *sec*-Butyl acetate

Explanation

The Committee has not previously evaluated *sec*-butyl acetate; however, at its eleventh and forty-ninth meetings, in 1968 and 1999, it evaluated one of its isomers *n*-butyl acetate (Annex 1, references *14* and *131*). SCF (1997) *(1)* considered *sec*-butyl acetate acceptable as a previous cargo for edible fats and oils primarily on the basis that it is easily removed by tank cleaning. More recently, the EFSA (2012) Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that *sec*-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils on the basis that the metabolites (i.e. acetic acid and 2-butanone) were previously assessed by the Panel as acceptable previous cargoes for edible fats and oils *(16)*. In addition, the primary metabolite *sec*-butanol was not found to be genotoxic and did not represent a toxicological concern at exposure levels that might occur in fats and oils from the transport of *sec*-butyl acetate as a previous cargo.

For the present assessment, previous assessments (monographs) produced by JECFA, EFSA, SCF, the WHO International Programme on Chemical

Safety (WHO/IPCS), and national and regional governmental authorities were identified by searching their respective websites. This was followed by a comprehensive search to identify any critical new data for the assessment of human health risk on PubMed and PubChem. The search terms used were *sec*-butyl acetate and synonyms (e.g. 2-butyl acetate), CAS number (105-46-4) and toxicity. Given the paucity of relevant information concerning the oral toxicity of *sec*-butyl acetate, secondary searches for relevant information were performed on the metabolites of *sec*-butyl acetate (i.e. acetic acid, *sec*-butanol and 2-butanone) in an effort to supplement the toxicological information on this compound. The cut-off date for inclusion in this report was 4 January 2021. The data concerning acetic acid are summarized in section 3.5.1.

Chemical and technical considerations

The chemical and technical considerations for *sec*-butyl acetate are summarized in Table 13.

Assessment

Biochemical aspects

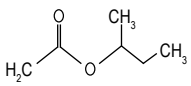
Following oral exposure, *sec*-butyl acetate is expected to be rapidly absorbed into the systemic circulation, then hydrolysed within minutes to acetic acid and *sec*-butanol in the blood, liver, small intestine and respiratory tract *(17)*. *sec*-Butanol is then expected to undergo rapid metabolism by alcohol dehydrogenase, primarily to 2-butanone, and to be excreted either by exhalation or in the urine, or to undergo further metabolism to 3-hydroxy-2-butanone and 2,3-butanediol (*18–20)*.

Toxicological studies

The acute toxicity of *sec*-butyl acetate after oral exposure is low. In rats, an oral LD_{50} of 3200 mg/kg bw was reported *(17)*. For *sec*-butanol, the oral LD_{50} was greater than 2000 mg/kg bw in rats and rabbits *(21)*. No short-term or long-term oral toxicity data are available for *sec*-butyl acetate.

Owing to the lack of information on the reproductive and developmental toxicity and short-term and long-term oral toxicity of *sec*-butyl acetate, the Committee also considered the summary results of a two-generation reproductive and developmental toxicity study on the primary metabolite, *sec*-butanol *(22)* (Cox et al., 1975, as cited by the US EPA *(22)*; original study unpublished). Male and female rats were given *sec*-butanol in drinking-water at concentrations of 0, 0.3, 1.0 or 3.0% for 8 weeks prior to mating, and during gestation of two separate F1 generations. The doses of the F0 generation up to day 10 after the birth of the F1A pups were reported based on average daily intakes as 538, 1644 or 5089 mg/

Table 13
Chemical and technical considerations for *sec*-butyl acetate

Name: Butyl acetate, *sec*- (sec-butyl acetate)	
CAS number	**Alternative CAS numbers**
105-46-4	None
Chemical details	*Sec*-butyl acetate; butan-2-yl acetate; 2-butyl acetate; *sec*-butyl ethanoate Colourless liquid with a fruity scent. It produces highly flammable, irritating vapour.  Molar mass: 116.16 g/mol Melting point: −99 °C Boiling point: 112 °C Slightly soluble in water, soluble in ethanol and diethyl ether
Route(s) of synthesis	The most common process for manufacturing *sec*-butyl acetate is esterification of acetic acid with *sec*-butanol using sulfuric acid as a catalyst. An alternative production route is esterification of *sec*-butanol with acetic anhydride.
Composition	May contain acetic acid up to 0.2% as an impurity. No impurities of concern have been identified.
Uses	Used as a solvent in nitrocellulose lacquers, thinners, enamels, leather finishes, acyclic polymers and vinyl resins, and as a flavouring substance for food.
Analytical methods	None reported for previous cargoes. Potential methods for its determination in fats and oils include GC-FID and GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Hydrolyses to acetic acid and *sec*-butanol, which, in the presence of acid, may participate in transesterification with lipids, producing a mixture of fatty acid *sec*-butyl esters and glycerol. However, the reactions are slow, requiring an excess of alcohol and temperatures above 100 °C.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

kg bw per day in males and 594, 1771 and 4571 mg/kg bw per day in females (intakes were not reported for subsequent weeks). Maternal toxicity (reduced body weight gain) accompanied by developmental effects (increased fetal death, and reduced fetal and pup body weight) and possible effects on male reproductive performance (i.e. effects on male copulatory success), was reported following exposure to the highest drinking-water concentration (doses of 5089 mg/kg bw per day in males and 4571 mg/kg bw per day in females). Although reduced pup body weights were observed in F1A pups at the 1% dose on postnatal days (PND) 4 and 21, reduction in pup body weights at the same dose in the F2 generation was not observed. Based on these results, the Committee identified a NOAEL of 594 mg/kg bw per day (0.3% *sec*-butanol) based on decreased pup body weight in the F1A generation. In a review in 2003, the US EPA, using its Benchmark Dose Software (BMDS, version 1.3.1), calculated a lower 95% confidence limit on the

BMD for a 5% response ($BMDL_{05}$) of 657 mg/kg bw per day based on decreased pup weights in the F1A pups on PND 21 *(22)*.

The Committee concluded that *sec*-butyl acetate, *sec*-butanol and 2-butanone are non-genotoxic in vitro and in vivo.

Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to *sec*-butyl acetate that would indicate that this substance is or contains a known food allergen.

sec-Butanol and 2-butanone did not induce skin sensitization reactions in the guinea-pig maximization tests *(21, 23, 24)*.

Impurities

No impurities of concern were identified.

Assessment of dietary exposure

sec-Butyl acetate is naturally present in vinegar at concentrations up to 67 mg/kg *(17)*. Maximum mean exposure to *sec*-butyl acetate from vinegar consumption, calculated based on data provided by the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids *(25)*, was estimated to range from 0.01 mg/kg bw per day for infants to 0.03 mg/kg bw per day for the elderly.

sec-Butyl acetate has a fruity odour *(17)* and is approved for use as a flavouring agent (09.323) in Europe *(26, 27)*. Exposure to *sec*-butyl acetate from it use as a flavouring agent in Europe was estimated to be 0.07 mg/kg bw per day for adults. No data are available on exposure of infants and young children to *sec*-butyl acetate from use as a flavouring agent, but these exposures are expected to be low given that most infant formulas and foods for young children do not contain fruity flavouring.

No data were found on concentrations of *sec*-butyl acetate in food oils due to carryover from previous cargoes. A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances. Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day, based on a worst-case concentration of 100 mg/kg and an oil intake of 3 g/kg bw per day by infants and young children who are high consumers (See section 3.4.3, Dietary exposure assessment for previous cargo chemical substances). Exposures to *sec*-butyl acetate from vinegar and from flavouring agent uses are not expected to add significantly to the estimated *sec*-butyl acetate exposure of 0.3 mg/kg bw per day from previous cargoes, for infants and young children.

Evaluation

No information regarding the short-term and long-term toxicity of *sec*-butyl acetate was identified; however, for *sec*-butanol, the Committee identified a $BMDL_{05}$ of 657 mg/kg bw per day based on reduced offspring body weight from a two-generation reproductive and developmental toxicity study in rats (summarized by US EPA *(22)*). *sec*-Butyl acetate is naturally present in vinegar and is approved for use as a flavouring agent in Europe. The Committee estimated that exposure to *sec*-butyl acetate from vinegar consumption and its use as a flavouring agent is approximately 0.1 mg/kg bw per day. A comparison of the $BMDL_{05}$ of 657 mg/kg bw per day for *sec*-butanol with the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day for *sec*-butyl acetate as a previous cargo plus its presence in the diet (0.1 mg/kg bw per day) yields a margin of exposure (MOE) of 1643, which is considered sufficient to address the uncertainties in the database.

There are no data on allergenicity upon oral exposure to *sec*-butyl acetate that indicate that it is or it contains a known food allergen.

sec-Butyl acetate hydrolyses to acetic acid and *sec*-butanol which, in the presence of acid, may participate in transesterification with lipids, producing a mixture of fatty acid *sec*-butyl esters and glycerol. However, the reactions are slow, requiring an excess of alcohol and temperatures above 100 °C.

Therefore, *sec*-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.5.3 *tert*-Butyl acetate

Explanation

The Committee has not previously evaluated *tert*-butyl acetate; however, at its eleventh and forty-ninth meetings, the Committee evaluated butyl acetate (not specifically *tert*-butyl acetate) (Annex 1, references *14* and *131*). SCF (1997) *(1)* considered *tert*-butyl acetate acceptable as a previous cargo for edible fats and oils primarily on the basis that it is easily removed by tank cleaning. More recently, the EFSA CONTAM Panel (2012) *(28)* concluded that *tert*-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils on the basis that the available data on *tert*-butyl acetate and its primary metabolites (i.e. acetate and *tert*-butanol), do not give rise to concerns about systemic toxicity, developmental toxicity, genotoxicity and allergenicity.

For the present assessment, previous assessments (monographs) produced by JECFA, EFSA, SCF, WHO/IPCS, and national and regional governmental authorities were identified by searching their respective websites. This was followed by a comprehensive search to identify any critical new data for the assessment of human health risk on PubMed and PubChem. The search terms

used were *tert*-butyl acetate and synonyms (e.g. *t*-butyl acetate), CAS number (540-88-5), toxicity and toxicokinetics. The results were screened for relevance, specifically concerning the oral route of exposure. Given the paucity of relevant information concerning the oral toxicity of *tert*-butyl acetate, a secondary search for relevant information on metabolites (i.e. *tert*-butanol and acetic acid) was conducted to supplement this assessment. The cut-off date for inclusion in this report was 29 December 2020. The data concerning acetic acid are summarized in section 3.5.1.

Chemical and technical considerations

The chemical and technical considerations for *tert*-butyl acetate are summarized in Table 14.

Assessment

Biochemical aspects

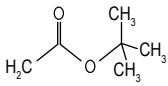
There are no published studies on the biochemical aspects of *tert*-butyl acetate following oral exposure and no physiologically-based pharmacokinetic models were identified; however, based on its physicochemical properties, *tert*-butyl acetate is expected to be readily absorbed and distributed systemically following oral exposure *(17)*. Since limited kinetic data are available for *tert*-butyl acetate following oral exposure, the available kinetic data following inhalation exposure were considered.

Based on the kinetic data in rats following inhalation exposure to *tert*-butyl acetate *(29)*, metabolism of *tert*-butyl acetate is likely to occur by both carboxylesterase and cytochrome P450 pathways resulting primarily in the production of *tert*-butanol and acetic acid. The biochemical aspects as well as the toxicological data on *tert*-butanol were also considered in the evaluation of methyl tertiary butyl ether (MTBE) as a previous cargo for edible fats and oils at the ninetieth meeting of the Committee.

Toxicological studies

The acute toxicity of *tert*-butyl acetate following oral exposure is low. The oral LD_{50} in rats was reported to be 3420 mg/kg bw *(17)*. No short-term or long-term oral toxicity data are available for *tert*-butyl acetate. According to the results of a developmental toxicity study in rats by Yang et al. *(30)*, *tert*-butyl acetate exhibits developmental toxicity (reduced fetal body weight and increased incidence of skeletal variations) at doses ≥800 mg/kg bw per day. On the basis of this study, the Committee identified a NOAEL for maternal toxicity of 800 mg/kg bw per day and a NOAEL of 400 mg/kg bw per day for embryo-fetal development.

Table 14
Chemical and technical considerations for *tert*-butyl acetate

Name: Butyl acetate, *tert*- (*tert*-butyl acetate)	
CAS number	**Alternative CAS numbers**
540-88-5	None
Chemical details	*tert*-Butyl acetate; acetic acid *tert*-butyl ester; *t*-butyl acetate; *tert*-butyl ethanoate Colourless liquid with a fruity odour. It produces highly flammable irritating vapour.  Molar mass: 116.16 g/mol Boiling point: 97.8 °C Practically insoluble in water; soluble in ethanol, ethyl ether, chloroform and acetic acid.
Route(s) of synthesis	Produced from isobutylene reacting with acetic acid with vanadium pentoxide impregnated silica as catalyst or by esterification of *tert*-butanol and acetic acid.
Composition	No impurities of concern have been identified. May contain acetic acid up to 0.2% as an impurity.
Uses	Used as a solvent in adhesives, sealants and paints and as a gasoline additive.
Analytical methods	None reported for previous cargoes. Potential methods for its determination in fats and oils include GC-FID and GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Hydrolyses to acetic acid and *tert*-butanol, which in the presence of acid may participate in transesterification with lipids producing a mixture of fatty acid *tert*-butyl esters and glycerol. However, the reactions are slow, requiring an excess of alcohol and temperatures above 100 °C.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

Owing to the lack of information on the short-term and long-term oral toxicity of *tert*-butyl acetate, the Committee also reviewed selected information on the oral toxicity of *tert*-butanol. Following chronic oral exposure via drinking-water *(31)*, *tert*-butanol produces significant effects in the kidneys of male (renal tubule adenoma or carcinoma) and female rats (dose-responsive increase in severity of chronic progressive nephropathy) at doses as low as 90 and 180 mg/kg bw per day, respectively. In female rats, dose-responsive increases in absolute kidney weights at 15 months, inflammation (suppurative) of the kidneys and transitional epithelial hyperplasia were also observed. In male rats, these renal effects are most likely attributable to the binding of *tert*-butanol to α2u-globulin in the kidneys *(31–33)* and the Committee considered this mechanism to be male rat-specific and not relevant to humans. The Committee considered that although chronic progressive nephropathy is a commonly diagnosed rat-specific condition *(34)*, the lesions associated with chronic progressive nephropathy in the female rats (such as tubular degeneration and glomerular sclerosis) also occur in the human kidney *(32)*. Moreover, there is evidence of a dose responsive increase in the incidence of nephropathy following only 13 weeks of exposure to *tert*-butanol,

in relatively young female rats *(31)*. At significantly higher concentrations in drinking-water, and following long-term exposure, *tert*-butanol also induces non-neoplastic effects in the liver of male mice (fatty changes in hepatocytes at 2070 mg/kg bw per day) and in the thyroid of male (follicular cell hyperplasia at 1040 mg/kg bw per day) and female mice (follicular cell hyperplasia at 1020 mg/kg bw per day) *(31)*. Associated increases in neoplasia in the thyroid of male (follicular cell adenoma or carcinoma at 1040 mg/kg bw per day) and female mice (follicular cell adenoma at 2070 mg/kg bw per day) were also noted.

The Committee concluded that the available information on *tert*-butyl acetate in vitro and in vivo does not raise concerns for genotoxicity and that the neoplastic effects observed in rats and mice exposed to *tert*-butanol via drinking-water probably occur via a non-genotoxic mode of action and at much higher doses than those expected from oral exposure to *tert*-butyl acetate as a previous cargo. The Committee noted that the neoplastic effects in the thyroid occur at much higher doses than the non-neoplastic (i.e. renal toxicity) effects in rats. This conclusion is supported by observations from the 13-week drinking-water studies in mice and rats where clear evidence of renal toxicity was observed in male and female animals without any evidence of thyroid toxicity at much higher doses *(31)*.

Allergenicity

The Committee did not identify any reports that indicated that *tert*-butyl acetate or *tert*-butanol elicits an allergic response upon oral exposure. There are also no data available that indicate that *tert*-butyl acetate would contain a known food allergen.

Impurities

No impurities of concern were identified.

Assessment of dietary exposure

No data were found on concentrations of *tert*-butyl acetate in food from any source. A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances. Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day, based on a worst-case concentration of 100 mg/kg and an oil intake of 3 g/kg bw per day by infants and young children who are high consumers (see section 3.4.3, Dietary exposure assessment for previous cargo chemical substances).

Evaluation

No information regarding the short-term and long-term toxicity of *tert*-butyl acetate was identified; however, the Committee identified a LOAEL of 180 mg/

kg bw per day *(31)* based on renal effects observed in female rats chronically exposed to a metabolite of *tert*-butyl acetate (i.e. *tert*-butanol) in drinking-water. The LOAEL for *tert*-butanol is lower than the NOAEL of 400 mg/kg bw per day of *tert*-butyl acetate for developmental toxicity and represents a conservative metric for risk assessment of *tert*-butyl acetate. No data were found on concentrations of *tert*-butyl acetate in food from any source. A comparison of the LOAEL of 180 mg/kg bw per day with the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day for *tert*-butyl acetate as a previous cargo yields a MOE of 600, which is considered sufficient to address the uncertainties in the database.

There are no data on allergenicity upon oral exposure to *tert*-butyl acetate that indicate that it is or it contains a known food allergen.

tert-Butyl acetate hydrolyses to acetic acid and *tert*-butanol, which, in the presence of acid, may participate in transesterification with lipids producing a mixture of fatty acid *tert*-butyl esters and glycerol. However, the reactions are slow, requiring an excess of alcohol and temperatures above 100 °C.

Therefore, *tert*-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.5.4 *n*-Pentane

Explanation

The Committee has not previously evaluated *n*-pentane. SCF considered *n*-pentane acceptable as a previous cargo in 1997 based on a previous decision concerning the use of *n*-pentane in the manufacture of plastic materials intended to come into contact with foodstuffs *(35)*. In the SCF (1995) opinion, *n*-pentane was considered acceptable as a food contact material due to its volatile nature and the unlikelihood of its presence in the finished product. EFSA (2012) *(2)* concluded that *n*-pentane meets the criteria for acceptability as a previous cargo for edible fats and oils, based on evidence suggesting that it exhibits a low systemic toxicity potential, and lacks mutagenic and carcinogenic potential. However, EFSA also concluded that there were inadequate data in humans and animals regarding the oral toxicity of *n*-pentane to establish a HBGV.

For the present assessment, previous assessments (monographs) produced by JECFA, SCF or EFSA were identified by searching their respective websites. This was followed by a comprehensive search to identify any critical new data for the assessment of human health risk on PubChem and PubMed and considering previous reviews by national and regional governmental authorities. The search terms used were the common name (*n*-pentane), CAS number (109-66-0), toxicity and toxicokinetics. The results were screened for relevance, specifically regarding the oral route of exposure. In an effort to supplement the

Table 15
Chemical and technical considerations for *n*-pentane

Name: *n*-Pentane	
CAS number	**Alternative CAS numbers**
109-66-0	None
Chemical details	*n*-Pentane; pentan; amyl hydride Clear colourless liquid with a petroleum-like odour. Forms highly flammable vapour–air mixtures.  Molar mass: 72.15 g/mol Melting point: −129.7 °C Boiling point: 36 °C Insoluble in water; soluble in most organic solvents.
Route(s) of synthesis	Produced by distillation from natural gasoline or naphtha, by dehydration and subsequent hydrogenation of 2- and 3-pentanol and from 2-bromopentane by Grignard reaction.
Composition	May contain residual sulfur, benzene and other aromatics. Technical grade pentane may contain branched and cyclic hydrocarbons of similar molecular mass.
Uses	Used as an additive in motor and aviation fuel, as a propellant and solvent in cosmetics and as a blowing agent to make foamed food packaging materials.
Analytical methods	None identified for previous cargoes. Pentane may be analysed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	It is not expected to react with edible fats and oils.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

toxicity information on short-term and long-term oral exposure, a secondary search was conducted for isopentane (CAS No: 78-78-4), an isomer of *n*-pentane. The cut-off date for inclusion in this report was 29 December 2020.

Chemical and technical considerations

The chemical and technical considerations for *n*-pentane are summarized in Table 15.

Assessment

Biochemical aspects

No studies on the biochemical aspects of *n*-pentane following oral exposure were identified. However, kinetic data in rats following whole-body exposure via inhalation indicated that *n*-pentane is readily absorbed and distributed systemically to various tissues, with a higher affinity for fat. It is rapidly metabolized to pentanols and pentanone, and exhaled as carbon dioxide *(36–38)*. The rapid metabolism and excretion of *n*-pentane suggest there is little potential for tissue accumulation *(39)*.

Toxicological studies

n-Pentane exhibits low acute oral toxicity in rats, with an estimated LD_{50} of > 2000 mg/kg bw *(39)*. Only one study, with limited reliability, which investigated the short-term toxicity of *n*-pentane was identified. No other studies on the short-term or long-term toxicity of *n*-pentane were identified. In a developmental toxicity study, pregnant rats (25 per group) were administered *n*-pentane via gavage at doses of 0, 100, 500 and 1000 mg/kg bw per day from gestation day (GD) 6 to GD 15 *(39)*. Maternal and developmental toxicity was not evident at any dose. Based on these results, the Committee identified a NOAEL of 1000 mg/kg bw per day, for the maternal and developmental toxicity of *n*-pentane.

To address the limitations in the short-term toxicity database for *n*-pentane, the Committee also considered the results of a one-generation reproductive toxicity test with isopentane (an isomer of *n*-pentane). In this study, male and female rats were administered 0, 100, 300 or 1000 mg/kg bw per day via oral gavage *(40)*. No evidence of treatment-related reproductive or developmental toxicity was observed at any dose. Effects were limited to the F0 generation. Transient salivation immediately following dosing was observed in males given doses of 300 and 1000 mg/kg bw per day and in females given the 1000 mg/kg bw per day dose (probably due to the irritating properties of isopentane). Male F0 generation rats treated with 1000 mg/kg bw per day also exhibited decreased body weight gain and slightly reduced food consumption. Following terminal necropsy, increased absolute and relative adrenal gland weights were observed in males and females in the 1000 mg/kg bw per day dose group. Male rats treated with 1000 mg/kg bw per day exhibited increased relative weights of the brain, liver, kidneys and testes. Although no histopathological lesions were noted in female rats, male rats treated with 1000 mg/kg bw per day showed an increased incidence of renal tubular degeneration or regeneration. No other effects were reported in the other F0 generation males or females. Based on effects observed in the F0 generation rats at the highest dose, the Committee identified a NOAEL of 300 mg/kg bw per day for isopentane.

The Committee concluded that *n*-pentane is non-genotoxic in vitro and in vivo.

Allergenicity

The Committee did not identify any reports that indicated that *n*-pentane elicits an allergic response upon oral exposure. There are also no data available that indicate that *n*-pentane would contain a known food allergen.

Impurities

Total aromatics (benzene, toluene and xylene) may be present as impurities in *n*-pentane at < 5 mg/kg; benzene and toluene may each be present at < 3 mg/kg, and sulfur may be present at < 1 mg/kg (Shell Chemicals Online). Assuming that the maximum concentrations of these substances are present as impurities in *n*-pentane, exposures associated with exposure to pentane at 0.3 mg/kg bw per day in oil as carryover from previous cargoes (see section 3.4.3, Dietary exposure assessment for previous cargo chemical substances) are 0.0015 µg/kg bw per day for aromatics, 0.0009 µg/kg bw per day for benzene or toluene, and 0.0003 µg/kg bw per day for sulfur.

The major route of exposure to benzene is inhalation, rather than diet *(41, 42)*. Estimated exposures to inhaled benzene in the United Kingdom range from 0.71 µg/kg bw per day for children living in rural areas to 14.12 µg/kg bw per day for adult smokers in urban areas who work adjacent to busy roads *(42)*. Estimates of dietary exposure range from 1.4 to 2.8 µg per day *(41)*, equivalent to 0.02–0.05 µg/kg bw per day for adults weighing 60 kg. The estimated maximum dietary exposure to benzene present in fats and oils when *n*-pentane is carried as a previous cargo, i.e. 0.0009 µg/kg bw per day, is minimal compared to total benzene exposure.

The estimated maximum exposure to toluene present in fats and oils when *n*-pentane is carried as a previous cargo, 0.0009 µg/kg bw per day, is less than 1% of the 0.119 µg/kg bw per day mean total dietary exposure to toluene estimated for the Belgian population *(43)*. The presence of sulfur as an impurity in *n*-pentane is not a safety concern, as sulfur is present in methionine, an essential amino acid, and is ubiquitous in the diet *(44)*.

Naphthenes (< 1%) and *n*-hexane (< 0.1%) may also be present in *n*-pentane (Shell Chemicals Online). Maximum exposures to naphthene and hexane associated with an *n*-pentane exposure of 0.3 mg/kg bw per day from previous cargoes are 0.003 mg/kg bw per day and 0.0003 mg/kg bw per day, respectively. No estimates of exposure to naphthenes were identified. The exposure to *n*-hexane present in fats and oils when *n*-pentane is carried as a previous cargo is expected to be low compared to exposure from other sources (for example see Environment and Climate Change Canada *(45)*).

Assessment of dietary exposure

n-Pentane may be used as a blowing agent in the production of foamed plastic food packaging (21 CFR 178.3010). However, no data were available on *n*-pentane concentrations in polystyrene packaging or on migration of *n*-pentane from packaging into food.

No data were found on concentrations of *n*-pentane in food oils due to carryover from previous cargoes. A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances. Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day, based on a worst-case concentration of 100 mg/kg and an oil intake of 3 g/kg bw per day by infants and young children who are high consumers (see section 3.4.3, Dietary exposure assessment for previous cargo chemical substances).

Evaluation

No reliable information regarding the short-term and long-term toxicity of *n*-pentane was identified; however, the Committee identified a NOAEL of 1000 mg/kg bw per day for *n*-pentane based on developmental toxicity testing in rats. The Committee also identified a NOAEL of 300 mg/kg bw per day for an isomer (isopentane) following short-term oral exposure in a one-generation toxicity test in rats (12 and 10 weeks of exposure in males and females, respectively). A comparison of the NOAEL of 300 mg/kg bw per day for isopentane with the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day yields a MOE of 1000, which is sufficient to address the uncertainties in the database.

There are no data on allergenicity upon oral exposure to *n*-pentane that indicate that it is, or it contains a known food allergen.

n-Pentane as a previous cargo is not expected to react with edible fats and oils to form any reaction products.

Exposure to impurities in *n*-pentane is not anticipated to contribute significantly to background exposures. Therefore, *n*-pentane meets the criteria for acceptability as a previous cargo for edible fats and oils.

3.5.5 Cyclohexane

Explanation

The Committee evaluated cyclohexane as an extraction solvent for foodstuffs in 1980 and noted a paucity of toxicological data relating to long-term oral exposure of animals and humans and that the available data indicated that it had a low order of toxicity (Annex 1, reference *50*). No toxicological monograph was prepared; however, since early findings of haematopoietic injury described in the literature may be attributed to benzene contamination, specifications were prepared (Annex 1, reference *50*). SCF (1981) *(46)* evaluated cyclohexane as an extraction solvent in foodstuffs and could not establish an ADI for humans. In 1996, SCF evaluated cyclohexane as a previous cargo and considered it acceptable on the basis that it was previously approved as an extraction solvent for flavouring agents *(47)*. EFSA (2012) *(2)* concluded that cyclohexane meets the criteria for acceptability

as a previous cargo for edible fats and oils based on low systemic toxicity via all routes, negative genotoxicity results in vitro and in vivo, and absence of allergenic potential.

For the present assessment, previous assessments (monographs) produced by JECFA, SCF or EFSA were identified by searching their respective websites. This was followed by a comprehensive search to identify any critical new data for the assessment of human health risk on PubChem and PubMed and considering previous reviews by national and regional governmental authorities. The search terms used were the common name (cyclohexane), CAS number (110-82-7), toxicity and toxicokinetics. In an effort to supplement the toxicity information on short-term and long-term oral exposure, a similar comprehensive literature search was conducted for methylcyclohexane (a structural analogue of cyclohexane). The cut-off date for inclusion in this report was 29 December 2020.

Chemical and technical considerations

The chemical and technical considerations for cyclohexane are summarized in Table 16.

Assessment

Biochemical aspects

Following oral exposure, cyclohexane undergoes hydroxylation to cyclohexanol and is excreted in the urine as glucuronide conjugates, or expired as carbon dioxide or unchanged parent compound *(48)*. Cyclohexane administered orally to rats is eliminated from the plasma and tissues with a measured elimination half-life of 10–15 hours *(49)*. Toxicokinetic information on methylcyclohexane, a structural analogue of cyclohexane, indicates that methylcyclohexane also undergoes hydroxylation and excretion as a glucuronide or is expired as carbon dioxide or unchanged parent compound *(50)*. However, owing to its lower volatility, it is estimated that significantly less unchanged methylcyclohexane is excreted in the expired air following ingestion compared to cyclohexane *(50)* and greater systemic exposure to methylcyclohexane may be expected compared to cyclohexane. Both cyclohexane and methylcyclohexane have a high affinity for adipose tissues.

Toxicological studies

Cyclohexane exhibits low acute oral toxicity in rodents, with an estimated LD_{50} of > 5000 mg/kg bw *(58)*. Methylcyclohexane exhibits a slightly higher acute oral toxicity with reported oral LD_{50} values of ≥1200 mg/kg bw (PubChem[4]). Based

[4] https://pubchem.ncbi.nlm.nih.gov/compound/Methylcyclohexane#section=NIOSH-Toxicity-Data

Table 16
Chemical and technical considerations for cyclohexane

Name: Cyclohexane	
CAS number 110-82-7	**Alternative CAS numbers** None
Chemical details	Cyclohexane; hexamethylene; hexanaphthene Colourless, clear, flammable liquid, with characteristic odour  Molar mass: 84.16 g/mol Melting point: 4–7 °C Boiling point: 80.7 °C Insoluble in water; soluble or miscible with ethanol, methanol, diethyl ether, acetone, benzene and carbon tetrachloride.
Route(s) of synthesis	Produced by hydrogenation of benzene in either the liquid or the vapour phase in the presence of hydrogen or by fractional distillation of petroleum.
Composition	Cyclohexane produced by hydrogenation of benzene may contain residues of benzene, whereas cyclohexane from petroleum contains residues of hydrocarbons of similar volatility, mainly C5–C7, possibly including benzene. Cyclohexane may contain residues of sulfur and polycyclic aromatic hydrocarbons.
Uses	Used as a solvent for lacquers and resins, as a paint and varnish remover, as an extraction solvent in the preparation of flavouring agents, in industrial recrystallization of steroids, and in the manufacture of adipic acid and caprolactam that is used in the production of polyamide (nylon) for food packaging materials.
Analytical methods	None identified for previous cargoes. Cyclohexane can be analysed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	It is not expected to react with edible fats and oils.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

on oral LD_{50} values in rabbits, Treon et al. *(51)* suggested that toxicity decreases from ketone (e.g. cyclohexanone) to alcohol (e.g. cyclohexanol) to hydrocarbon (cyclohexane) and that the methylated compounds (i.e. methylcyclohexane, methycyclohexanol and methylcyclohexanone) are more hazardous than the corresponding non-methylated compounds (i.e. cyclohexane, cyclohexanol and cyclohexanone). No information on the short-term or long-term oral toxicity of cyclohexane via the oral route was identified. Following short-term exposure via inhalation, mice and rats showed increased liver weights and centrilobular hypertrophy at concentrations of between 6000 and 7000 ppm *(52)*. Bernard et al. *(53)* observed evidence of nephrotoxicity in female rats following exposure to 400 mg/kg bw per day via intraperitoneal injection, 5 days/week for 2 weeks, and suggested that the nephrotoxic effects of cyclohexane are likely to be due to cyclohexanol.

In a guideline-compliant 28-day repeated-dose oral toxicity study (OECD 407 *(54)*) methylcyclohexane was administered via gavage to groups of rats at doses of 0, 100, 300 and 1000 mg/kg bw per day *(49)*. At a dose of 1000 mg/kg bw per day, male and female animals showed transient salivation after dosing, changes in clinical chemistry parameters, and histopathological changes in the liver (hepatocellular hypertrophy) and kidney (hyaline droplet degeneration). Effects observed at the intermediate dose of 300 mg/kg bw per day were restricted to the males and consisted of transient salivation after dosing, increased body weight and food consumption, and hyaline droplet degeneration in the kidney. With the exception of slight hyaline droplet degeneration observed in one male, no other toxicologically relevant effects were observed in animals dosed at 100 mg/kg bw per day.

In a combined repeated-dose toxicity study with the Reproduction/Developmental Toxicity Screening Test (OECD 422), rats were administered methylcyclohexane daily via gavage at doses of 0, 62.5, 250 and 1000 mg/kg bw per day *(55)*. Once again, male and female animals exposed to 1000 mg/kg bw per day showed transient salivation after dosing and evidence of liver (increased absolute and relative organ weights) and kidney effects (increased relative organ weight). Toxicologically significant effects observed at 250 mg/kg bw per day (i.e. hyaline droplets in the renal tubules) were limited to male rats. No significant effects on reproduction or development were noted at any dose. According to the OECD summary of this study *(54)*, immunohistochemical examination for α2u-globulin in the kidneys of rats in the control and high-dose (1000 mg/kg bw per day) groups, revealed similar levels of α2u-globulin (positive controls confirmed assay function). Although α2u-globulin-related nephropathy in male rats is a common effect associated with repeated exposures to hydrocarbon solvents *(56)*, the Committee concluded that the evidence on α2u-globulin-related effects in rats exposed to methylcyclohexane is incomplete. In the absence of definitive evidence for α2u-globulin-associated effects, and considering that hyaline droplet accumulation was also observed in female rats in the high-dose group, the renal effects observed in male rats were considered relevant for the current evaluation.

The Committee also concluded that cyclohexane is non-genotoxic in vitro and in vivo.

Allergenicity

The Committee did not identify any reports that indicated that cyclohexane or methylcyclohexane elicits an allergic response upon oral exposure. There are also no data available that indicate that cyclohexane would contain a known food allergen.

Impurities

Cyclohexane transported as a previous cargo may contain benzene, but is unlikely to contain more than 0.1% benzene *(16)*. The estimated exposure to benzene present at a concentration of 0.1% in cyclohexane carried as a previous cargo is 0.3 µg/kg bw per day. The major route of exposure to benzene is inhalation, rather than diet *(42, 57)*. Estimated exposures to benzene in the United Kingdom range from 0.71 µg/kg bw per day for children in rural areas to 14.12 µg/kg bw per day for adult smokers in urban areas who work adjacent to busy roads *(42)*. Estimates of dietary exposure range from 1.4 to 2.8 µg/day *(57)*, equivalent to 0.02–0.05 µg/kg bw per day for adults weighing 60 kg. The estimated maximum dietary exposure to benzene present in fats and oils when cyclohexane is carried as a previous cargo, 0.3 µg/kg bw per day, is below the estimated range of inhalation exposures, although it is high compared to estimated total dietary exposures.

Cyclohexane may also contain residues of polycyclic aromatic hydrocarbons (PAH), but these residues have neither been characterized nor quantified, and exposures to PAH substances in cyclohexane carried as a previous cargo therefore cannot be estimated. The Committee noted that some PAH and benzene are carcinogens.

Assessment of dietary exposure

Cyclohexane may be used as an extraction solvent in the preparation of flavouring agents from natural flavouring materials, at levels up to 1 mg/kg in food in the EU (2009/32/EC) or as a diluent in colour additive mixtures in the United States (21 CFR 73.1). However, no estimates of cyclohexane concentrations in foods or of exposure from these sources were identified.

No data were found on concentrations of cyclohexane in food oils due to carryover from previous cargoes. A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances. Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day, based on a worst-case concentration of 100 mg/kg and an oil intake of 3 g/kg bw per day by infants and young children who are high consumers (see section 3.4.3, Dietary exposure assessment for previous cargo chemical substances).

Evaluation

No information regarding the short-term and long-term toxicity of cyclohexane was identified; however, cyclohexane exhibits relatively low systemic toxicity following short-term exposure via inhalation. The Committee identified a NOAEL of 62.5 mg/kg bw per day from two short-term oral toxicity studies with the structural analogue methylcyclohexane. Cyclohexane may be used as an extraction solvent for flavouring agents or as a diluent in colour additive mixtures.

However, no estimates of cyclohexane concentrations in foods or of exposure from these sources were identified. A comparison of the NOAEL of 62.5 mg/kg bw per day with the estimated generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day yields a MOE of 208. The Committee noted that this MOE is based on a potentially more toxic compound *(51)* and a sensitive critical effect (hyaline droplets in the renal tubules of male rats). In consideration of the conservative nature of both the exposure and hazard metrics used, the Committee concluded that this MOE is sufficient to address the uncertainties in the database.

There are no data on allergenicity upon oral exposure to cyclohexane that indicate that it is or it contains a known food allergen.

Cyclohexane as a previous cargo is not expected to react with edible fats and oils.

Although exposure to cyclohexane as a result of transporting cyclohexane as a previous cargo does not appear to be a health concern, there is uncertainty concerning the purity or "grade" of cyclohexane that will be transported as a previous cargo. Since cyclohexane may contain carcinogenic impurities in amounts that could significantly increase dietary exposure, the Committee could not reach a conclusion on the safety of transporting cyclohexane as a previous cargo for edible fats and oils until the nature and the quantities of these impurities in cyclohexane has been clarified.

A toxicological monograph including dietary exposure and chemical and technical considerations was prepared for all previous cargo substances considered at this meeting.

3.5.6 Future work and recommendations

The Committee recommended that sufficient chemical information that allows the evaluation of acetic anhydride and cyclohexane transported as previous cargoes be made available prior to the next evaluation. At a minimum this information should address the following:

- product grade(s) and composition, including characterization and levels of impurities arising from all methods of manufacture.

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4. Revision of specifications: Steviol glycosides

At its eighty-seventh meeting (Annex 1, reference *243*) the Committee reviewed information on the methods of manufacture and the identity and purity of steviol glycosides. The following manufacturing processes were included in the evaluation: aqueous extraction from the leaves of *Stevia rebaudiana* Bertoni; fermentation of selected strains of *Yarrowia lipolytica* or *Saccharomyces cerevisiae*; enzymatic modification of steviol glycosides extracted from *S. rebaudiana* Bertoni; and enzymatic glucosylation of steviol glycosides extracted from *S. rebaudiana* Bertoni. The Committee noted that the reviewed products are ≥ 95% steviol glycosides on the dried basis; the remainder being residues of starting material and food-grade processing aids used in production. A framework was adopted for specifications for steviol glycoside products based on the method of production. Individual specifications for steviol glycosides from each of the manufacturing processes identified were developed as four annexes within the new *(Framework for) steviol glycosides*.

The Committee, at its eighty-seventh meeting, also determined that no safety issues existed for steviol glycosides produced by any one of these methods where the products are ≥ 95% steviol glycosides as prescribed by the specifications. The Committee reviewed the ADI of 0–4 mg/kg bw established at its sixty-ninth meeting for steviol glycosides (expressed as steviol) (Annex 1, reference *190*) and applied it to steviol glycosides produced by the four methods described in the annexes of the specifications monograph. The Committee recognized that new methods or the modification or combination of current production methods could be developed in the future and decided that, if the final product meets the current specification of ≥ 95% steviol glycosides, the Committee would evaluate the method of manufacture and possible impurities. When appropriate, modifications would be introduced into the relevant annex or, alternatively, a new specifications annex would be developed. At the same meeting Annex 4 was added to the framework for enzyme modified glucosylated steviol glycosides. The specifications for Annex 4 were made tentative pending receipt of an assay method with supporting validation and data from production batches of enzyme modified glucosylated steviol glycosides. The *(Framework for) steviol glycosides* prepared by the Committee at its eighty-seventh meeting replaces all existing specifications monographs for steviol glycosides, including those for *Rebaudioside A From Multiple Gene Donors Expressed in Yarrowia lipolytica* and *Steviol Glycosides From Stevia rebaudiana Bertoni.*

The *(Framework for) steviol glycosides* was on the agenda at the present meeting for revision of the methods of assay for steviol glycosides and for glucosylated steviol glycosides. The Committee received a validated method of assay for steviol glycosides using high-performance liquid chromatography

with ultraviolet or diode array detection (HPLC-UV/DAD) coupled in series to a mass spectrometer. Major steviol glycosides are identified by comparison to commercially available reference standards and quantified using external standards. Mass spectrometry is used to identify minor steviol glycosides. The Committee also received supporting validation data from three laboratories and information from five batches of commercial steviol glycosides tested using the proposed method. The Committee additionally received a method of assay for glucosylated steviol glycosides based on Chinese national standard GB2760-2014. This assay is a multi-step procedure to: determine the percentage of dextrin and total steviol glycosides by column separation; determine the percentage of parent steviol glycosides and α-glucosyl steviol glycosides by HPLC-UV/DAD; identify individual α-glucosyl steviol glycosides by comparison with an example chromatogram; and quantify α-glucosyl steviol glycosides as the total peak area. The Committee reviewed verification data for the proposed method and batch data from 10 separate lots of enzyme modified glucosylated steviol glycosides.

The Committee replaced the existing assay for steviol glycosides in the (*Framework for) steviol glycosides* (Appendix B) with the HPLC-UV-MS technique utilizing external reference standards. The Committee additionally replaced the assay method in Annex 4 (enzyme modified glycosylated steviol glycosides) with the submitted HPLC-UV technique and removed the tentative status of Annex 4. An updated table of chemical information for steviol glycosides from *S. rebaudiana* Bertoni replaced Appendix A; and Annexes 1, 2 and 3 were revised to include the harmonized solubility parameters and a reference to Appendix B (the assay for steviol glycosides). The Committee noted that the revised *(Framework for) steviol glycosides* specifications monograph, including the appendices and four annexes, replaces the tentative specifications prepared at its eighty-seventh meeting. All specifications for steviol glycoside products evaluated by JECFA are now incorporated in the *(Framework for) steviol glycosides* prepared at the present meeting.

The Committee received additional information and literature describing a method of assaying steviol glycosides with quantification using 19-*O*-β-D-galactopyranosyl-13-*O*-β-D-glucopyranosyl-steviol (the 19-galactosyl ester of steviolmonoside) as internal standard. The Committee noted the reported method characteristics as a potential improvement on the HPLC-UV methods using external standards. However, the proposed internal standard is not currently commercially available and the level of international collaboration in developing the related method appeared to be low compared to that for the other steviol glycosides method submission. The Committee, therefore, noted that industry and interested stakeholders could collaborate in a comprehensive comparison of available methods requiring external standards with the internal standard method once a commercial supply of a suitable internal standard can be assured.

5. Future work and recommendations

Ergot alkaloids

The Committee recommended the following:

- additional data on the EAs to allow for the derivation of toxic equivalency factors (TEFs);
- additional data on the occurrence of EAs (at least for the 12 considered at this meeting) in wheat and wheat-based products and in rye and rye products from WHO regions and clusters where no data were submitted for this evaluation;
- the establishment of sampling plans for EAs.

Previous cargoes

1) The Committee reiterated the recommendations made at the ninetieth meeting that the Codex Committee on Fats and Oils (CCFO) consider revising Criterion no. 2 in RCP-36-1987 as adopted by CAC 34 (2011).
   - Based on the consumption of fats and oils by infants and young children, there is no health concern for the general population from dietary exposure to previous cargo chemical substances if the ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to 0.3 mg/kg bw per day. Substances for which there is no numerical ADI or TDI should be evaluated on a case-by-case basis (e.g. margin of exposure (MOE) approach).
   - Where there are additional sources of dietary exposure to the previous cargo chemical substances, they should be considered in the exposure assessment.
2) The Committee recommended that sufficient chemical information that allows the evaluation of acetic anhydride and cyclohexane transported as previous cargoes be made available prior to the next evaluation. At a minimum this information should address the following:
   - product grade(s) and composition, including characterization and levels of impurities arising from all methods of manufacture.

Annex 1

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Annex 2

Summary of toxicological and dietary exposure information

Food and Agriculture Organization of the United Nations

World Health Organization

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Ninety-first meeting
Virtual meeting, 1–12 February 2021

SUMMARY AND CONCLUSIONS
Issued on 5 March 2021

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held on a virtual online platform from 1 to 12 February, with an additional day for the report adoption on 25 February 2021. The purpose of the meeting was to evaluate the acceptability of certain substances as previous cargoes and the safety of certain food contaminants, as well as to revise the specifications on steviol glycosides. The present meeting was the ninety-first in a series of similar meetings.
If conditions had permitted, the ninety-first meeting of JECFA would have been held at FAO headquarters in Rome, Italy. Because of the travel restrictions and lock-downs due to the COVID-19 pandemic in many countries, the joint FAO/WHO JECFA secretariat was unable to convene a physical meeting. Therefore, the meeting was held as a video-conference.

In view of the time differences in the countries of origin of the invited experts, the only possible time for a video-conference was restricted to a 4-hour time slot (12:00–16:00 CEST) each day. This allowed only 40% of the usual daily length (8–10 hours) of a JECFA meeting. In an effort to regain some additional meeting time, the ninety-first JECFA meeting was extended by 3 days, adding Monday 1 February and Friday 12 February 2020, and Thursday 25 February.

Dr R. Cantrill served as Chairperson and Dr D. Benford served as Vice-Chairperson.

Ms K.B. Laurvick and Dr U. Mueller served as joint rapporteurs.

The Committee evaluated the contaminants cadmium and ergot alkaloids, and 5 substances that may occur as previous cargoes, as well as revising the specifications for steviol glycosides. The tasks before the Committee were (a) to undertake toxicological evaluations and dietary exposure assessments in relation to certain contaminants in food and (b) to revise the specifications for certain food additives.

This document summarizes the conclusions of the ninety-first meeting of JECFA. More details than are normally made available in a summary report are included on cadmium. This decision was made on an exceptional basis to facilitate the deliberations of the upcoming Codex Committee on Contaminants in Food.

Toxicological and dietary exposure monographs on the contaminants and additives considered will be published in WHO Food Additives Series No. 82.

More information on the work of JECFA is available at:
http://www.fao.org/food-safety/resources/publications/en/
and
https://www.who.int/foodsafety/en/

Toxicological and dietary exposure information and conclusions

Contaminants evaluated

Cadmium (exposure assessment from all food sources)[1]

Explanation

Cadmium was evaluated by the Committee at its sixteenth, thirty-third, forty-first, fifty-fifth, sixty-first, sixty-fourth, seventy-third and seventy-seventh meetings. At the sixty-first and sixty-fourth meetings, the Committee noted that the estimated total mean dietary exposure to cadmium from all foods, derived from per capita data from the five GEMS/Food regional diets, ranged from 40% to 60% of the provisional tolerable weekly intake (PTWI) applicable at that time of 7 µg/kg bw. The seven commodity groups that contributed significantly to total mean dietary exposure to cadmium were rice, wheat, root vegetables, tuber vegetables, leafy vegetables, other vegetables and molluscs (40–85% of the total mean dietary exposure to cadmium across the five regional diets).

At its seventy-third meeting in 2011, the Committee re-evaluated cadmium and established a provisional tolerable monthly intake (PTMI) of 25 µg/kg bw, reflecting the long half-life of cadmium in humans. Reported national estimates of mean dietary exposure to cadmium from all foods for adults ranged from 2.2 to 12 µg/kg bw per month, or 9–48% of the PTMI. For European children up to 12 years of age, this estimate was 11.9 µg/kg bw per month or 47% of the PTMI. High percentile dietary exposures to cadmium for adults from Europe, Lebanon and the USA were reported to range from 6.9 to 12.1 µg/kg bw per month (28–48% of the PTMI), and from 20.4 to 22.0 µg/kg bw per month (82–88% of the PTMI) for children aged 0.5–12 years from Australia and the United States of America (USA). Data on cadmium occurrence and consumption of foods containing cocoa and its derivatives were included in all 2011 estimates. Although not all estimates of dietary cadmium exposure evaluated at the seventy-third meeting reported the major contributing foods, for those estimates that did report this information, cereals and cereal products and vegetables were consistently reported as major contributors, with seafood and meat, including offal, also reported in some studies. None of the studies reported cocoa products as major contributors to dietary cadmium exposure.

At its seventy-seventh meeting in 2013, the Committee conducted an assessment of dietary exposure to cadmium from cocoa and cocoa products at the request of the sixth session of the Codex Committee on Contaminants in Foods (CCCF). The Committee considered the exposure to cadmium from foods containing cocoa and its derivatives in the context of overall dietary exposure. The estimates of mean dietary exposure to cadmium from foods containing cocoa and its derivatives ranged from 0.005 to 0.39 µg/kg bw per month or 0.2–1.6% of the PTMI across the 17 GEMS/Food cluster diets, assuming a body weight of 60 kg. Mean dietary exposure estimates for individual cocoa products based on national food consumption data ranged from 0.001 to 0.46 µg/kg bw per month or 0.004–1.8% of the PTMI. The cocoa products included were cocoa beverages, cocoa powder and other cocoa products. The highest high exposure (P97.5) was estimated at 12 µg/kg bw per month for European children 7–11 years of age solely due to the consumption of cocoa powder. Combining the highest P97.5 dietary exposure estimate for adults and children out of the three cocoa products with the mean dietary exposure estimates for both age groups from the whole diet, the Committee estimated a total dietary exposure of 7.4–17.2 µg/kg bw per month or 30–69% of the PTMI for adults and 23.9 µg/kg bw per month or 96% of the PTMI for children aged 0.5–12 years. The Committee noted that these estimates of total dietary cadmium exposure very likely overestimated the exposure, because the estimates from the whole diet also included a contribution from cocoa and cocoa products.

At the request of the thirteenth session of CCCF for more comprehensive occurrence data for cadmium in food, the JECFA Secretariat issued a call for data on cadmium in chocolate and cocoa-derived products in 2019. The submitted data included a wider geographical range of occurrence data for cadmium in cocoa products than considered at the seventy-seventh meeting of the Committee. The occurrence data also showed a higher mean concentration for cadmium in cocoa products than previously noted by the Committee. As a result, the JECFA Secretariat considered it appropriate to revise the dietary exposure assessment of cadmium to include not only chocolate and cocoa products but the contribution from all food sources. At the present meeting the Committee reassessed cadmium exposure to include the contribution of all food sources, particularly cocoa products.

[1] More details than are normally made available in a summary report are included on cadmium. This decision was made on an exceptional basis to facilitate the deliberations of the upcoming Codex Committee on Contaminants in Food.

Data submitted or available to the Committee

The GEMS/Food contaminants database was queried for records relating to cadmium in any food. The database query was restricted to records submitted since the previous assessment of dietary cadmium exposure from the whole diet by the Committee in 2011. Data submitted since 1 January 2011 originated from 27 countries or country groups (WHO European Region, WHO African Region), representing 10 of the 17 GEMS/Food cluster diets. It should be noted that for several of the countries or clusters the available data were limited in quantity or restricted to a narrow range of foods. For example, the sole country providing data from cluster G09 (Indonesia) submitted analytical results for 30 samples of cocoa products only. Five clusters (G07, G08, G10, G11 and G15) cover the countries of Europe; however, most of the contaminant concentration data available for these countries were only identified at the level of the WHO European Region and it was not possible to examine differences in contamination profile between these clusters using these data.

The final data set contained 277 292 records, of which 216 373 (78%) were from the WHO European Region. A considerable body of non-European data was available for cluster G10, submitted by Canada (n = 21 501), Japan (n = 5332) and the USA (n = 5887). Records were widely spread across different food types, with the most commonly analysed food types being edible pig offal (7.3%), marine fish (6.9%) and cattle meat (3.7%).

Given the focus of the current assessment on cadmium in cocoa and cocoa products, an overview of these data as included in the dietary exposure assessment was prepared. In total, 6957 records for cocoa and cocoa products were available, representing 2.5% of all records in the final data set. These records related to five groups of cocoa products: cocoa beans (n = 108), cocoa beverage (n = 20), cocoa butter (n = 20), cocoa mass (n = 218), cocoa powder (n = 2583) and chocolate (n = 4008). As for the whole database, the main single source of records for cocoa products was the WHO European Region, accounting for 2293 records (33%).

The Committee additionally evaluated published data on dietary exposure to cadmium at a national level. Since the evaluation of cadmium at the seventy-third meeting of the Committee in 2011, a number of national evaluations of chronic dietary exposure have been published. The Committee evaluated 44 national studies conducted worldwide in 32 countries and a country grouping, as reported in the literature. Studies evaluated were from Australia, Bangladesh, Benin, Brazil, Cameroon, Canada, Chile, China, Denmark, Europe, France, French Polynesia, Germany, Hong Kong Special Administrative Region of China, Ireland, Islamic Republic of Iran, Italy, Japan, Republic of Korea, Mali, the Netherlands, New Zealand, Nigeria, Poland, Serbia, Spain, Sri Lanka, Sweden, Thailand, the United Kingdom, the United States of America and Viet Nam. Evaluation was restricted to studies that included most of the foods commonly eaten in the country.

Given the large number of national estimates of dietary cadmium exposure available from the literature, their coverage of countries across the world, and their consistency, the Committee considered that deriving less refined international and national estimates of dietary exposure was inappropriate. The GEMS/Food cluster diets were used only to examine the contribution of cocoa products to dietary cadmium exposure.

National estimates of dietary exposure

The mean dietary exposure to cadmium from the total diet at a national level ranged from 0.6 µg/kg bw per month for adults in the Sikasso region of Mali (2.4% of the PTMI) up to 24 µg/kg bw per month in children aged 4–11 years in China (96% of the PTMI). The maximum reported high percentile estimate of dietary cadmium exposure was 66 µg/kg bw per month in boys aged 8 years from Australia (260% of the PTMI). However, this estimate was based on a one-day 24-hour dietary recall (24HDR), which may have inflated the high percentile estimate. The highest high percentile estimate of dietary cadmium exposure based on multiple-day dietary records was for children aged 4–11 years in China (48.2 µg/kg bw per month; 190% of the PTMI). High percentile estimates of adult dietary cadmium exposure were only occasionally above the PTMI and were typically 20–60% of the PTMI. The main sources of cadmium exposure were grain and grain-based products, vegetables, and fish and seafood.

Temporal trends in dietary cadmium exposure

Owing to differences in study design and study location, it is not possible to identify any trends in dietary exposure to cadmium across the Committee evaluations (sixty-first, sixty-fourth, seventy-third and current). Most studies continue to report estimated mean dietary exposure to cadmium approximately in the range of 10–40% of the health-based guidance value, and sometimes higher. Similarly, the major foods contributing to dietary cadmium exposure have not changed, with cereals, vegetables and seafood, especially molluscs being consistent major contributors across evaluations. None of the Committee evaluations have identified cocoa products as major contributors to dietary cadmium exposure.

Contribution of cocoa products to dietary exposure

Where relevant information was included in the published national estimates of dietary exposure, the contribution of cocoa products to the total mean dietary exposure to cadmium ranged from 0.2 to 9%.

Further estimates of the contribution of cocoa products to dietary cadmium exposure were derived using the GEMS/Food cluster diets and global estimates of mean concentrations of cadmium derived from all extracted data in the GEMS/Food contaminants database (277 292 records). Across cluster diets, cocoa products contributed 0.1–5.9% of dietary cadmium exposure. Clusters with the highest contributions to dietary cadmium exposure from cocoa products were the "westernized" clusters (G07, G08, G10 and G15), including predominantly European and North American countries. Contributions for these clusters ranged from 3.4–5.9%, with the greatest contribution for G07. These contributions reflect the higher consumption of chocolate and, more particularly, cocoa powder in the countries within these clusters, as the cadmium concentrations in foods were assumed not to differ between clusters.

The major producers of cocoa are African countries (Cameroon, Côte d'Ivoire, Ghana and Nigeria), Indonesia and South and Central American countries (Brazil, Colombia, Dominican Republic, Ecuador and Peru). These countries are represented by the clusters G03, G05, G09 and G13. Interestingly, cocoa products were generally very low contributors to dietary cadmium exposure (<1%) in these regions.

The potential impact on the contribution of cocoa products to dietary cadmium exposure of consuming products sourced from a single geographical region (GEMS/Food cluster) was explored for the cluster diet (G07) with the greatest contribution from cocoa products to cadmium exposure. In addition, sufficient information for such an analysis was also available from the European dietary exposure assessment, carried out by the European Food Safety Authority (EFSA).[2] Based on these data, the Committee conducted a more detailed analysis of the impact of consumption of cocoa products from a single geographical region on dietary cadmium exposure for different age groups in Europe. The results of these analyses are summarized in Table 1. This analysis suggests that there are potential scenarios under which cocoa products would be the main contributor to dietary cadmium exposure.

Table 1
Impact of the source of cocoa products consumed on the contribution of cocoa products to dietary cadmium exposure, GEMS/Food cluster G07 and European countries

	Contribution of cocoa products to dietary cadmium exposure (%) dependent on the source of cocoa products consumed			
Population[a]	**All[b]**	**G03[c]**	**G05[c]**	**G09[c]**
Literature national estimate	0.1–9.4			
Cluster G07c	5.9	0.9	9.8	3.8
European countries				
Infants	0.2	0.1	1.3	0.4
Toddlers	4.2	1.2	19.7	7.0
Other children	9.4	3.9	39.4	17.6
Adolescents	9.4	4.2	39.5	17.9
Adults	4.6	1.4	21.1	7.6
Elderly	2.6	0.7	12.6	4.2
Very elderly	2.8	0.8	13.7	4.7

[a] Infants: 12 weeks–11 months; toddlers: 12–35 months; other children: 3–9 years; adolescents: 10–17 years; adults: 18–64 years, elderly: 65–74 years; very elderly: ≥ 75 years.
[b] For the GEMS/Food cluster G07, "all" refers to the total data set on cadmium concentrations in cocoa products submitted to the GEMS/Food contaminants database. For literature and European estimates *(1)*, "all" refers to the cadmium concentration data used in the original analyses.
[c] Cluster G03 includes African countries, G05 includes mainly South and Central American.

Impact of established and proposed maximum limits for cadmium on cocoa product rejection rates and dietary cadmium exposure

The Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed includes maximum limits (MLs) for cadmium in:

[2] EFSA. Cadmium dietary exposure in the European population: European Food Safety Authority. EFSA J. 2012;10:2551.

- chocolate containing or declaring ≥ 50% to < 70% total cocoa solids on a dry matter basis of 800 µg/kg; and
- chocolate containing or declaring ≥ 70% total cocoa solids on a dry matter basis of 900 µg/kg.

At the thirteenth meeting of CCCF in 2019, further MLs were discussed and it was proposed to derive MLs proportional to the cocoa solids content of the cocoa products:

- ML of 300 µg/kg for chocolates containing or declaring <30% total cocoa solids on a dry matter basis;
- ML of 500 µg/kg for chocolates containing or declaring ≥30% to <50% total cocoa solids on a dry matter basis; and
- ML of 1500 µg/kg for cocoa powder (100% total cocoa solids on a dry matter basis, sold for final consumption).

Of the 4008 records in the GEMS/Food contaminants database related to chocolate, it was only possible to establish the percentage of cocoa solids for 638 (15.9%). These records were virtually all from countries in cluster G05 (South/Central America). The proportion of samples that exceeded the established or proposed ML ranged from 2.1% for chocolate with a ≥30 to <50% cocoa solids content to 16.3% for cocoa powder. Virtually all cocoa powder samples with cadmium concentrations above the ML were from countries in cluster G05 (South/Central America), resulting in a substantially higher potential rejection rate for cocoa powder samples from this cluster (405 of 1345 samples, 30.1%).

A summary of potential rejection rates for chocolate and cocoa powder from application of established and proposed MLs and the impact of applying the MLs on mean cadmium concentrations is provided in Table 2.

Table 2
Proportion of chocolate samples in different cocoa solids content classes and cocoa powder from different sources exceeding the established or proposed Codex maximum limit (ML) and the impact on mean cadmium concentration (medium bound)

	Chocolate, classified by cocoa solids content (%)[a]				Cocoa powder			
	<30	≥30 to <50	≥50 to <70	≥70	All	G03	G05	G09
ML (µg/kg)	300	500	800	900	1500	1500	1500	1500
Number of samples	114	187	251	86	2583	74	1345	9
Number of samples with cadmium concentration > ML (%)	3 (2.6)	4 (2.1)	27 (10.7)	4 (4.7)	420 (16.3)	0 (0.0)	405 (30.1)	0 (0.0)
MB mean, all samples	121	180	474	318	971	141	1600	609
MB mean, sample ≤ ML only (µg/kg)	110	172	418	255	502	141	814	609

G03: mainly African countries; G05: mainly South/Central American countries; G09: mainly South-East Asian countries; LOD: limit of detection; MB: medium bound, analytical results below the limit of detection (LOD) are substituted by a value equal to LOD/2; ML: maximum limit.
[a] Samples for which the cocoa solids content was available were almost all from countries in cluster G05.

Using the data across all clusters with sufficient information to allow application of the MLs, the mean contribution of cocoa products to dietary cadmium exposure was 2.2% without application of the MLs and 1.5% with application of MLs (see Table 3). Application of the MLs resulted in a mean reduction in dietary cadmium exposure of 0.7% across all clusters with reductions ranging from 0.0% (cluster G16) to 2.4% (cluster G07).

Application of the MLs had the greatest impact on dietary cadmium exposure when it was assumed that cocoa powder was sourced entirely from countries in cluster G05. This is not surprising as, for clusters G03, G05 and G09, only cocoa powder samples from cluster G05 had cadmium concentrations above the ML (30.1%, see Table 2). For cocoa products sourced from countries in cluster G03 and G09, application of the MLs had a negligible impact on dietary cadmium exposure, as the changes in exposure were only due to changes in the mean cadmium concentration for chocolate. The results of these analyses are summarized in Table 3.

Table 3
Proportion of chocolate samples in different cocoa solids content classes and cocoa powder from different sources exceeding the established or proposed Codex maximum limit (ML) and the impact on mean cadmium concentration (medium bound)

Source of cocoa products[a]	Potential rejection rate (%) for cocoa powder samples from application of ML[b]	Mean contribution (range) of cocoa products to dietary cadmium exposure, GEMS/Food cluster diets (%)		Mean reduction (range) in dietary cadmium exposure due to application of MLs, GEMS/Food cluster diets[c] (%)
		Without MLs applied	With MLs applied	
All[d]	16.3	2.2 (0.1–6.6)	1.5 (0.1–4.3)	0.7 (0.0–2.4)
Cluster G03	0.0	1.1 (0.0–2.9)	1.1 (0.0–2.6)	0.1 (0.0–0.3)
Cluster G05	30.1	2.9 (0.2–9.3)	1.9 (0.1–5.7)	1.1 (0.0–3.8)
Cluster G09	0.0	1.7 (0.1–5.0)	1.6 (0.1–4.8)	0.1 (0.0–0.3)

ML: maximum limit, both proposed and established MLs were applied in this analysis; G03: mainly African countries; G05: mainly South/Central American countries; G09: mainly South-East Asian countries.

[a] Cocoa products included in the GEMS/Food cluster diets are cocoa beans, cocoa butter, cocoa mass, cocoa powder and chocolate

[b] Potential rejection rates for chocolate are not given, as submitted data with sufficient information to allow application of MLs were only received from countries in cluster G05. The total rejection rate for chocolate samples was 4.9%

[c] The percentages in this column are the percentage decreases in the estimated dietary cadmium exposure due to application of the MLs, rather than the difference in the contribution from cocoa products

[d] "All" refers to the total data set on cadmium concentrations in cocoa products submitted to the GEMS/Food contaminants database with sufficient information to apply the MLs.

Evaluation

The Committee assessed information related to exposure to cadmium from all food sources, with a particular focus on cocoa products. Information assessed was restricted to the period since the previous assessment of dietary exposure to cadmium in 2011. The Committee summarized dietary cadmium exposure estimates from 44 national studies conducted worldwide in 32 countries and a country grouping as reported in the literature. The mean dietary exposure to cadmium from the whole diet ranged from 0.6 µg/kg bw per month (2.4% of the PTMI) for adults in the Sikasso region of Mali up to 24 µg/kg bw per month (96% of the PTMI) in children aged 4–11 years in China. These children from China also had the highest high percentile estimate of dietary cadmium of 48.2 µg/kg bw per month (190% of the PTMI). High percentile estimates of adult dietary cadmium exposure were only occasionally above the PTMI and were typically 20–60% of the PTMI. Consistent with the previous evaluations of the Committee, the present evaluation identified the main sources of dietary cadmium exposure in these national studies as cereals and cereal-based products, vegetables, and fish and seafood. Of the 44 studies reviewed, only nine reported the contribution of cocoa products to the total mean dietary exposure to cadmium, which ranged from 0.2 to 9%.

Given the large number of national estimates of dietary cadmium exposure available from the literature, their coverage of countries across the world, and their consistency, the Committee considered that deriving less refined international and national estimates of dietary exposure was unnecessary.

Based on data on the concentration of cadmium in foods submitted to the GEMS/Food contaminants database since 1 January 2011, the Committee examined the contribution of cocoa products to the mean dietary exposure to cadmium using the GEMS/Food clusters diets. Analyses using these data showed that the contribution of cocoa products to the dietary exposure to cadmium was consistent with the estimates based on national dietary exposure studies, ranging from 0.1% to 5.9%. The highest contributions were calculated for European and North American countries, reflecting the higher consumption of chocolate and cocoa powder in these countries.

The potential impact of consumption of cocoa products from a single geographical region, as represented by GEMS/Food clusters was examined. For the cluster with the greatest contribution to dietary cadmium exposure from cocoa products (G07, mainly European countries, 5.9%) this contribution would decrease to 0.9% or increase to almost 10% if cocoa products were sourced only from countries in cluster G03 (Africa) or G05 (South/Central America), respectively. The Committee carried out a similar analysis using data (mean concentrations of cadmium in cocoa products, dietary cadmium exposure estimates and contributions of cocoa products to dietary exposure) for European countries reported by EFSA.[3] In the EFSA study, the age group with the greatest contribution to dietary cadmium exposure from cocoa products was children aged 3–9 years (contribution 9.4%). From the Committee's analysis, if this age group were to

[3] EFSA. Cadmium dietary exposure in the European population: European Food Safety Authority. EFSA J. 2012;10:2551.

consume cocoa products sourced solely from cluster G03 (Africa), dietary cadmium exposure would decrease modestly (16.8 to 15.8 µg/kg bw per month), while the contribution from cocoa products would decrease to 3.9%. If this group were to consume cocoa products sourced solely from cluster G05 (South/Central America), dietary cadmium exposure would increase to 25.1 µg/kg bw per month, with cocoa products contributing 39% of dietary cadmium exposure.

CCCF has proposed MLs for chocolate with proportions of total cocoa solids of <30% and ≥30% to <50% on a dry matter basis and for cocoa powder with 100% total cocoa solids on a dry matter basis. These MLs are proposed in addition to exi sting MLs for chocolate with ≥50% to <70% and ≥70% total cocoa solids on a dry matter basis. Cocoa solids content information was available for a limited subset (15.9%) of the chocolate records in the GEMS/Food contaminants database. Comparing the cadmium concentrations in chocolate and cocoa powder in the GEMS/Food contaminants database to the existing and proposed MLs showed that 2.1–10.7% of the chocolate samples and 16.3% of the cocoa powder samples had concentrations higher than the MLs and could potentially be rejected by importing countries through application of the MLs. Applying these MLs compared to not applying them resulted in an average decrease in the contribution of cocoa products (including also cocoa beans, cocoa butter and cocoa mass) to the dietary exposure to cadmium of 0.7% across all clusters.

At its seventy-third meeting in 2011, the Committee established a PTMI of 25 µg/kg bw, reflecting the long half-life of cadmium in humans. The PTMI was not reviewed at the current meeting. The national exposure estimates were predominantly below this PTMI, with some exceptions for young children or adults living in China. The Committee noted that the current JECFA PTMI for cadmium is based on long-term bioaccumulation in the kidney, with steady-state not achieved until after 45–60 years of exposure. The Committee concluded that dietary exposure above the PTMI for limited periods may be of lesser concern in younger age groups. However, there may be a health concern in areas where the cadmium exposure during adulthood exceeds the PTMI.

The Committee concluded that major contributors to dietary cadmium exposure were cereals and cereal products, vegetables and seafood. The contribution of cocoa products to dietary cadmium exposure was minor in comparison (0.1–9.4% for national studies and estimates based on GEMS/Food cluster diets), even in countries in which the consumption of cocoa products is relatively high.

Application of both established and proposed MLs for chocolate and cocoa powder may result in substantial rejection rates (up to 30%) for products from some regions, but has only a minor impact (mean decrease across clusters of 0.7%, range 0.0–2.4%) on total dietary cadmium exposure.

A dietary exposure monograph was prepared.

Ergot alkaloids

The Committee identified the pharmacological effect of ergometrine maleate on the uterus, causing uterine contractions in humans during late pregnancy and postpartum, as the critical effect for the evaluation of ergot alkaloids (EAs) in the diet.

The Committee established an acute reference dose (ARfD), based on the following considerations:

1. The lowest oral therapeutic dose of 0.2 mg ergometrine maleate (equivalent to 2.5 µg/kg bw, expressed as ergometrine) is considered a pharmacological effect level in the most sensitive individuals, i.e. those with high absorption.
2. Of the EAs that have been used as drugs, ergometrine is known to have the highest potency for uterine contractions and its uterotonic effect increases towards the end of pregnancy.

In selecting an uncertainty factor (UF) for extrapolation from the pharmacological effect level at the therapeutic dose (LOEL) to a NOEL, the Committee took into consideration that the data relate to a short-lived, reversible, pharmacological effect, seen within a very sensitive subpopulation (women in late pregnancy or postpartum). A UF of 2 was considered appropriate for extrapolating from a pharmacological LOEL to a NOEL.

To derive an ARfD from a NOEL based on human data, in the absence of additional information, the default UF would normally be 10. However, for a substance that reversibly interacts with specific receptors, as is the case here, with a pharmacological effect that is predominantly dependent on its maximum plasma concentration (i.e. Cmax), a UF for toxicokinetic differences is considered unnecessary. The Committee therefore applied the UF of 3.16 to cover possible interindividual toxicodynamic differences.

Applying a composite UF of 6.3 (2×3.16) results in an acute reference dose of 0.4 µg ergometrine/kg bw ($2.5 \div 6.3 = 0.4$). The Committee noted that it is appropriate to establish a group acute reference dose for EAs but concluded that the available data are not sufficient to establish toxic equivalency factors (TEFs) for different EAs. Therefore, the ARfD is established as a group ARfD for the simple sum of total EAs in the diet.

This ARfD would also be protective for other potentially sensitive subgroups in the population, such as children, based on similar calculations in relation to adverse effects (gastrointestinal symptoms) in that group following unintentional exposure to ergometrine maleate.

Limited data from two 4-week studies on ergotamine tartrate and α-ergocryptine in rats allowed the determination of a reference point (BMDL10) of 1.3 mg/kg bw for EAs, based on muscular degeneration in the tail, secondary to vasoconstriction. The Committee noted that the human pharmacological effect level of 2.5 µg/kg bw and its derived NOEL provided a much more sensitive reference point for derivation of an ARfD than the BMDL10 value from a downstream toxic effect in animals.

As a first approach to establishing a TDI, the Committee considered the data from repeated-dose animal studies and selected the lowest BMDL10 value of 0.6 mg/kg bw per day calculated for ergotamine, based on tail muscular atrophy, secondary to vasoconstriction, observed in the 13-week study in rats as reference point. Applying a default UF of 100 for intra- and inter-species differences, a UF of 2 for extrapolation from a 13-week study to chronic exposure and an additional UF of 3 to take into account the limitations of the available toxicity data would indicate derivation of a TDI of 1 µg/kg bw per day.

The Committee considered that a TDI should not be higher than the ARfD and decided to establish a group TDI for the sum of total EAs in the diet at the same value as the group ARfD of 0.4 µg/kg bw per day.

The Committee noted that some estimates of the mean (0.46–0.47 µg/kg bw per day) and high percentile (0.56–0.86 µg/kg bw per day) chronic dietary exposure in children and some estimates of the high percentile acute dietary exposure in children (0.65–0.98 µg/kg bw per day) and in adults (0.49 µg/kg bw per day) exceeded the EAs group health-based guidance value (HBGV), and that this may indicate a human health concern.

Previous cargoes	Margins of exposure and other conclusions on toxicology and dietary exposure
Solvents/reactants (Group 1)	
Acetic anhydride	No information regarding the short-term and long-term toxicity of acetic anhydride was identified. However, upon evaluation of the available information, the Committee noted that it had previously allocated a group acceptable daily intake (ADI) "not specified" to acetic anhydride's immediate hydrolysis product, i.e. acetic acid and its potassium and sodium salts. Since acetic anhydride is anticipated to be rapidly hydrolysed to acetic acid during tank washing, within the edible oil cargo and after ingestion, the group ADI "not specified" for acetic acid and its potassium and sodium salts is considered directly relevant for this assessment of acetic anhydride. The United States National Research Council estimated that mean exposure to acetic acid from all food sources is 2.1 g/day for persons above 2 years of age, which is equivalent to 35 mg/kg bw per day for adults based on a body weight of 60 kg. It is not expected that exposure to acetic acid present due to hydrolysis of acetic anhydride in carryover from previous cargoes would add significantly to total exposures to acetic acid. Therefore, acetic anhydride at the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day would only contribute marginally to the overall dietary exposure to acetic acid and is not expected to result in adverse effects on human health. The Committee concluded that considering the widespread presence of acetic acid in the diet, it is unlikely that acetic anhydride present in low concentrations such as when transported as a previous cargo will produce an allergic response. Acetic anhydride acetylates free hydroxyl groups without a catalyst, but esterification is more complete in the presence of acids, so acetic anhydride and acetic acid could react with alcohols (for example mono- and diglycerides) forming acetates. Reaction rates are likely to be slow at ambient temperature. Although exposure to acetic anhydride and acetic acid as a result of transporting acetic anhydride as a previous cargo does not appear to be a health concern, there is uncertainty concerning the purity or "grade" of acetic anhydride that is transported as a previous cargo. Since acetic anhydride may contain impurities (e.g. diketene), which are potentially genotoxic, the Committee could not reach a conclusion on the safety of transporting acetic anhydride as a previous cargo for edible fats and oils until the nature and quantities of these impurities have been clarified.

***sec*-Butyl acetate**	No information regarding the short-term and long-term toxicity of *sec*-butyl acetate was identified; however, for *sec*-butanol, the Committee identified a BMDL05 of 657 mg/kg bw per day based on reduced offspring body weight from a two-generation reproductive and developmental toxicity study in rats. *sec*-Butyl acetate is naturally present in vinegar and is approved for use as a flavouring agent in Europe. The Committee estimated that exposure to *sec*-butyl acetate from vinegar consumption and its use as a flavouring agent is approximately 0.1 mg/kg bw per day. A comparison of the BMDL05 of 657 mg/kg bw per day for *sec*-butanol with the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day for *sec*-butyl acetate as a previous cargo plus its presence in the diet (0.1 mg/kg bw per day) yields a margin of exposure (MOE) of 1643, which is considered sufficient to address the uncertainties in the database. There are no data on allergenicity upon oral exposure to *sec*-butyl acetate that indicate that it is or it contains a known food allergen. *sec*-Butyl acetate hydrolyses to acetic acid and sec-butanol, which in the presence of acid may participate in transesterification with lipids, producing a mixture of fatty acid *sec*-butyl esters and glycerol. However, the reactions are slow, requiring an excess of alcohol and temperatures above 100 °C. Therefore, *sec*-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.
***tert*-Butyl acetate**	No information regarding the short-term and long-term toxicity of *tert*-butyl acetate was identified; however, the Committee identified a LOAEL of 180 mg/kg bw per day based on renal effects observed in female rats chronically exposed to a metabolite of *tert*-butyl acetate (i.e. *tert*-butanol) in drinking-water. The LOAEL for *tert*-butanol is lower than the NOAEL of 400 mg/kg bw per day of *tert*-butyl acetate for developmental toxicity and represents a conservative metric for risk assessment of *tert*-butyl acetate. No data were found on concentrations of *tert*-butyl acetate in food from any source. A comparison of the LOAEL of 180 mg/kg bw per day with the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day for *tert*-butyl acetate as a previous cargo yields a MOE of 600, which is considered sufficient to address the uncertainties in the database. There are no data on allergenicity upon oral exposure to *tert*-butyl acetate that indicate that it is or it contains a known food allergen. *tert*-Butyl acetate hydrolyses to acetic acid and *tert*-butanol, which in the presence of acid may participate in transesterification with lipids producing a mixture of fatty acid *tert*-butyl esters and glycerol. However, the reactions are slow, requiring an excess of alcohol and temperatures above 100 °C. Therefore, *tert*-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.
***n*-Pentane**	No reliable information regarding the short-term and long-term toxicity of *n*-pentane was identified; however, the Committee identified a NOAEL of 1000 mg/kg bw per day for *n*-pentane based on developmental toxicity testing in rats. The Committee also identified a NOAEL of 300 mg/kg bw per day for an isomer (isopentane) following short-term oral exposure in a one-generation toxicity test in rats (12 and 10 weeks of exposure in males and females, respectively). A comparison of the NOAEL of 300 mg/kg bw per day for isopentane with the generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day yields a MOE of 1000, which is sufficient to address the uncertainties in the database. There are no data on allergenicity upon oral exposure to *n*-pentane that indicate that it is, or it contains a known food allergen. *n*-Pentane as a previous cargo is not expected to react with edible fats and oils to form any reaction products. Exposure to impurities in n-pentane is not anticipated to contribute significantly to background exposures. Therefore, *n*-pentane meets the criteria for acceptability as a previous cargo for edible fats and oils.
Cyclohexane	No information regarding the short-term and long-term toxicity of cyclohexane was identified; however, cyclohexane exhibits relatively low systemic toxicity following short-term exposure via inhalation. The Committee identified a NOAEL of 62.5 mg/kg bw per day from two short-term oral toxicity studies with the structural analogue methylcyclohexane. Cyclohexane may be used as an extraction solvent for flavouring agents or as a diluent in colour additive mixtures. However, no estimates of cyclohexane concentrations in

	foods or of exposure from these sources were identified. A comparison of the NOAEL of 62.5 mg/kg bw per day with the estimated generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day yields a MOE of 208. The Committee noted that this MOE is based on a potentially more toxic compound and a sensitive critical effect (hyaline droplets in the renal tubules of male rats). In consideration of the conservative nature of both the exposure and hazard metrics used, the Committee concluded that this MOE is sufficient to address the uncertainties in the database. There are no data on allergenicity upon oral exposure to cyclohexane that indicate that it is or it contains a known food allergen. Cyclohexane as a previous cargo is not expected to react with edible fats and oils. Although exposure to cyclohexane as a result of transporting cyclohexane as a previous cargo does not appear to be a health concern, there is uncertainty concerning the purity or "grade" of cyclohexane that will be transported as a previous cargo. Since cyclohexane may contain carcinogenic impurities in amounts that could significantly increase dietary exposure, the Committee could not reach a conclusion on the safety of transporting cyclohexane as a previous cargo for edible fats and oils until the nature and the quantities of these impurities in cyclohexane has been clarified.
Revision of specifications	
Steviol glycosides	The Committee replaced the existing assay for steviol glycosides in the *(Framework for) steviol glycosides* (Appendix B) with the HPLC-UV-MS technique utilizing external reference standards. The Committee additionally replaced the assay method in Annex 4 (enzyme modified glycosylated steviol glycosides) with the submitted HPLC-UV technique and removed the tentative status of Annex 4. An updated table of chemical information for steviol glycosides from Stevia rebaudiana Bertoni replaced Appendix A; and Annexes 1, 2 and 3 were revised to include the harmonized solubility parameters and a reference to Appendix B (the assay for steviol glycosides). The Committee noted that the revised *(Framework for) steviol glycosides* specifications monograph, including the appendices and four annexes, replaces the tentative specifications prepared at its eighty-seventh meeting. All specifications for steviol glycoside products evaluated by JECFA are now incorporated in the *(Framework for) steviol glycoside*s prepared at the present meeting.

Annex 3

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

91st JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
1–12 February 2021

Virtual meeting: 12:00–16:00 (Geneva time)

1. Opening

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

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

4. Adoption of the agenda

5. Matters of interest arising from previous Sessions of the Codex Committee on Contaminants in Food (CCCF), Codex Committee on Food Additives (CCFA) and Codex Committee on Fats and Oils (CCFO)

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

7. Evaluations

    7.1. Cadmium (exposure assessment from all food sources)
    7.2. Ergot alkaloids
    7.3. Previous cargoes – solvents and reactants
    7.4. Steviol glycosides (revision of specifications)

8. Other matters to be considered (general considerations).

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

10. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of certain food additives
Eighty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1027, 2020 (106 pages)

Evaluation of certain veterinary drug residues in food
Eighty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1023, 2020 (116 pages)

Evaluation of certain food additives
Eighty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1020, 2019 (109 pages)

Evaluation of certain food additives
Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1014, 2019 (156 pages)

Evaluation of certain veterinary drug residues in food
Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1008, 2017 (150 pages)

Safety evaluation of certain food additives
Eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 75, 2018 (244 pages)

Evaluation of certain food additives
Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1007, 2017 (92 pages)

Safety evaluation of certain contaminants in food
Eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 74, 2018 (897 pages)

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)

Further information on these and other WHO publications can be obtained from
WHO Press, World Health Organization ▪ 1211 Geneva 27, Switzerland ▪ www.who.int/bookorders
tel.: +41 22 791 3264; **fax:** +41 22 791 4857; **email:** bookorders@who.int

Evaluation of certain food additives and contaminants

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

The report contains a summary of the Committee's evaluations of the following contaminants: cadmium (exposure assessment from all food sources); ergot alkaloids and previous cargoes – solvents and reactants. This is followed by a revision of the specifications for steviol glycosides and a summary of future work and recommendations made by the Committee.

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