WHO FOOD ADDITIVES SERIES: 82

Prepared by the ninety-first meeting of the
Joint FAO/WHO Expert Committee
on Food Additives (JECFA)

Safety evaluation of certain contaminants in food

Food and Agriculture Organization of the United Nations

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CONTENTS

PREFACE

The monographs contained in this volume were prepared at the ninety-first meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met online in November 2020. These monographs summarize the data on selected food contaminants reviewed by the Committee.

The ninetieth report of JECFA has been published by WHO as WHO Technical Report No. 1036. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication. JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Foods and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and dietary exposure and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The monographs contained in this volume are based on working papers that were prepared by WHO and FAO experts. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in WHO concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of or dietary exposure to the compounds evaluated in this publication should be addressed to: WHO Joint Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (jecfa@who.int).

SAFETY EVALUATIONS OF SPECIFIC CONTAMINANTS IN FOOD

Cadmium: dietary exposure assessment

First draft prepared by
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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 Global Environment Monitoring System (GEMS)/Food Contamination Monitoring and Assessment Programme regional diets, ranged from 40% to 60% of the provisional tolerable weekly intake applicable at that time of 7 µg/kg body weight (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 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 (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 chocolates 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.

2. Food consumption and dietary exposure assessment

2.1 Concentrations in food used in the dietary exposure estimates

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 African Region, WHO European 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 extracted data set was cleaned by removal of records of aggregate analyses (summary of multiple analyses), records with no result recorded and records relating to analysis of animal feed. The units of measurement were standardized to μg/kg. After consideration of the range of reported limits of detection (LODs), a cut-off was made at 20 μg/kg. When considering the full distribution of reported LODs, this value was approximately two standard deviations above the mean LOD.

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 were 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%).

Cadmium concentrations for most food types were consistent with expectations; however, the data set contained 714 records for cadmium in "sugar", most of which came from the WHO European Region. This data set contained a subset of records of very high concentrations (up to 12 000 μg/kg). Information from the literature does not support such high concentrations of cadmium in sugar and the records under this descriptor were excluded from the analyses of dietary cadmium exposure.

2.2 Food consumption data used in the dietary exposure estimates

The Committee used the GEMS/Food cluster diets to investigate the contribution of different food types, particularly cocoa products, to dietary cadmium exposure. The consumption cluster diets provide mean per capita consumption values based on FAO food balance sheet data for raw commodities and some semi-processed commodities for 17 clusters of countries (Sy et al., 2013). Clusters G01 and G06 include primarily Middle Eastern, central Asian and north African countries; clusters G03, G13 and G16 include primarily African countries; cluster G02 includes countries in west Asia and the Balkan region of Europe; cluster G04 includes Middle Eastern and Caribbean countries; cluster G09 includes countries in Africa and Asia; clusters G07, G08, G10, G11 and G15 include European and North American countries and developed countries from Asia and the Pacific (Australia, Japan, New Zealand, the Republic of Korea); clusters G05 and G12 consist mainly of South and Central American countries; and clusters G14 and G17 include Caribbean, Asian and Pacific Island states (see Appendix).

In addition to national estimates of dietary exposure published in the literature, the Committee may derive national estimates of dietary exposure using food consumption information from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss), in combination with summary concentration data from the GEMS/Food contaminants database. Previously, national estimates of chronic dietary exposure were derived by the Committee when:

- national food consumption information was available through CIFOCOss;
- suitable concentration data were submitted to the Committee (GEMS/Food contaminants database); and
- no existing recent dietary exposure assessment for cadmium was available for the country from the literature.

These criteria were not fully met for any country with food consumption information in CIFOCOss or concentration data on cadmium in the GEMS/Food contaminants database. Consequently, the Committee decided not to derive additional national estimates of dietary exposure to cadmium.

2.3 National estimates of chronic dietary exposure from the literature

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. Studies were identified from the scientific literature (PubMed and Web

of Science) or the grey literature using the search terms: cadmium AND (food OR diet*) AND (exposure OR intake OR "total diet"). The studies summarized below are those that were considered by the Committee to include most of the main contributors to dietary cadmium exposure. Studies that only considered a single food type or a narrow range of food types were not summarized. The Committee considered evaluations from Australia, Bangladesh, Benin, Brazil, Cameroon, Canada, Chile, China, Denmark, Europe, France, French Polynesia, Germany, Hong Kong Special Administrative Region (SAR) 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 USA and Viet Nam (Table 1).

(a) Australia

Dietary exposure to cadmium was assessed as part of the twenty-fifth Australian Total Diet Study (ATDS) (FSANZ, 2019). Foods (n = 88) were sampled in all Australian states and territories on two occasions. Individual food purchases were combined to give either four or eight food type composites. Food samples were analysed for cadmium by inductively-coupled plasma-mass spectrometry (ICP-MS), following acid digestion. The limit of reporting (LOR) for all sample types was 5 µg/kg. Median concentrations for each food type were used to estimate dietary exposure. For foods with a low proportion of results above the LOR, lower-bound (LB)–upper-bound (UB) estimates of the median were determined, with results below the LOR either substituted by zero or the LOR, respectively. Individual food consumption information for the population aged 2 years or above was taken from the 2011–2012 Australian National Nutrition and Physical Activity Survey. Food consumption information was only used if respondents (n = 7735) had completed two 24-hour dietary recall (24HDR) surveys. A model diet was used for infants (9 months old). Mean (90th percentile) LB–UB estimates of dietary cadmium exposure for all respondents aged 2 years and over were 2.5–6.6 (4.8–11) µg/kg bw per month. For 9-month-old infants the corresponding dietary exposure estimates were 2.8–16 (5.7–33) µg/kg bw per month. Cereals and cereal products and root vegetables were the main contributors to dietary exposure for all age groups. Hot chocolate beverages, cocoa, chocolate and fudge contributed 4–6% of estimated dietary exposure.

A study carried out in Western Australia conducted an initial 24HDR and food frequency questionnaire with 22 families with children aged 5–6 years, to identify foods for further study (Callan et al., 2014). Samples of foods and beverages (n = 253) were purchased from supermarkets between April and July 2011. Food samples were prepared by normal household methods, but not cooked. Samples were analysed for cadmium by ICP-MS, following nitric

acid digestion. LOD and limit of quantification (LOQ) were 3 and 10 µg/kg for solid and liquid food samples. Geometric mean concentrations were calculated for each food group. It is unclear how analytical results below the LOQ were handled. Food consumption information was derived from the 2008 Child and Adolescent Physical Activity and Nutrition Survey, a state-based survey involving administration of a single 24HDR to 653 children aged 8–17 years. Data were used to calculate mean, 5th and 95th percentile (P95) food consumption for the food groups. Mean (P95) estimates of dietary cadmium exposure ranged from 0.2 (1.0) for 16-year-old males or females to 0.4 (2.2) µg/kg bw per day (12 and 66 µg/kg bw per month) for 8-year-old males. It is likely that the use of food consumption data from a single 24HDR will have inflated the P95 estimates of dietary exposure. The main contributors to dietary exposure were fish and seafood products (36%), cereals (31%), and vegetable products and dishes (18%). Although it was reported that cadmium concentrations in cocoa powder and chocolate were among the highest in the foods analysed, contributions of these foods to dietary exposure were not explicitly reported. However, they do not appear to be main contributors to dietary exposure.

(b) **Bangladesh**

The study from Bangladesh was slightly unusual in that the dietary cadmium exposure of the Bangladeshi population was estimated based on analysis of Bangladeshi foods imported into the United Kingdom (Al-Rmalli et al., 2012). Food samples were collected from Bangladeshi shops during 2008 and 2009. Samples were analysed for cadmium by ICP-MS, following nitric acid/hydrogen peroxide digestion. The sensitivity of the method was not reported and there was no indication of how analytical results below the LOD were handled. Food consumption information was taken from the literature. Rice consumption was assumed to be equivalent to 500 g of uncooked rice per day. A default body weight of 60 kg was used. Estimated dietary cadmium exposure was 34.6 µg/day or 17.3 µg/kg bw per month for a 60-kg Bangladeshi. The main contributors to dietary exposure were steamed rice (54%) and green vegetables (35%). No information on dietary exposure from cocoa products was provided.

(c) **Benin**

As part of a multicountry total diet study in sub-Saharan Africa, foods were collected at two locations in Benin (Littoral and Borgou) and combined to give 40 food group composites, representing 13 food groups (Ingenbleek, 2019). Samples were analysed for a range of contaminant elements, including cadmium, by ICP-MS, following nitric acid digestion. LOQs were in the range 0.2–1.0 µg/kg. Cadmium was quantified in 63% of composite samples. LB–UB estimates of

the mean concentration were derived for each food group. Food consumption information was obtained from a household budget survey including 2494 households across the two sampling locations in Benin. Household food intake was converted to intake per adult male equivalent (AME). LB–UB mean (P95) estimates of dietary cadmium exposure for Littoral and Borgou were 0.05–0.05 (0.10–11) and 0.04–0.05 (0.09–0.10) μg/kg bw per day, respectively. These exposure estimates correspond to 1.5–1.5 (3.0–3.3) μg/kg bw per month and 1.2–1.5 (2.7–3.0) μg/kg bw per month, respectively. The main contributors to dietary exposure were tomatoes, rice, pasta, yams and smoked fish. No information on the contribution to exposure from cocoa products was reported.

(d) **Brazil**

Dietary cadmium exposure was determined for a cohort (n = 64) of 1–4-year-old children from day care centres in São Paulo, Brazil (Leroux et al., 2018). For each child, a weekday 24-hour duplicate diet sample was collected and analysed for cadmium by acid digestion followed by ICP-MS. Portion sizes were recorded at the time of collection of the duplicate diet samples. Mean dietary cadmium exposure for male and female children was estimated to be 0.08 and 0.09 μg/kg bw per day, respectively. These exposures are equivalent to 2.4 and 2.7 μg/kg bw per month, assuming the single day sampled was representative of the children's normal dietary patterns. The duplicate diet method does not allow assessment of the contributions of individual food types to overall dietary exposure.

(e) **Cameroon**

Individual food samples (n = 1773) of 203 different food items were composited into 64 analytical samples, representing 10 food categories (Gimou et al., 2014). Samples were analysed for cadmium by ICP-MS following acid digestion. The LOQ was 1 μg/kg, with 17% of samples containing cadmium concentrations below the LOQ. Of these, 11% were below the LOD and the remaining 6% were between the LOD and LOQ. LB and UB estimates of the mean cadmium concentration were determined for each food category. Dietary exposure to cadmium was derived using UB estimates of the mean concentration. Food consumption data were obtained from the second Cameroonian Household Budget Survey. The survey recorded expenditure for 11 553 households over a 2-week period. Food expenditure was converted to food consumption for "adult equivalents". A default body weight of 60 kg was used. Mean and P95 UB estimates of dietary cadmium exposure were 4.7 and 8.2 μg/kg bw per month, respectively. Cereals and cereal products contributed 54% of overall dietary exposure, with fruits, vegetables and oilseeds (13%) and fish (11%) being the other main contributors. The category "sugar and cocoa products" accounted for 1% of dietary cadmium exposure.

As part of a multicountry total diet study in sub-Saharan Africa, foods were collected at two locations in Cameroon (Duala and North) and combined to give 50 food group composites, representing 13 food groups (Ingenbleek, 2019). Samples were analysed for a range of contaminant elements, including cadmium, by ICP-MS, following nitric acid digestion. LOQs were in the range 0.2–1.0 µg/kg. Cadmium was quantified in 58% of composite samples. LB–UB estimates of the mean concentration were derived for each food group. Food consumption information was obtained from a household budget survey including 1398 households across the two sampling locations in Cameroon. Household food intake was converted to intake per AME. LB–UB mean (P95) estimates of dietary cadmium exposure for Duala and North were 0.08–0.08 (0.17–0.17) and 0.05–0.05 (0.10–0.10) µg/kg bw per day, respectively. These exposure estimates correspond to 2.4–2.4 (5.1–5.1) µg/kg bw per month and 1.5–1.5 (3.0–3.0) µg/kg bw per month, respectively. The main contributors to dietary exposure were rice, green leaves, wheat and bread, peanuts and maize. No information on the contribution to exposure from cocoa products was reported for either study location.

(f) **Canada**

Dietary exposure to cadmium for First Nations peoples residing on-reserve in the province of Ontario was determined using a total diet study approach (Juric, 2016). The study was conducted under the umbrella of the 2011–2012 First Nations Food, Nutrition and Environment Study. Cadmium was determined in tap water from 20 participating communities and in 419 composite food samples, representing 141 different traditional food items, by ICP-MS following acid digestion. The LODs were 0.005 µg/L for water and 1 µg/kg for food samples. The cadmium concentrations for market foods were taken from the Canadian Total Diet Study (Health Canada, 2020). Analytical results below the LOD were substituted by a concentration equal to the LOD (UB). Food consumption information was taken from a single-pass 24HDR. A total of 1429 individuals, aged 19 years and over, participated. The overall mean and P95 estimates of dietary cadmium exposure were 3.9 and 9.6 µg/kg bw per month, respectively. Dietary cadmium exposure was slightly higher for traditional food consumers (6.0 and 11.1 µg/kg bw per month for the mean and P95, respectively). Consumption of potatoes accounted for 18% of dietary cadmium exposure, followed by plain pasta (9%). Contributions to exposure from cocoa products were not reported.

(g) **Chile**

A total diet study was conducted to assess dietary exposure of the adult (18–65 years) population of Valdivia, Chile to cadmium (Munoz et al., 2017). Food

items from food stores in Valdivia were sampled on three different occasions. The number of food samples taken was not reported. Samples were analysed for cadmium by flame atomic absorption spectrophotometry (AAS), following acid digestion. The LOD was 2 μg/kg. Protocols for handling analytical results below the LOD were not reported. Food consumption information was derived from a single-pass 24HDR questionnaire administered to 382 adult residents of Valdivia during 2012. A default body weight of 70 kg was used. The mean estimate of dietary cadmium exposure was 18.1 μg/day (7.8 μg/kg bw per month for a 70-kg adult). Bread (27%) contributed most to cadmium exposure, followed by non-alcoholic beverages (21%) and cereals (11%). No information on the contribution of cocoa products was reported.

(h) China

Dietary cadmium exposure was estimated for a cohort of Shanghainese over 40 years of age (He et al., 2013). Concentrations of cadmium in food were taken from a Chinese national cadmium exposure survey carried out in 2000 and a survey carried out in Shanghai during 2002 and 2007. Samples were analysed for cadmium by graphite furnace atomic absorption spectrometry (GF-AAS). Performance characteristics of the analytical method were not reported in this publication. Food consumption information for the recruited cohort (n = 267) came from a food frequency questionnaire (FFQ), with associated assessment of portion sizes. Mean dietary cadmium exposure was estimated to be 12.8 μg/day (6.4 μg/kg bw per month, assuming a 60 kg body weight), with a 90th percentile estimate of 20.6 μg/day (10.3 μg/kg bw per month, assuming a 60 kg body weight). The main contributors to dietary cadmium exposure were vegetables (40%) and rice (38%). No information on the contribution from cocoa products was reported.

In a large national study, 228 687 individual food samples were collected in 31 provinces, autonomous regions and municipalities across China between 2001 and 2015 (Song et al., 2017). Samples were analysed for cadmium in laboratories in the various areas. Although no information was provided on the analytical methods used, LODs were reported to be in the range of 0.01 to 100 μg/kg, with 35.7% of analytical results below the LOD. Foods were aggregated into 32 food groups. For calculating mean cadmium concentrations, analytical results below the LOD were substituted by a value of LOD/2, except for milk and eggs, for which over 60% of results were below the LOD and a UB estimate of the mean was derived. Food consumption data were derived from the 2002 Chinese National Nutrition and Health Survey. This survey involved administration of 24HDR questionnaires on three consecutive days to 67 608 study participants. Individual body weights were also determined. Mean and P95 estimates of dietary cadmium exposure were determined for all participants and for five population subgroups.

The overall mean and P95 dietary exposure estimates were 15.3 and 33.0 µg/kg bw per month, respectively. Rice (56%), wheat flour (12%) and leafy vegetables (11%) were the main contributors to mean dietary exposure. No information on the contribution from cocoa products was reported.

A total diet study was conducted in Shenzhen province to estimate adult dietary exposure to cadmium (Wang et al., 2018). Individual food samples (*n* = 276) from 13 food groups were analysed for cadmium by ICP-MS following acid digestion. The LOD and LOQ were 0.2 and 1.0 µg/kg, respectively. Overall, 12% of samples had analytical results below the LOD. For these results a value of LOD/2 was substituted. Median cadmium concentrations for each food group were used for estimating dietary exposure. Food consumption information was derived from a survey of 662 adult (18+ years) residents of Shenzhen, which took the form of a 3-day food diary. Individual body weights were recorded and used for estimation of dietary exposure. Dietary exposure was estimated for mean and P95 consumers in three age groups (18–39, 40–50 and 60+ years) and separately for males and females. Mean dietary exposure estimates were in the range of 8.3–11 µg/kg bw per month, whereas P95 estimates were in the range of 12–14 µg/kg bw per month. The main contributors to mean dietary exposure were vegetables (33%), rice and rice products (19%) and fish, seafood and shellfish (19%). No information on the contribution from cocoa products was reported, although these products would have been included, along with others, in an "others" food group that contributed 4% of estimated dietary exposure.

Results of the fifth China Total Diet Study (TDS) were used to estimate dietary exposure to cadmium for residents of 20 regions of China (Wei et al., 2019; Xiao et al., 2020). Respondents of the fifth TDS included male residents aged 18–45 years, with an average body weight of 63 kg. Food consumption information was obtained from 3-day (2 weekdays and 1 weekend day) 24-hour household dietary surveys. More than 200 foods were sampled and classified into 13 categories. Foods were prepared for consumption and analysed for cadmium by ICP-MS. The mean dietary exposure to cadmium for the 20 different regions ranged from 7.3 to 186.1 µg/day (3.7 to 93 µg/kg bw per month for a 60-kg adult). The overall average exposure was 32.7 µg/day (16.4 µg/kg bw per month for a 60-kg adult). The main dietary sources of cadmium were cereals and vegetables. No information was provided on how dietary exposure was calculated, how left-censored data were managed or on the contribution from cocoa products.

Dietary exposure to cadmium was estimated for residents of Guangzhou City in China (Zhang et al., 2018). For this purpose, foods were sampled in 12 districts of Guangzhou City during 2013–2015. A total of 4039 single-species food samples were collected belonging to 11 food groups. Food samples were analysed for cadmium by GF-AAS following nitric acid/hydrogen peroxide digestion. The LOD and LOQ were 1 and 3 µg/kg, respectively. Food consumption data were

obtained from a survey among urban and rural residents of Guangzhou City in 2011 aged 3–88 years (n = 2976). Data were obtained using a 3-day, 24HDR. Mean dietary exposure was calculated by multiplying the average amount of food consumed per kg body weight across the three recording days by the mean concentration of cadmium in each food group. High exposure was calculated by multiplying the mean concentration by the P95 food consumption level. The mean and P95 estimates of dietary cadmium exposure were 14.4 and 41 μg/kg bw per month. The food groups that contributed most to the mean exposure were cereals (50.2%; mainly rice), laver (19.2%) and vegetables (13%). No information on the contribution from cocoa products was reported.

(i) **Denmark**

The Danish National Food Institute (DTU Food) carries out periodic risk assessments of chemical contaminants in the diet (DTU Food, 2015). Food samples were collected during the period 2004–2011, on a project-by-project basis. Analyses were carried out in two regional laboratories, with foods prepared according to normal household practices, but not cooked. Samples were analysed for cadmium by ICP-MS, following nitric acid digestion. While not specifically stated, it appears that LODs were in the range 1.2–8.6 μg/kg. Between 1 and 384 samples of individual food types were analysed. Results below the LOD were included as indicative values in the calculation of mean concentrations of food types. Food consumption data were collected as a part of DANSDA (DAnish National Survey of Diet and physical Activity) in 2005–2008. Participants (n = 2700, aged 4–75 years) completed a 7-day food diary. Individual estimates of dietary exposure were derived for each participant in the food consumption survey. The mean and P95 estimates of dietary cadmium exposure for the Danish population were 0.18 and 0.38 μg/kg bw per day (5.4 and 11.4 μg/kg bw per month), respectively. The main contributors to dietary exposure were cereals and cereal products (49%) and vegetables and vegetable products (34%). No information on the contribution from cocoa products was reported.

(j) **Europe**

The European Food Safety Authority (EFSA) calculated dietary exposure to cadmium in Europe in 2012 (EFSA, 2012). In these calculations, individual food consumption data from European countries were combined with a merged data set of cadmium occurrence data in foods from 22 European Union Member States, three European Economic Area or other countries, and some food business operators, mainly sampled during the period 2003–2011. Most of the analytical results were obtained from the Slovak Republic, followed by Germany, France, Romania, Spain and Denmark between 2003 and 2007. EFSA calculated LB,

medium-bound (MB) and UB exposure estimates for infants (<1 year), toddlers (1–2 years), other children (3–9 years), adolescents (10–17 years), adults (18–64 years), elderly people (65–74 years) and the very elderly (75+ years). In the MB estimates, concentrations below the LOD or LOQ were substituted by a value of LOD/2. The MB median mean dietary exposure across dietary surveys within an age group ranged from 1.62 μg/kg bw per week (6.9 μg/kg bw per month) in the elderly to 4.80 μg/kg bw per week (20.6 μg/kg bw per month) in toddlers. The median P95 of exposure ranged from 2.89 μg/kg bw per week (12.4 μg/kg bw per month) for the very elderly to 6.59 μg/kg bw per week (28.2 μg/kg bw per month) for infants. The food categories contributing most to the LB estimates of exposure, being least influenced by left-censored data and LODs, were grains and grain-based products (27%), vegetables and vegetable products (16%), and starchy roots and tubers (13%). Cocoa products were included in the assessment based on cadmium concentrations in cocoa powder (n = 732), chocolate (n = 1558) and cocoa beverages (n = 2196). The contribution of cocoa products to the total dietary exposure distributions ranged from 0.2% for infants to 9.4% for other children, with the greatest component of this contribution from the consumption of chocolate.

(k) France

The second French TDS involved collection of 1319 food samples, covering 41 food groups, between 2007 and 2009 (Arnich et al., 2012). Samples were prepared for consumption and analysed for cadmium by ICP-MS, following acid digestion. The method LOQ was 1 μg/kg. Analytical results below the LOD or LOQ were reported for 21% of samples. Food consumption information was derived from the second individual and national food consumption survey (INCA2). The survey involved completion of a 7-day food diary by 3362 individuals (1918 adults aged 18–79 years and 1444 children aged 3–17 years). Mean concentrations of cadmium for the 41 food groups were calculated as MB. Mean (P95) estimates of dietary cadmium exposure for children and adults were 0.24 (0.45) and 0.16 (0.27) μg/kg bw per day, respectively. This equates to 7.2 (13.5) and 4.8 (8.1) μg/kg bw per month, respectively. For adults, the main contributors to mean dietary exposure were bread and dried bread products (22%), potatoes and potato products (12%), pasta (6%) and crustaceans and molluscs (6%). Chocolate contributed 1–2% of dietary cadmium exposure, depending on the age group considered.

A study was carried out to determine dietary and non-dietary exposure to inorganic species, including cadmium, for French children aged 3–6 years (Glorennec et al., 2016). Concentration data on cadmium in foods were taken from the second French TDS and food consumption data from the INCA2 study. Mean and P95 estimates of dietary cadmium exposure were 0.31 and 0.52 μg/

kg bw per day, respectively (9.3 and 15.6 µg/kg bw per month, respectively). The main foods contributing to cadmium exposure were potatoes and similar (14%), bread and dry bread (10%), other vegetables (8%) and pasta (8%). Chocolate contributed 2% of dietary cadmium exposure.

A further study was carried out to assess dietary exposure for French children <3 years of age (Jean et al., 2018). Food samples (n = 291), including infant foods (n = 219) common foods and bottled water (n = 72) were collected during 2011 and 2012 in central France. Samples were analysed for cadmium by ICP-MS, following acid digestion. LOD and LOQ were 0.3 and 0.5 µg/kg, respectively. Cadmium was detected in 65% of samples. LB–UB estimates of the mean concentration were calculated for each food type. Food consumption information was taken from three consecutive 1-day food diaries completed for 705 children, collected by the Syndicat Français des Aliments de l'Enfance et de la Nutrition Clinique. Dietary cadmium exposure was estimated for four age subgroups: 1–4 months, 5–6 months, 7–12 months and 13–36 months. LB–UB estimates of mean (90th percentile) dietary cadmium exposure ranged from 0.39 to 0.67 (1.4 to 1.4) µg/kg bw per week for 1–4-month-old infants to 2.3–2.4 (3.8–4.0) µg/kg bw per week. The dietary exposure estimates were converted to monthly estimates by multiplying by 30/7 (Table 1). Infant formula was the main contributor to dietary exposure for the 1–4-month cohort (58–72%), whereas for the 13–36-month cohort potatoes and potato products (23–24%), vegetables (17–18%) and pasta (10%) were the main contributors. Cocoa products were not among the foods considered.

(l) French Polynesia

As part of a case–control study to examine associations between thyroid cancer and exposure to heavy metals, dietary exposure to several heavy metals, including cadmium, was estimated (Zidane et al., 2019). Locally produced foods were sampled at seven locations in French Polynesia between 2011 and 2013, with five individual subsamples composited to give 124 food samples for analysis. Samples were lyophilized and analysed for cadmium by ICP-MS, following digestion. Measures of method performance characteristics and the technique for handling analytical results below the LOD were not reported. Food consumption was determined for 229 cases and 373 controls by application of an adapted version of the European Prospective Investigation into Cancer and Nutrition (EPIC) questionnaire, which collected information on frequency of consumption of 66 foods in the previous year as well as on serving sizes. The age distribution of cases and controls was not reported, but it is likely that they were adults. Mean dietary cadmium exposure did not differ between cases and controls and was estimated to be 0.07 µg/kg bw per day (2.1 µg/kg bw per month). The maximum estimated

dietary exposure to cadmium was 1.6 µg/kg bw per day (48 µg/kg bw per month). No information on the contribution of foods, including cocoa products, to dietary exposure was reported.

(m) Germany

As part of the LExUKon project, dietary cadmium exposure was estimated for the adult German population (aged 14–80 years) (Schwarz et al., 2014). Data on cadmium in foods were taken mainly from the German Food Monitoring Programme (GFMP) for the years 1993–2007 supplemented by data from targeted surveys carried out after 2003. MB estimates of mean concentrations were calculated. Food consumption information was derived from the German National Nutrition Survey II (NVS II). The survey included three different methods: a dietary history interview, two 24HDR questionnaires and two 4-day food diaries. The mean estimate of dietary cadmium exposure for the full cohort (n = 15 371) was 1.46 µg/kg bw per week (6.3 µg/kg bw per month). A "high-end" estimate of dietary exposure was calculated using P95 consumption amounts for two main food groups and mean consumption amounts for the remaining food groups. The high-end estimate of dietary cadmium exposure for the general population was 2.35 µg/kg bw per week (10.1 µg/kg bw per month). The main contributors to dietary exposure were vegetables and cereals. No information on the contribution from cocoa products was reported.

Data from the same sources were used to specifically examine dietary cadmium exposure from consumption of cocoa products (Fechner et al., 2019). Data from the NVS II were re-examined to extract information on cocoa consumption. Mean and P95 consumption estimates for all cocoa were 0.028 and 0.098 g/kg bw per day, respectively. Results from analyses of samples of cocoa powder were available from the GFMP for the years 2008–2012. Combining the mean concentration of cadmium in cocoa products with the median and P95 estimates of cocoa consumption gave estimates of dietary exposure of 0.019 and 0.131 µg/kg bw per week (0.081 and 0.56 µg/kg bw per month, respectively). When considered in association with the estimates from Schwarz et al. (2014), these findings are consistent with other studies in suggesting that, at mean or median estimates of dietary exposure, cocoa products account for about 1% of dietary cadmium exposure. Other scenarios were considered (P95 concentration, cocoa from particular locations), but they do not appear to be relevant to the current assessment.

(n) Hong Kong Special Administrative Region (SAR), China

Dietary exposure to cadmium was estimated as part of the first Hong Kong SAR TDS (Chen et al., 2014). A total of 1800 food samples were collected during 2010

and 2011 and combined into 600 composite samples of 150 food types, prepared as consumed. Samples were analysed for cadmium by ICP-MS, following acid digestion. LODs were 0.4 and 2 µg/kg for water and tea and general foods, respectively. The corresponding LOQs were 2 and 10 µg/kg. Overall, 58% of analytical results were above the LOD and an MB approach to mean calculation was adopted. Information on food consumption was derived from the first Hong Kong Population-based Food Consumption Survey. The survey included two 24HDR and an FFQ administered to approximately 5000 adults (aged 20–84 years). Mean and P95 estimates of dietary cadmium exposure for the whole adult cohort were 8.3 and 19 µg/kg bw per month, respectively. The main contributors to dietary exposure were vegetables and their products (36%), fish and seafood and their products (26%), and cereals and their products (21%). No information on the contribution of cocoa products was reported.

(o) Iran, Islamic Republic of

Although this study in the Islamic Republic of Iran did not look at all dietary sources of cadmium, the likely main contributors to dietary exposure were included (cereals and vegetables) (Heshmati et al., 2020). Samples (n = 50 per food) of potatoes, onions, tomatoes, lettuces, leeks, carrots, wheat and rice were collected from retail outlets in Hamadan Province, western Islamic Republic of Iran. Samples were analysed for cadmium by GF-AAS, following acid digestion. LOD and LOQ were 0.22 and 0.68 µg/kg, respectively. It was not stated how results below the LOD were treated in the calculation of mean cadmium concentrations for each food. Mean daily consumption for each food was reported, but the method for deriving these estimates was not. A default body weight of 70 kg was used. Mean estimated daily intakes were reported for each food type. The sum exposure to cadmium across food types was 0.49 µg/kg bw per day (14.7 µg/kg bw per month). The main contributors to dietary exposure were the cereals: wheat and rice. Cocoa products were not included in this study.

(p) Ireland

A TDS was carried out by the Food Safety Authority of Ireland (FSAI) during 2012–2014 (FSAI, 2016). Samples of 141 food types from 27 food categories were collected. Foods were prepared for consumption and were analysed for cadmium by ICP-MS, with typical LOD and LOQ of 5 and 17 µg/kg, respectively. The food consumption data used for adults were derived from the National Adult Nutrition Survey, which investigated habitual food and beverage consumption in a representative sample (n = 1500) of adults aged 18 years and over during 2008–2010. Assessment of children used the National Children's Food Survey, which investigated habitual food and drink consumption in 594 children aged

5–12 years, during 2003–2004, using a 4-day food diary. LB–UB mean (97.5th percentile) estimates of dietary cadmium exposure were derived separately for children and adults; 7.2–9.6 (14.1–17.7) µg/kg bw per month and 4.8–6.6 (9.9–12.6) µg/kg bw per month, respectively. Cereals and vegetables were the main contributors to dietary exposure for both adults and children. No information on the contribution of cocoa products was reported.

(q) Italy

A subnational estimate of dietary cadmium exposure was determined in the Emilia-Romagna region of northern Italy (Filippini et al., 2018). Foods for inclusion in the study were selected on the basis of previous studies. Foods (n = 890) were collected during 2016–2017 and analysed for cadmium by ICP-MS, following nitric acid/hydrogen peroxide digestion. Very low LOD and LOQ of 0.007 and 0.02 µg/kg, respectively, were reported. Overall, 25 samples (3%) contained concentrations of cadmium below the LOD. Food consumption information was derived by administering a semi-quantitative FFQ of 188 food items, developed for the European Prospective Investigation into Cancer and Nutrition (EPIC) study, to 719 adults (aged 18–87 years). The median estimate of dietary cadmium exposure was 0.5 µg/kg bw per week (2.1 µg/kg bw per month). The main contributors to dietary exposure were cereals (55%) and vegetables (19%). No information on the contribution of cocoa products was reported.

(r) Japan

Dietary exposure to cadmium was estimated for a cohort (n = 296) of Japanese children (aged 3–6 years) from Miyagi prefecture during 2001–2004 (Watanabe et al., 2013). Twenty-four-hour duplicate diet portions were collected and analysed by ICP-MS. A limit of determination of 0.1 µg/kg was reported, but it is uncertain whether this was an LOD or an LOQ. Age, height and weight were recorded for each child. The geometric mean dietary cadmium exposure was 11.8 µg/day (0.60 µg/kg bw per day or 18 µg/kg bw per month). No discernible pattern was apparent when dietary exposure was considered by individual age (3, 4, 5 or 6 years) and sex. The duplicate diet approach does not allow information on the contribution of specific food types to dietary exposure to be determined.

(s) Korea, Republic of

Concentrations of cadmium in 118 foods were obtained from the database of the Korean Research Project on the Integrated Exposure Assessment of Hazardous Substances for Food Safety (Kim et al., 2014). In total, 3823 analytical results, representing the 118 core foods, were considered suitable for inclusion in the study. The results in the database for cadmium resulted from ICP-MS

analysis of food collected from seven cities in the Republic of Korea. Analytical results below the LOD (0.2 μg/kg) were substituted by a value equal to LOD/2 (MB). Consumption data were obtained from two non-consecutive 24HDR questionnaires administered to 457 child–caregiver pairs of children aged 0–6 years. Body weight was also recorded. The mean body weight for the participating children was 16.2 kg. Individual estimates of dietary exposure were calculated for each of the two 24HDR for each participant. The distribution of habitual dietary exposures was then derived using the Iowa State University method, through C-SIDE software. The mean and P95 estimates of dietary cadmium exposure for the cohort were 0.38 and 0.75 μg/kg bw per day, respectively (11.4 and 22.5 μg/kg bw per month, respectively). The main contributors to dietary cadmium exposure were cereals, fish, shellfish and seaweed. No information on the contribution of cocoa products was reported.

(t) Mali

As part of a multicountry TDS in sub-Saharan Africa, foods were collected at two locations in Mali (Bamako and Sikasso) and combined to give 50 food group composites, representing 13 food groups (Ingenbleek, 2019). Samples were analysed for a range of contaminant elements, including cadmium, by ICP-MS, following nitric acid digestion. LOQs were in the range of 0.2–1.0 μg/kg. Cadmium was quantified in 42% of composite samples. LB–UB estimates of the mean concentration were derived for each food group. Food consumption information was obtained from a household budget survey including 2333 households across the two sampling locations in Mali. Household food intake was converted to intake per AME. LB–UB mean (P95) estimates of dietary cadmium exposure for Bamako and Sikasso were 0.07–0.07 (0.10–0.11) and 0.02–0.02 (0.04–0.04) μg/kg bw per day, respectively. These exposure estimates correspond to 2.1–2.1 (3.0–3.3) μg/kg bw per month and 0.6–0.6 (1.2–1.2) μg/kg bw per month, respectively. The main contributors to dietary exposure were rice, peanuts and millet. No information on the contribution of cocoa products was reported.

(u) The Netherlands

Dietary exposure to cadmium was estimated for children of 2–6 years (n = 1279) and people aged 7–69 years (n = 3819) (Sprong & Boon, 2015). Concentrations of cadmium in food and drinking water were obtained from Dutch monitoring programmes. Concentrations in cocoa beans were not available from these programmes and were derived from a study performed by EFSA in 2012 (EFSA, 2012). Information on food consumption was obtained on two non-consecutive days using a food diary or 24HDR, as part of the Dutch National Food

Consumption Survey. Based on LB, MB and UB mean concentrations, median and P95 dietary exposure estimates were calculated using a statistical model that corrects for inter-individual variability. The MB estimates ranged from 0.40–0.55 µg/kg bw per day (12–16.5 µg/kg bw per month) for children aged 2–6 years and from 0.18–0.47 µg/kg bw per day (5.4–14.1 µg/kg bw per month) for people aged 7–69 years. Corresponding ranges for the P95 dietary exposure were 0.58–0.81 µg/kg bw per day (17.4–24.3 µg/kg bw per month) and 0.30–0.77 µg/kg bw per day (9.0–23.1 µg/kg bw per month), respectively. Overall, mean MB dietary exposure across all ages was 0.25 µg/kg bw per day (7.5 µg/kg bw per month). Cereals (38–40%), potatoes (16–18%) and vegetables (11–13%) contributed most to the exposure in both populations. Cocoa beans contributed a maximum of 1% to the total dietary exposure distribution.

(v) New Zealand

The eighth New Zealand TDS sampled 132 different food types on two occasions during 2016 (Pearson et al., 2018). Eight composite samples of each food type were analysed after preparation for consumption. Samples were analysed for cadmium by ICP-MS following acid digestion. Limits of reporting (LORs) ranged from 0.05 µg/kg for drinking water to 2 µg/kg for high-fat samples. Cadmium was detected at concentrations above the LOR in 68% of samples. Food consumption information was derived from simulated typical diets for 10 population subgroups. Mean LB–UB estimates of dietary cadmium exposure ranged from 4.4–4.6 µg/kg bw per month for adult females of Pacific Island ethnicity to 12.4–12.8 µg/kg bw per month for toddlers (aged 1–3 years). The main contributors to dietary exposure were vegetables, grains and "additional meat and shellfish". The contribution from the latter food category is a reflection of the high concentrations of cadmium in bivalve molluscs (oysters and mussels). Although the contribution to dietary exposure from cocoa products was not explicitly reported, it can be calculated from available data and was in the range of 1% (adult males and females) to 8% (children).

(w) Nigeria

As part of a multicountry TDS in sub-Saharan Africa, foods were collected at two locations in Nigeria (Lagos and Kano) and combined to give 54 food group composites, representing 13 food groups (Ingenbleek, 2019). Samples were analysed for a range of contaminant elements, including cadmium, by ICP-MS, following nitric acid digestion. LOQs were in the range of 0.2–1.0 µg/kg. Cadmium was quantified in 61% of composite samples. LB–UB estimates of the mean concentration were derived for each food group. Food consumption information was obtained from a household budget survey including 1066

households across the two sampling locations. Household food intake was converted to intake per AME. LB–UB mean (P95) estimates of dietary cadmium exposure for Lagos and Kano were 0.09–0.09 (0.18–0.18) and 0.04–0.05 (0.11–0.12) μg/kg bw per day, respectively. These exposure estimates correspond to 2.7–2.7 (5.4–5.4) μg/kg bw per month and 1.2–1.5 (3.3–3.6) μg/kg bw per month, respectively. The main contributors to dietary exposure were rice, beef, and wheat and bread. No information on the contribution of cocoa products was reported.

(x) Poland

A duplicate daily diet study was carried out for a cohort of 850 university students, recruited over the period 2006–2010 from the south-east of Poland (Marzec et al., 2014). This study appears to be a variant on the more usual duplicate diet approach. Students initially completed a 24HDR questionnaire. Representative duplicate diets were then assembled from locally obtained foods. Samples were analysed for cadmium by flame AAS, following ashing and acid dissolution. The LOD and LOQ were 0.2 and 0.5 μg/kg, respectively. Separate estimates of dietary exposure were derived per year (2006, 2007, 2008 and 2010), sex (male and female) and for each of three universities. Estimates of mean dietary exposure were in the range of 76 (females, Catholic University, 2010) to 330 (males, Medical University, 2008) μg/week. Based on a conservative mean body weight of 60 kg, these values equate to 5.4 and 24 μg/kg bw per month.

A similar study was conducted in 2011–2013, with an initial 24HDR (583 participants, aged 19–30 years) used to formulate six "market baskets" for each combination of year (2011, 2012 and 2013) and sex (Koch et al., 2016). Foods were prepared for consumption. Analyses for cadmium were performed by ICP-MS, following nitric acid digestion. The LOD for cadmium was 0.12 μg/kg. Mean estimates of dietary cadmium exposure across the year–sex cohorts were in the range of 12.7–21.6 μg/day. Based on a default body weight of 60 kg, this range equates to 6.4–10.8 μg/kg bw per month. No assessment of contributing foods was conducted.

Both Polish studies were duplicate diets, an approach that does not allow derivation of information on the contribution of specific food types to dietary exposure.

(y) Serbia

Food samples (n = 114) were collected in January 2012 and March 2013 from supermarkets in Novi Sad, Serbia (Skrbic et al., 2013). Food types were selected on the basis of a total Serbian market basket prepared by the Statistical Office of the Republic of Serbia. The market basket was also the source of the consumption information used in the dietary exposure assessment. Samples were analysed for

cadmium by GF-AAS, following nitric acid/hydrogen peroxide digestion. The LOD and LOQ were reported to be the same; 0.3 µg/kg. However, this is almost certainly a typographical error, and this is likely to be the LOD. Results below the LOD were substituted by a value equal to LOD/2 (MB). The estimated dietary exposure to cadmium from adult consumption of the Serbian market basket was 11.5 µg/day (5.8 µg/kg bw per month, for a default body weight of 60 kg). The main contributors to dietary exposure were white bread (54%), sugar (16%) and potatoes (9%). Chocolate contributed 0.7% of dietary exposure to cadmium.

(z) Spain

Dietary exposure to cadmium was estimated for the region of Valencia in Spain (Marín et al., 2017). In 2010–2011, a TDS was performed including collection of 8100 food samples. Food samples were aggregated in groups of 10 to give 810 samples for analysis for cadmium by ICP-MS. LOQs were in the range 4–10 µg/kg. Food consumption data were obtained from 1478 subjects (195 children aged 6–15 years and 1281 adults aged 16–95 years) using a 24HDR on three non-consecutive days. LB and UB estimates of exposure to cadmium were calculated for children and adults. Mean LB–UB exposure estimates for children were 1.26–2.89 µg/kg bw per week (5.4–12.4 µg/kg bw per month) and for adults 0.77–1.78 µg/kg bw per week (3.3–7.6 µg/kg bw per month). For high consumers (99th percentile), the corresponding estimates were 5.74–9.10 and 4.62–6.31 µg/kg bw per week (24.6–39.0 and 19.8–27.0 µg/kg bw per month). Cereals (38%) contributed most to the exposure to cadmium, followed by fish (29%). No information on the contribution of cocoa products was reported.

A further Spanish dietary exposure assessment for cadmium was conducted in the region of Catalonia (Gonzalez et al., 2019). Foods were sampled in 12 cities in Catalonia. For each food type (approximately 70 food types aggregated into 15 food groups), three composite samples were prepared from 20 individual samples. Composite samples were analysed for cadmium by ICP-MS, following nitric acid/hydrogen peroxide digestion. The LOD was reported as 2 µg/kg, although the method of determining the LOD was not reported. For calculation of mean concentrations, analytical results below the LOD were substituted by a value of LOD/2 (MB). Food consumption information was derived from national dietary surveys (ENALIA and ENALIA-2), which included 1862 children and adolescents, 623 adults, 310 "seniors" and 157 pregnant women. The surveys included two non-consecutive 24HDRs and an FFQ. The survey cohort was reclassified into eight age-based subgroups. It should be noted that the terms "toddlers" and "infants" appear to have been transposed in this publication, as toddlers are referred to as being aged 6–11 months and infants as 12–36 months. The following summary uses the term infants for 6–11-month-

olds and toddlers for 12–36-month-olds. Mean body weights for each subgroup were derived from the literature. Mean estimates of dietary exposure were only presented graphically but ranged from (approximately) 3 μg/day (infants) to 10.1 μg/day (adolescents, aged 10–17 years). These dietary exposure estimates equate to 10.7 (infants) and 5.9 (adolescents) μg/kg bw per month, based on the body weights given in the publication. For the adult population, with a dietary exposure of 6.1 μg/day or 2.4 μg/kg bw per month, the main contributors to dietary exposure were fish and seafood (37%), bread and cereals (30%), and potatoes (14%). Chocolate contributed 1% of adult dietary exposure, despite being the food type with the second-highest mean cadmium concentration.

(aa) **Sri Lanka**

Dietary exposure to cadmium (and lead and arsenic) was estimated for a cohort of people with chronic kidney disease (CKD) from a region in central northern Sri Lanka (Jayalal et al., 2019). Food samples (n = 277) were collected from 70 households in the region associated with CKD cases. Food samples were analysed for cadmium by ICP-MS, following acid digestion. The LOQ was reported as 0.3 μg/L; however, it is uncertain what this refers to as the LOQ was reported on a volume basis, and only 9% of samples were reported to contain cadmium concentrations above the LOQ. The minimum reported cadmium concentration was 31 μg/kg, suggesting a LOQ of about 30 μg/kg, which is quite high. Samples with cadmium concentrations below the LOQ were substituted by a value of LOQ/2 for calculation of means (MB). Food consumption information was elicited from 87 CKD patients using a semi-quantitative FFQ. However, it appears that this information was not used to estimate dietary exposure and instead a model diet was used, based on the consumption of 3 kg of rice and 1 kg of vegetables per week. A default body weight of 60 kg was used. Mean dietary cadmium exposure was estimated to be 83.7 μg/week (6.0 μg/kg bw per month). No information on the contributions of specific foods to dietary exposure was reported.

(bb) **Sweden**

Data on occurrence of cadmium in foods were taken from Swedish national monitoring programmes or Swedish market basket surveys performed during 1999–2008 (Sand & Becker, 2012). Concentrations below the LOD were substituted by a value of LOD/2 for calculation of food category mean concentrations. Food consumption information was taken from a 1997–1998 survey carried out by the National Food Agency in collaboration with the Swedish Statistical Agency (Riksmaten 97-98). The survey involved the collection of 7-day food diaries for 1211 Swedish adults (aged 17–80 years). Median and P95 estimates of dietary cadmium exposure were 0.97 and 1.7 μg/kg bw per week, respectively (4.2 and

7.3 µg/kg bw per month, respectively). The main contributors to median dietary exposure were potatoes (25%) and wheat flour (24%). No information on the contribution of cocoa products was reported.

(cc) Thailand

A study was undertaken in four locations in Thailand during the period 2011–2013 (Kluengklangdon et al., 2017). One hundred households were recruited in each location (total 400 households, representing 1241 people) and duplicate portions of meals collected on each of 4 days. A sample of drinking water was also collected from each household. The food and water samples were analysed for cadmium by GF-AAS or ICP-optical emission spectrometry (ICP-OES). MB estimate of dietary cadmium exposure across the study population was 7.3 µg/kg bw per month. The duplicate diet approach does not allow information on the contribution of specific food types to dietary exposure to be determined.

(dd) United Kingdom

Dietary exposure to cadmium in the United Kingdom was estimated based on the 2014 TDS (FSA, 2019). In this study, foods belonging to 28 food groups were sampled and samples were analysed for cadmium by ICP-MS. Food consumption data were obtained from the National Diet and Nutrition Survey Rolling Programme (NDNSRP) in which data are collected using a 4-day food diary. No information on the treatment of samples with non-detect results was reported. The mean and P97.5 of exposure were estimated for children aged 1.5 to 3 years as being the age group with the highest exposure. The mean and P97.5 of exposure were 2.24–3.78 µg/kg bw per week and 3.78–5.95 µg/kg bw per week, respectively (9.6–16.2 and 16.2–25.5 µg/kg bw per month, respectively). The "miscellaneous cereals" food group made the highest contribution to total mean exposure and was followed by bread and potatoes. No information on the contribution of cocoa products to cadmium exposure was reported.

(ee) United States of America

Dietary exposure to cadmium of children 1–6 years of age was estimated based on cadmium concentrations from the TDS of the Food and Drug Administration (FDA) and on food consumption data from What We Eat In America, the food survey portion of the National Health and Nutrition Examination Study (NHANES) (Spungen, 2019). Food samples ($n = 2923$) representing 268 food types were collected during 2014–2016, representing key food groups in the US diet. Foods were analysed for cadmium by ICP-MS, following microwave digestion. LODs and LOQs were in the range of 0.04–1.2 and 0.3–11 µg/kg, respectively. Cadmium was detected in 65% of food samples analysed. Food consumption

data were obtained from a 24HDR administered on two non-consecutive days. Mean and upper level (90th percentile) 2-day average exposures were estimated for the total age group (n = 3103) and for two age subgroups of 1–3 years (n = 1717) and 4–6 years (n = 1386). LB, UB and hybrid estimates of dietary exposure were derived. The hybrid approach assigned a value of zero to analytical results below the LOD if no cadmium had been detected in that food type during 2009–2016, whereas a value of LOD/2 was assigned otherwise. Hybrid estimates of mean dietary exposure were 0.41, 0.43 and 0.38 µg/kg bw per day (12.3, 12.9 and 11.4 µg/kg bw per month) for 1–6-year-olds, 1–3-year-olds and 4–6-year-olds, respectively. Corresponding 90th percentile hybrid estimates were 0.66, 0.70 and 0.59 µg/kg bw per day (19.8, 21.0 and 17.7 µg/kg bw per month) for the same three age groups. The main contributors to the mean dietary exposure were grains (31.8%), mixtures (29.1%; e.g. hamburgers, pizza, lasagna, soups) and vegetables (21.8%). No information on the contribution of cocoa products was reported.

Dietary exposure was also estimated for individuals aged 2 years and older (n = 12 523) from the NHANES 2007–2012 (Kim et al., 2019). Cadmium concentrations were obtained from the TDS from 2006–2013, which included 260 individual foods. The TDS determined cadmium by GF-AAS, at the time these analyses were carried out, although this was not reported in the publication. LODs and LOQs were not reported. Food consumption data were collected with 24HDR during two non-consecutive days. No information on treatment of samples with non-detect results was reported. The weekly dietary exposure to cadmium was estimated for six age groups and ranged from 0.43 µg/kg bw per week (1.8 µg/kg bw per month) for 70+ year-olds to 0.94 µg/kg bw per week (4.0 µg/kg bw per month) for 2–10-year-olds. Mean weekly exposure for the whole population was 0.54 µg/kg bw per week (2.3 µg/kg bw per month). The main contributors to cadmium exposure were cereals and bread (34%), leafy vegetables (20%) and potatoes (11%). No information on the contribution of cocoa products was reported.

(ff) **Viet Nam**

An average diet for residents of Hanoi was determined from previous food consumption surveys, with foods aggregated into 22 food groups (Marcussen et al., 2013). Samples of foods from each food group (n = 14 per food group) were collected during 2007–2009. The edible portion of each food sample was analysed for cadmium by ICP-MS, following nitric acid/hydrogen peroxide digestion. The LOD was 16 µg/kg. LB–UB estimates of mean cadmium concentration were calculated for each food group. Average diets were determined from several surveys, including a three seasonal period 24HDR household survey (250 households). LB–UB estimates of mean dietary cadmium exposure for the Hanoi

population were 0.68–0.70 µg/kg bw per day (20.4–21.0 µg/kg bw per month), with 90% of exposure due to consumption of rice. No information was provided on cadmium exposure due to the consumption of cocoa products.

(gg) Summary

The national estimates of dietary exposure to cadmium described above are summarized in Table 1. 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 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.

Where included in the exposure assessment, the contribution of cocoa products to the total mean dietary exposure to cadmium ranged from 0.2 to 9%. Owing to differences in methodologies used to assess the exposure (e.g. typical diets, FFQ, 24HDR), comparisons between studies should made with caution.

2.4 International estimates of chronic dietary exposure

The GEMS/Food cluster diets that were used for international estimates of chronic dietary exposure to cadmium are based on food availability, rather than actual food consumption. Consequently, these estimates of dietary exposure will be less "refined" than the estimates of dietary cadmium exposure from the literature. 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, together with the limitations of the GEMS/Food contaminants database with respect to detailed data on cadmium concentration from many regions, the Committee considered that deriving less refined estimates of dietary exposure was not necessary, and therefore the international estimates are not reported. However, these estimates were used to provide a suitably consistent basis to examine the contribution of cocoa products and other food sources to dietary exposure to cadmium.

Table 1
Summary of national estimates of dietary exposure to cadmium from the literature

Country	Food concentration data used	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer)[a] in µg/kg bw per month[b]	The main contributors	Contribution from cocoa products (% of dietary exposure)	Reference
Australia	Median of composites	Individual (2 × 24HDR)	Infant (9 months)	2.8–16 (5.7–33)[c]	Cereals and cereal products, root vegetables	55	FSANZ, 2019
			2–5 years	5.5–14 (9.9–20)		6	
			6–12 years	4.3–9.5 (8.2–15)		6	
			13–18 years	2.9–6.3 (5.6–9.6)		4	
			19+ years	2.0–5.8 (3.7–8.8)		4	
			2+ years	2.5–6.6 (4.8–11)			
Australia	Survey mean	24HDR	Males		Fish and seafood products, cereals, vegetable products and dishes	–	Callan et al., 2014
			8 years	12 (66)			
			12 years	9 (42)			
			13 years	6 (39)			
			16 years	6 (30)			
			Females				
			8 years	12 (60)			
			12 years	9 (39)			
			13 years	6 (36)			
			16 years	6 (30)			
Bangladesh	Survey mean	Literature values	General population	17.3	Steamed rice, green vegetables	–	Al-Rmalli et al., 2012
Benin	LB–UB survey mean	Household budget	Adult male equivalents		Tomatoes, rice, pasta, yams, smoked fish	–	Ingenbleek, 2019
			Littoral	1.5–1.5 (3.0–3.3)			
			Borgou	1.2–1.5 (2.7–3.0)			
Brazil	Duplicate diet	Portion size of duplicate diet	Children (1–4 years)		–	–	Leroux et al., 2018
			Males	2.4			
			Females	2.7			
Cameroon	Mean of composites	Household expenditure	Adult equivalents	4.7 (8.2)[d]	Cereals and cereal products	1	Gimou et al., 2014

Country	Food concentration data used	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer)[a] in µg/kg bw per month[b]	The main contributors	Contribution from cocoa products (% of dietary exposure)	Reference
Cameroon	LB–UB survey mean	Household budget	Adult male equivalents Duala North	 2.4–2.4 (5.1–5.1) 1.5–1.5 (3.0–3.0)	Rice, green leaves, wheat/bread, peanuts, maize	–	Ingenbleek, 2019
Canada (First Nations)	UB mean of composites	24HDR	Adults (19+ years)	3.9 (9.6)	Root vegetables, cereals and cereal products	–	Juric, 2016
Chile	Survey mean	24HDR	Adults (18–65 years)	7.8	Bread, non-alcoholic beverages	–	Munoz et al., 2017
China (Shanghai)	Mean from previous survey	FFQ + portion sizes	Adults (>40 years)	0.4 (10.3)[d]	Vegetables, rice	–	He et al., 2013
China	Survey means for 32 food categories	3 × 24HDR	4–11 years 12–17 years, male 12–17 years, female 18+ years, male 18+ years, female General population	24.2 (48.2) 17.9 (35.7) 15.7 (31.5) 13.8 (27.4) 13.6 (27.8) 15.3 (33.0)	Rice, wheat flour, leafy vegetables	–	Song et al., 2017
China	Food group medians	3-day food diary	18–39 years male 18–39 years female 40–59 years male 40–59 years female 60+ years male 60+ years female	9.9 (14) 11 (13) 9.9 (12) 9.4 (12) 8.3 (12) 8.3 (13)	Vegetables, rice and rice products, fish, seafood and shellfish	–	Wang et al., 2018
China	Survey mean	–	General population	15.6[i]	Rice, vegetables, aquatic foods and potatoes	–	Wei et al., 2019; Xiao et al., 2020
China (Guangzhou City)	Survey mean	3-day 24HDR	3–88 years	14.4 (41)	Cereals, vegetables and laver	–	Zhang et al., 2018
Denmark	Survey mean	7-day food diary	General population (4–75 years)	5.4 (11.4)	Cereals and cereal products, vegetables and vegetable products	–	DTU Food, 2015

Table 1 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer)[a] in µg/kg bw per month[b]	The main contributors	Contribution from cocoa products (% of dietary exposure)	Reference
Europe	MB mean of monitoring data	Individual (2–7 days; dietary/ food record; 24HDR)	Infants (0–1 years) Toddlers (1–2 years) Other children (3–9 years) Adolescents (10–17 years) Adults (18–64 years) Elderly (65–74 years) Very elderly (≥ 75 years)	11.5 (28) 20.6 (28) 16.8 (27) 9.9 (18) 7.6 (13) 6.9 (12) 7.2 (12)	Grains and grain-based products, vegetables and vegetable products, starchy roots and tubers	0.2–9.4	EFSA, 2012
France	Survey MB mean	7-day food diary	Children (3–17 years) Adults (18–79 years)	7.2 (13.5) 4.8 (8.1)	Crustaceans and molluscs (adults only)	2 1	Arnich et al., 2012
France	Survey MB mean	7-day food diary	Children (3–6 years)	9.3 (15.6)	Potatoes and potato products, bread and dried bread products, other vegetables, pasta,	2	Glorennec et al., 2016
France	Survey LB–UB mean	3-day food diary	1–4 months 5–6 months 7–12 months 13–36 months	1.7–2.9 (5.9–5.9)[c] 6.8–7.6 (12.5–13.4) 9.7–10.4 (16.2–17.3) 8.7–9.3 (14.7–15.5)	Infant formula, vegetables and vegetable-based meals, potatoes and potato products, meat/fish-based meals	–	Jean et al., 2018
French Polynesia	Survey, summary statistic not reported	FFQ	Adult	2.1	–	–	Zidane et al., 2019
Germany	MB mean of monitoring data	Various methods	General population (14–80 years)	6.3 (10.1)[e]	Vegetables, cereals	–	Schwarz et al., 2014
Germany	MB mean of monitoring data	Various methods	General population (14–80 years)	0.081 (0.56)[f]	Only cocoa products included	–	Fechner et al., 2019
Hong Kong SAR, China	MB mean of food type composites	2 × 24HDR	Males 20–29 years 30–39 years 40–49 years	 7.5 (18) 8.6 (25) 8.0 (18)	Vegetables and their products, fish, seafood and their products, cereals and their products	–	Centre for Food Safety, 2013

Country	Food concentration data used	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer)[a] in µg/kg bw per month[b]	The main contributors	Contribution from cocoa products (% of dietary exposure)	Reference
			50–59 years	8.0 (20)			
			60–69 years	8.0 (17)			
			70–84 years	6.8 (16)			
			All males	7.9 (19)			
			Females				
			20–29 years	8.9 (21)			
			30–39 years	9.4 (21)			
			40–49 years	9.3 (20)			
			50–59 years	8.2 (16)			
			60–69 years	7.6 (18)			
			70–84 years	7.1 (15)			
			All females	8.7 (19)			
			All combined	8.3 (19)			
Iran, Islamic Republic of	Survey mean	Not stated	Adult	14.7	Wheat, rice	–	Heshmati et al., 2020
Ireland, Republic of	Survey mean	4-day food diary	Children (5–12 years) Adults (18+ years)	7.2–9.6 (14.1–17.7)[g] 4.8–6.6 (9.9–12.6)	Cereals, vegetables	–	FSAI, 2016
Italy (northern)	Survey median	FFQ	Adults (18–87 years)	2.1	Cereals, vegetables	–	Filippini et al., 2018
Japan	Duplicate diet	Duplicate diet	Children (3–6 years)	18	–	–	Watanabe et al., 2013
Korea, Republic of	Database mean	2 × 24HDR	Children (0–6 years)	11.4 (22.5)	Cereals, fish and shellfish, seaweeds	–	Kim et al., 2014
Mali	LB–UB survey mean	Household budget	Adult male equivalents Bamako Sikasso	 2.1–2.1 (3.0–3.3) 0.6–0.6 (1.2–1.2)	Rice, millet, peanuts	_	Ingenbleek, 2019
Netherlands	MB mean of monitoring data	Individual (2 × food diary, 2 × 24HDR)	2–6 years 7–69 years	12–17 (17–24)[f] 5.4–14 (9.0–23)	Cereals, potatoes, vegetables	1	Sprong & Boon, 2015
New Zealand	Survey mean	Simulated typical diets	Infant Toddler Children	9.5–12.6 12.4–12.8 12.0–12.4	Vegetables, cereals, shellfish	2 4 8	Pearson et al., 2018

Table 1 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer)[a] in µg/kg bw per month[b]	The main contributors	Contribution from cocoa products (% of dietary exposure)	Reference
			Teenage boy	7.7–7.9		6	
			Teenage girl	6.9–7.1		6	
			Young male	6.8–7.0		3	
			Adult male (PI)	5.8–6.0		2	
			Adult female (PI)	4.4–4.6		2	
			Adult male	6.5–6.7		1	
			Adult female	5.1–5.3		1	
Nigeria	LB–UB survey mean	Household budget	Adult male equivalents		Beef, rice, wheat/bread	–	Ingenbleek, 2019
			Lagos	2.7–2.7 (5.4–5.4)			
			Kano	1.2–1.5 (3.3–3.6)			
Poland	Mean of "analogue" duplicate diets	24HDR	University students	5.4–24[h]	–	–	Marzec et al., 2014
Poland	Mean of "analogue" duplicate diets	24HDR	University students (19–30 years)	6.4–10.8[h]	–	–	Koch et al., 2016
Serbia	MB survey mean	Market basket	Adults	5.8	White bread, sugar, potatoes	0.6	Skrbic et al., 2013
Spain (Valencia)	LB–UB survey	3 × 24HDR	6–15 years	5.4–12 (25–39)	Cereals and fish	–	Marin et al., 2017
			16–90 years	3.3–7.6 (20–27)			
Spain (Catalonia)	MB survey mean	2 × 24HDR and FFQ	Infants (6–11 months)	10.7	Fish and seafood, bread and cereals, potatoes (adults)	1 (adults)	Gonzalez et al., 2019
			Toddlers (12–36 months)	11.7			
			Children (3–9 years)	10.3			
			Adolescents (10–17 years)	5.9			
			Young adults (18–39 years)	3.0			
			Adults (40–64 years)	2.4			
			Seniors (65–74 years)	2.8			
			Pregnant women	2.4			

Country	Food concentration data used	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer)[a] in µg/kg bw per month[b]	The main contributors	Contribution from cocoa products (% of dietary exposure)	Reference
Sri Lanka	MB survey mean	Model diet	Adult male	6.0	Rice	–	Jayalal et al., 2019
Sweden	Monitoring data mean	7-day food diary	Adults (17–80 years)	4.2 (7.3)[f]	Potatoes, wheat flour	–	Sand & Becker, 2012
Thailand	Duplicate diet	Duplicate diet	General population	7.3	–	–	Kleungklangdon et al., 2017
United Kingdom	Survey mean	4 × food diary	1.5–3 years 4–10 years 11–18 years 19+ years	9.6–16 (16–26)[g] 8.3–12 (15–21) 4.5–6.6 (9.0–12) 3.6–5.7 (6.3–10)	Cereals, bread, potatoes	–	FSA, 2019
United States of America	Survey mean	2 × 24HDR	1–6 years 1–3 years 4–6 years	12 (20)[c] 13 (21) 11 (18)	Grains, mixtures, vegetables	–	Spungen, 2019
United States of America	Survey mean	2 × 24HDR	2–10 years 11–19 years 20–30 years 31–50 years 51–70 years 70+ years 2–70+ years	4.0 2.1 2.1 2.1 1.9 1.8 2.3	Cereals and bread, leafy vegetables and potatoes	–	Kim et al., 2018
Viet Nam	LB–UB survey mean	Average diet	Residents of Hanoi	20.4–21.0	Rice	–	Marcussen et al., 2013

24HDR, 24-hour dietary recall; PI, Pacific Island ethnicity; LB, lower bound; MB, middle bound; UB, upper bound.

[a] The high consumer estimate is at the 95th percentile unless stated otherwise.

[b] Dietary exposure estimates originally reported on a daily basis were converted to a monthly basis by multiplying by 30, estimates originally reported on a weekly basis were converted to a monthly basis by multiplying by 30/7.

[c] The high consumer estimate is at the 90th percentile.

[d] Upper bound estimates.

[e] A "high-end" estimate of dietary exposure was derived based on 95th percentile food consumption for two main food groups and mean food consumption for the remaining food groups.

[f] The measure of central tendency was the median (50th percentile).

[g] The high consumer estimate is at the 97.5th percentile.

[h] The study considered various combinations of sex, year and university. The range given is the range of dietary exposure estimates across the various scenarios.

[i] Calculated based on a reported body weight of 63 kg (Xiao et al., 2020).

Owing to the widespread nature of cadmium contamination across the food supply, it is important to include as many foods as possible in the dietary exposure assessment. Due to the very limited cadmium data from some clusters and the complete lack of data from others, international estimates of dietary cadmium exposure were derived using global mean concentrations for all food types. Cadmium exerts its adverse effects after a long period of exposure. Therefore, mean concentrations are the most appropriate metric for calculating dietary exposure, as fluctuations in concentrations are expected to level out to a mean concentration over time.

Concentration data were either reported as a numerical value above the LOQ, as an indicative value between the LOD and LOQ, or as undetectable. MB concentrations were used in the assessments due to the relatively low proportion of left-censored data (46%). Samples with concentrations reported as undetectable were assumed to contain cadmium at a concentration of LOD/2. All reported numerical values were used as such.

Global mean concentrations were derived by considering all available data in the GEMS/Food contaminants database. This meant that, for many food types, the mean cadmium concentrations were heavily weighted towards concentration data provided by the WHO European Region. Global mean concentrations are summarized in the Appendix.

Given the focus of the current assessment on cadmium in cocoa products, an overview of these data as included in the dietary exposure assessment is provided in Table 2. In total, 6957 records for 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 source of records for cocoa products was the WHO European Region, accounting for 2293 records (33%).

Mean levels ranged from 5.4 µg/kg in cocoa butter reported in the WHO European Region to 1601 µg/kg in cocoa powder reported in cluster G05 (Brazil, Chile, Colombia, Ecuador and Peru) (Table 2). Across clusters and the two WHO regions, concentrations ranged from 43 µg/kg in cocoa beverage to 971 µg/kg in cocoa powder. Note that most of the concentration data from European countries were identified at the level of the WHO European Region. The concentrations of two clusters including these countries, namely G07 and G10, may therefore not be representative for that cluster. The mean cadmium concentrations for cluster G07 were solely based on data from Australia and for cluster G10 on data from Canada, Japan and the USA. The mean concentrations of cluster G08 (Austria, Germany, Poland and Spain) were based on concentrations from Germany and Spain.

Table 2

Summary of the cadmium concentrations in cocoa products from the GEMS/Food contaminants database for the GEMS Food clusters and two WHO regions

Cocoa products	Statistics[a,b]	G02	G03	G04	G05	G06	G07	G08	G09	G10	WHO African Region	WHO European Region	Global
Cocoa beans	Number of individual records	–	31	–	–	1	–	18	–	–	–	58	108
	Perc < LOD (n)	–	0% (0)	–	–	0% (0)	–	0% (0)	–	–	–	14% (8)	7.4% (8)
	MB conc (µg/kg)												
	Mean	–	113	–	–	20	–	195	–	–	–	362	260
	Median	–	110	–	–	20	–	80	–	–	–	166	118
	P95	–	155	–	–	–	–	–	–	–	–	1427	1151
Cocoa beverage	Number of individual records	–	–	–	–	–	4	–	8	–	2	6	20
	Perc < LOD (n)	–	–	–	–	–	25% (1)	–	25% (2)	–	0% (0)	33% (2)	25% (5)
	MB conc (µg/kg)												
	Mean	–	–	–	–	–	7.2	–	64	–	48	38	43
	Median	–	–	–	–	–	6.1	–	65	–	48	40	40
	P95	–	–	–	–	–	–	–	–	–	–	–	111
Cocoa butter	Number of individual records	–	–	3	–	–	1	10	–	–	–	6	20
	Perc < LOD (n)	–	–	0% (0)	–	–	0% (0)	0% (0)	–	–	–	100% (6)	30% (6)
	MB conc (µg/kg)												
	Mean	–	–	21	–	–	10	77	–	–	–	5.4	44
	Median			14	–	–	10	20	–	–	–	5.0	20
	P95			–	–	–	–	–	–	–	–	–	73
Cocoa mass	Number of individual records	–	27	–	141	11	8	7	3	–	–	21	218
	Perc < LOD (n)	–	0% (0)	–	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	–	–	4.8% (1)	0.5% (1)
	MB conc (µg/kg)												
	Mean	–	82	–	1035	350	726	375	373	–	–	329	773
	Median		73	–	730	297	885	350	330	–	–	136	500
	P95		187	–	2810	753	–	–	–	–	–	1000	2592

Table 2 (continued)

Cocoa products	Statistics[a, b]	G02	G03	G04	G05	G06	G07	G08	G09	G10	WHO African Region	WHO European Region	Global
Cocoa powder	Number of individual records	–	74	–	1345	2	2	20	9	399	–	732	2583
	Perc < LOD (n)	–	0% (0)	–	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	–	3.4% (25)	1.0% (25)
	MB conc (µg/kg)												
	Mean	–	141	–	1601	471	117	650	609	391	–	228	971
	Median	–	140	–	1120	471	117	465	650	183	–	155	482
	P95	–	191	–	5295	–	–	1510	–	1258	–	754	3740
Chocolate	Number of individual records	4	44	64	1086	74	294	–	10	962	–	1470	4008
	Perc < LOD (n)	100% (4)	0% (0)	13% (8)	2.1% (23)	0% (0)	22% (66)	–	10% (1)	0.8% (8)	–	12% (170)	7.0% (280)
	MB conc (µg/kg)												
	Mean	10	15	381	388	54	39	–	111	117	–	110	184
	Median	10	13	21	259	37	19	–	85	76	–	52	84
	P95	–	31	121	878	126	172		–	310	–	337	576

conc, concentration; LOD, limit of detection; MB, medium bound; median: 50th percentile; n, number; P95, 95th percentile; perc, percentage.

[a] Concentrations below the LOD were assumed to contain cadmium at a level equal to LOD/2 (MB).

[b] The 95th percentile concentrations are only reported where at least 20 records were available.

Table 3
Contribution of cocoa products to dietary cadmium exposure

GEMS/Food Cluster	Description of countries in cluster	Contribution from cocoa products (%)[a]
G01	Middle East, Central Asia, North Africa	0.6
G02	Balkans, Central Asia	1.5
G03	Africa, Paraguay	0.8
G04	Caribbean, Middle East, Brunei Darussalam, French Polynesia	2.7
G05	South and Central America, Indian and Indian Ocean island states, Djibouti, South Africa, Macedonia, Malaysia, New Caledonia, Tajikistan, Trinidad and Tobago	0.7
G06	Middle East, Cuba, Egypt, Greece	0.9
G07	Northern Europe/Scandinavia, Australia, Bermuda, Uruguay	5.9
G08	Austria, Germany, Poland, Spain	4.5
G09	East Asia, South-East Asia, Guinea-Bissau, Sierra Leone	0.2
G10	Balkans, Baltic states, Canada, Italy, Malta, New Zealand, Republic of Korea, Russian Federation, United States of America	3.4
G11	Belgium, Netherlands	0.3
G12	Belize, Dominica	2.9
G13	Africa, Haiti	0.1
G14	Pacific and Indian Ocean island states	0.9
G15	Europe/Scandinavia	3.7
G16	Gabon, Rwanda, Uganda	0.1
G17	Samoa, Sao Tome and Principe	2.9

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

Based on the international estimates of dietary cadmium exposure, the main contributors (>10% in at least one cluster) to dietary exposure were potatoes (0.4–18% depending on the cluster), fresh vegetables (10–50%), wheat flour (1.1–19%), and cattle offal (1.5–11%). The contribution of cocoa products to dietary cadmium exposure for each GEMS/Food cluster is summarized in Table 3.

The clusters with the highest contribution to dietary exposure to cadmium from cocoa products were the "westernized" clusters (G07, G08, G10 and G15), including predominantly European and North American countries. Contributions in these clusters ranged from 3.4–5.9%, with the greatest contribution from 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; 3.5 million tonnes in 2020), Indonesia (0.65 million tonnes in 2020) and South and Central American countries (Brazil, Colombia,

Dominican Republic, Ecuador and Peru; 0.70 million tonnes in 2020).[1] These countries are represented by the clusters G03, G05, G09 and G13. Interestingly, cocoa products are generally very low contributors to dietary cadmium exposure (<1%) in these regions.

Overall, the contribution of cocoa products to the total mean cadmium exposure was consistent with the estimates based on national dietary exposure estimates (Table 1).

2.4.1 Temporal trends in dietary cadmium exposure

Owing to differences in study design and study location, it was 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.

2.4.2 Impact of cocoa product source on dietary cadmium exposure

The potential impact on the contribution of cocoa products to dietary exposure to cadmium of consuming products sourced from a single geographical region (GEMS/Food cluster) was explored for the cluster diet with the greatest contribution from cocoa products (G07) to cadmium exposure (see Table 2). Clusters for which suitable cadmium concentration data were available were G03 (including data from Cameroon, Côte d'Ivoire and Ghana) and G05 (including data from Brazil, Chile, Colombia, Ecuador and Peru). Although data on cadmium in foods were also submitted for cluster G13 by Nigeria, these data did not include cadmium concentrations in cocoa and cocoa products. A limited number of records (n = 30) were submitted by Indonesia (G09). Due to the importance of Indonesia as a cocoa producer, these data were also included in the analysis. A summary of the results of the analysis is presented in Table 4.

Given the higher concentrations of cadmium in cocoa powder and chocolate from countries in cluster G05 (Table 2), the contribution of cocoa products to the mean dietary exposure to cadmium was highest when using concentration data from this cluster (Table 4). However, the combination of food consumption information from the cluster with the highest consumption of cocoa products (G07) and the cadmium concentration information from the

[1] https://www.worldatlas.com/articles/top-10-cocoa-producing-countries.html

Table 4
Contribution of cocoa products[a] to dietary cadmium exposure for cluster G07,[b] depending on source (cluster) of cocoa and cocoa products

Source of cocoa and cocoa products	Contribution (%) of cocoa products to dietary cadmium exposure for cluster G07
Global	5.9
Cluster G03 (African countries)	0.9
Cluster G05 (South/Central American countries)	9.8
Cluster G09 (Indonesia)	3.8

[a] Cocoa products included in the GEMS/Food cluster diets are cocoa beans, cocoa butter, cocoa mass, cocoa powder and chocolate.
[b] Cluster G07 includes Australia, Bermuda, northern Europe and Uruguay.

cluster with the highest concentrations of cadmium in cocoa products (G05) still only resulted in cocoa products contributing approximately 10% of dietary cadmium exposure.

To examine the impact on dietary exposure to cadmium of cocoa products from different geographical sources, the national studies reviewed for this evaluation were considered to determine whether sufficient information was provided to allow substitution of concentration information from Table 2 and to examine the impact of such a substitution on dietary cadmium exposure and the contribution from cocoa products. The Committee concluded that sufficient information for such an analysis was also available from the European dietary exposure assessment, carried out by EFSA (EFSA, 2012). In this analysis, the mean concentrations of cadmium in cocoa powder and chocolate were 183 and 76 μg/kg, respectively. Reported contributions of individual food types to total dietary exposure were used to calculate the absolute contributions of cocoa powder and chocolate to dietary exposure. These absolute contributions were then scaled by the ratio of the concentrations used in the original analysis and the concentration in Table 2 to give revised absolute contributions from cocoa powder and chocolate. A revised dietary cadmium exposure and a revised proportional contribution from cocoa products was then calculated. The results of this analysis are summarized in Table 5.

If the European population was to consume cocoa products sourced solely from countries in cluster G05, dietary cadmium exposure might increase by up to 8 μg/kg bw per month, with the contribution of cocoa products to dietary exposure increasing approximately fourfold for the most highly exposed age group. Decreases in dietary cadmium exposure if cocoa products were sourced solely from countries in cluster G03 were relatively modest. This analysis suggests that there are a few potential scenarios under which cocoa products would be the main contributor to dietary exposure to cadmium.

Table 5
Impact on dietary cadmium exposure and the contribution of cocoa products to dietary exposure for European countries, depending on source (cluster) of cocoa products

	Source of concentration data for cadmium in cocoa products[b]							
	Original (EFSA 2012)		G03		G05		G09	
Age group[a]	DE	%cocoa	DE	%cocoa	DE	%cocoa	DE	%cocoa
Infants	11.5	0.2	11.5	0.1	11.6	1.3	11.5	0.4
Toddlers	20.6	4.2	19.9	1.2	24.6	19.7	21.2	7.0
Other children	16.8	9.4	15.8	3.9	25.1	39.4	18.5	17.6
Adolescents	9.9	9.4	9.4	4.2	14.9	39.5	11.0	17.9
Adults	7.6	4.6	7.3	1.4	9.2	21.1	7.8	7.6
Elderly	6.9	2.6	6.8	0.7	7.7	12.6	7.1	4.2
Very elderly	7.2	2.8	7.0	0.8	8.1	13.7	7.3	4.7

DE, dietary exposure in µg/kg bw per month; %cocoa, proportional contribution of cocoa products to dietary cadmium exposure.
[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] Cluster G03 includes African countries, G05 includes mainly South and Central American countries, and G09 includes mainly South-East Asian counties.

2.4.3 Impact of 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 (800 µg/kg); and
- chocolate containing or declaring ≥ 70% total cocoa solids on a dry matter basis (900 µg/kg).

At the thirteenth meeting of CCCF in 2019, further MLs were discussed and it was proposed to take a proportional approach to products with lower proportions of cocoa solids:

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

Of the 4008 records in the GEMS/Food contaminants database related to chocolate it is only possible to establish the percentage of cocoa solids for 638 (15.9%). These records are virtually all from countries in cluster G05 (South/

Table 6

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 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.

Central America). For the records with an identified proportion of cocoa solids, Table 6 summarizes the 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.

The proportion of samples that exceeded the established or proposed ML ranged from 2.1% for chocolate with a cocoa solids content of ≥ 30 to < 50%, to 16.3% for cocoa powder (Table 6). 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%).

Table 7 provides an analysis of the impact of applying these MLs on the contribution of cocoa products to dietary exposure to cadmium. The approach is similar to that presented in Table 4, with global cadmium concentrations (Appendix) matched to all foods in the cluster diets, except for chocolate and cocoa powder. For these foods, concentrations without application of the MLs were initially used, then concentrations with application of the MLs were used. For chocolate, concentrations used were the means of the four categories summarized in Table 6, weighted by the number of samples in each category. Resulting mean concentrations for cadmium in chocolate were 304 µg/kg, without application of MLs, and 269 µg/kg, with application of MLs. The corresponding concentrations for cocoa powder are available in Table 6.

Across all clusters, the average contribution of cocoa and cocoa products was 2.2% without application of the MLs and 1.5% with application of MLs. Application of the MLs resulted in a mean reduction in dietary cadmium exposure of 0.7% across all clusters. The reductions ranged from 0.0% (cluster G16) to

Table 7
Contribution of cocoa products to dietary cadmium exposure, with and without application of maximum limits for chocolate and cocoa powder, with cocoa products sourced from all relevant GEMS/Food clusters

GEMS/Food cluster	Contribution to dietary cadmium exposure from cocoa products (%)[a]		Reduction in estimated dietary cadmium exposure from application of MLs (%)[b]
	Without application of MLs	With application of MLs	
G01	0.8	0.6	0.2
G02	2.1	1.8	0.2
G03	0.9	0.6	0.3
G04	3.3	2.5	0.9
G05	0.7	0.4	0.3
G06	1.0	0.6	0.4
G07	6.6	4.3	2.4
G08	5.2	3.4	1.8
G09	0.2	0.1	0.1
G10	3.7	2.4	1.3
G11	0.3	0.3	0.1
G12	3.5	2.7	0.8
G13	0.1	0.1	0.1
G14	1.0	0.7	0.3
G15	4.6	3.4	1.2
G16	0.1	0.1	0.0
G17	3.0	1.6	1.3

ML, maximum limit; both proposed and established MLs were applied in this analysis.
[a] Cocoa products included in the GEMS/Food cluster diets are cocoa beans, cocoa butter, cocoa mass, cocoa powder and chocolate.
[b] The percentages in this column are the percentage decrease in the estimated dietary cadmium exposure due to application of the MLs, rather than the difference in the contribution from cocoa products.

2.4% (cluster G07). The contributions to dietary cadmium exposure without application of MLs differed slightly from the contributions shown in Table 3, as only a subset of the records concerning chocolate was accompanied by sufficient data to allow application of the MLs.

Table 8 summarizes an extension of the analysis in Table 7, assuming that the source of cocoa powder was solely from countries in the three main cocoa-producing regions; clusters G03, G05 and G09. The concentration data for this analysis are summarized in Table 6. Note that this analysis relates only to the source of cocoa powder, as the chocolate samples with associated information on cocoa solids are virtually all from cluster G05.

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

Table 8

Contribution of cocoa products to dietary cadmium exposure, with and without application of maximum limits for chocolate and cocoa powder, assuming cocoa powder is sourced from countries in a single GEMS/Food cluster[a]

	Reference								
	G03			G05			G09		
Species	ML–	ML+	DE[b]	ML–	ML+	DE[b]	ML–	ML+	DE[b]
G01	0.5	0.5	0.1	1.0	0.7	0.3	0.7	0.6	0.1
G02	2.0	1.8	0.2	2.1	1.9	0.3	2.0	1.8	0.2
G03	0.4	0.4	0.0	1.2	0.8	0.5	0.7	0.7	0.0
G04	2.1	1.9	0.2	4.2	2.9	1.3	2.8	2.6	0.2
G05	0.2	0.2	0.0	1.1	0.6	0.5	0.5	0.5	0.0
G06	0.4	0.4	0.0	1.5	0.9	0.6	0.8	0.7	0.0
G07	2.8	2.6	0.2	9.3	5.7	3.8	5.0	4.8	0.2
G08	2.4	2.2	0.2	7.1	4.5	2.8	4.0	3.8	0.2
G09	0.0	0.0	0.0	0.3	0.2	0.1	0.1	0.1	0.0
G10	1.6	1.4	0.1	5.3	3.2	2.1	2.8	2.7	0.1
G11	0.2	0.2	0.0	0.4	0.3	0.1	0.3	0.3	0.0
G12	2.5	2.3	0.2	4.3	3.1	1.2	3.0	2.9	0.2
G13	0.1	0.0	0.0	0.2	0.1	0.1	0.1	0.1	0.0
G14	0.6	0.6	0.0	1.3	0.9	0.4	0.8	0.8	0.0
G15	2.9	2.6	0.3	5.8	4.0	1.9	3.9	3.6	0.3
G16	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0
G17	0.7	0.6	0.0	4.7	2.5	2.2	2.0	1.9	0.0

G03, mainly African countries; G05, mainly South/Central American countries; G09, mainly South-East Asian countries; ML–, without application of the maximum limits; ML+, with application of the maximum limits, both proposed and established MLs were applied in this analysis; DE, decrease in dietary exposure estimate due to application of maximum limits.

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

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

above the ML (30.1%, see Table 6). For cocoa products sourced from countries in cluster G03 and G09, application of the MLs had a negligible impact on dietary exposure to cadmium, as the changes in exposure were only due to changes in the mean cadmium concentration for chocolate.

3. 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 (2012). 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.

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5. Appendix

Global mean cadmium concentrations used for international estimates of dietary exposure

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Alcoholic beverage NES	494	464	94	1.9
Almonds	192	54	28	13.2
Anise, badian, fennel, coriander	114	10	9	88.0
Apple	3 020	2666	88	2.7
Apple juice	1 855	1788	96	1.7
Apricot	393	318	81	2.8
Artichoke globe	96	76	79	5.6
Asparagus	772	307	40	11.4
Assorted (sub)tropical fruits	14	8	57	1.4

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Avocado	366	139	38	19.7
Bacon and ham	185	114	62	3.4
Bamboo shoots	5	1	20	3.1
Banana	465	422	91	2.3
Barley	712	210	29	14.2
Beans (dry)	405	288	71	6.4
Beans except broad bean and soya bean (green pods and immature seeds)	931	588	63	5.8
Beans, shelled (immature seeds)	257	145	56	3.4
Beans-based meals	67	17	25	4.7
Beer and beer-like beverage	931	906	97	1.4
Berries and other small fruits	465	317	68	5.9
Bird meat NES	176	153	87	5.0
Blueberries	243	181	74	2.5
Bottled water	908	804	89	4.2
Brazil nut	81	78	96	4.2
Bread and other cooked cereal products	2 759	335	12	26.1
Breakfast cereals	430	82	19	25.9
Broad bean (dry)	29	12	41	6.3
Buckwheat	442	21	5	38.1
Buffalo meat	141	138	98	2.7
Buffalo milk	39	39	100	3.5
Bulb vegetables	24	5	21	11.3
Butter and other animal fat emulsion	163	140	86	3.5
Cabbages and other brassicas	2 253	720	32	6.9
Camel, edible offal of	1	0	0	14.7
Canned fish	5	0	0	19.4
Caper buds	6	2	33	5.0
Cardoon	6	5	83	4.9
Carob	7	6	86	0.8
Carrots and turnips	2 972	697	23	24.3
Cashew nut	46	41	89	2.1
Cassava	70	31	44	2.5
Cattle fat	5	5	100	2.5
Cattle meat	10 340	9698	94	4.7
Cattle milk	3 347	3093	92	1.3
Cauliflower/broccoli	1 239	453	37	9.5
Celeriac	308	23	7	58.6
Celery (whole)	414	100	24	34.8
Celery leaves	29	5	17	25.8
Cephalopods	1 468	278	19	217
Cereal grains	233	37	16	34.1
Cereal starch	9	7	78	7.7
Cereal-based dishes	185	46	25	13.3
Cereal-based food for infants and young children	1 989	483	24	13.4
Cereals and cereal-based products NES	802	54	7	51.8

Table (continued)

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Cereals flour	292	58	20	22.7
Cheese and analogues	1 712	1497	87	3.1
Cherries	361	333	92	1.8
Chestnuts	13	9	69	5.2
Chicken eggs	860	775	90	2.3
Chicken fat	39	31	79	2.3
Chicken meat	4 958	4439	90	4.0
Chicken, edible offal of	3 338	555	17	34.0
Chick-pea (dry)	463	364	79	6.3
Chicory, roots	17	11	65	10.6
Chives	113	7	6	23.4
Chocolate	4 008	280	7	184
Cinnamon bark	44	1	2	259
Citrus fruits	44	33	75	3.2
Citrus juice	37	27	73	1.0
Cloves, buds	47	29	62	11.1
Cocoa beans	108	8	7	260
Cocoa beverage	20	5	25	43.2
Cocoa butter	20	6	30	43.8
Cocoa mass	218	1	0	773
Cocoa powder	2 583	25	1	971
Coconut	33	15	45	8.5
Coconut oil, refined	8	8	100	5.3
Coffee (beverage)	45	35	78	2.3
Coffee beans	120	20	17	2 460
Coffee beans, roasted	278	188	68	5.1
Coffee imitates beverage	5	5	100	5.0
Common bean (pods and/or immature seeds)	9	9	100	2.9
Composite food NES	636	68	11	55.1
Concentrated fruit juice	94	81	86	2.5
Confectionery	554	279	50	71.9
Corn salad	221	128	58	3.9
Cotton seed	1		0	190
Cowpea (dry)	1	0	0	56.0
Cranberries	37	18	49	6.7
Cream	143	90	63	4.4
Crustaceans	3 032	1017	34	499
Cucumbers and gherkins	1 073	715	67	3.0
Currants, red, black, white	124	26	21	4.9
Dandelion leaves	5	0	0	265
Date	138	124	90	4.3
Dietetic food for diabetics (labelled as such)	10	6	60	53.8
Dried fish	51	0	0	253

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Dried fruits	127	62	49	1150
Drinking water	6 614	6 284	95	0.6
Dry apricots	60	40	67	4.0
Duck meat	854	747	87	5.7
Duck, edible offal of	364	17	5	111
Edible offals (mammalians)	5 008	730	15	2 600
Eggplant	436	219	50	6.1
Egg-based meal (e.g. omelette)	1	1	100	3.0
Eggs and egg products NES	711	692	97	3.6
Endive	261	85	33	13.2
Extracts tea, mate, prep	219	70	32	68.1
Fats and oils NES	322	301	93	4.2
Fennel, bulb	82	58	71	5.1
Fermented milk products	366	311	85	3.1
Fig	24	18	75	9.5
Figs dried	24	11	46	13.6
Fish and seafood-based meals	701	264	38	33.2
Fish, sea food, amphibian reptile snail or insect NES	2 429	442	18	264
Fishes	310	218	70	12.4
Food for sports people (labelled as such)	296	170	57	44.4
Food for weight reduction	72	24	33	21.4
Freshwater fishes	3 116	2661	85	3.7
Fruit and fruit products NES	950	638	67	31.1
Fruit juice	2 653	1964	74	20.7
Fruit juice and herbal tea for infants and young children	207	135	65	6.0
Fruit nectar	796	719	90	3.0
Fruit or vegetable juice NES	222	186	84	8.4
Fruit, tropical, fresh NES	14	6	43	2.7
Fruiting vegetables	68	27	40	15.6
Galangal, rhizomes	2	0	0	2.0
Game meat	4 325	3084	71	12.4
Garlic	316	54	17	22.4
Ginger, root	81	8	10	47.4
Goat meat	338	320	95	3.8
Goat milk	276	266	96	1.4
Goose and guinea fowl meat	142	110	77	3.7
Goose, edible offal of	90	2	2	134
Gooseberries	68	25	37	2.9
Grape juice	556	496	89	1.5
Grape leaves	6	3	50	5.2
Grapefruit (inc. pomelos)	304	258	85	2.2
Grapefruit juice	143	136	95	2.8
Grapes	1 117	1018	91	3.8
Hazelnut	78	16	21	10.1
Herb, spice or condiment NES	873	492	56	19.2

Table (continued)

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Herbs	715	148	21	62.2
Honey	2 676	2082	78	17.3
Hops, dry	12	6	50	8.6
Horse meat	2 184	547	25	38.8
Horseradish	19	0	0	48.2
Ices and desserts	399	67	17	12.2
Indian mustard	28	1	4	165
Infant food	3 891	2 590	67	5.1
Kale	268	27	10	16.9
Kale, curly	35	5	14	46.8
Kiwi fruit	217	189	87	2.7
Lactose	6	6	100	5.0
Lard (of pigs)	8	8	100	4.3
Leafy vegetables	388	107	28	38.5
Leek	419	77	18	16.4
Legume vegetable	57	37	65	6.5
Legumes and pulses NES	21	10	48	116
Lemon juice	105	101	96	1.0
Lemons and limes (including citron)	572	535	94	2.6
Lemons and limes, edible oil refined	1	1	100	5.0
Lentils	876	648	74	6.9
Lettuce	2 505	504	20	29.0
Lima bean (young pods and/or immature beans)	50	48	96	5.3
Liquorice, roots	1	0	0	8.0
Liver product	80	18	23	16.2
Loquat	4	3	75	8.1
Macaroni	913	78	9	31.6
Maize	718	514	72	5.0
Maize flour	511	354	69	7.0
Maize oil, edible	147	147	100	3.9
Mandarin and mandarin-like hybrid	275	256	93	2.9
Mango juice	47	27	57	0.7
Mangoes, mangosteens, guavas	313	255	81	3.4
Maple sugar and syrups	2	1	50	10.0
Margarine	170	154	91	4.4
Marine fishes	19 023	12 270	65	10.7
Meat and meat products NES	705	356	50	30.4
Meat of cattle, goats, horses, pigs and sheep	184	143	78	4.8
Meat of cattle, pigs and sheep	23	12	52	0.8
Meat-based meals	503	131	26	7.5
Medical food (specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone; intended to be used under medical supervision)	242	175	72	7.6

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Medlar	2	2	100	1.8
Melons, except watermelon	388	166	43	7.0
Milk and dairy products NES	395	208	53	16.0
Milks	358	338	94	2.3
Millet	159	23	14	19.1
Mints	5	3	60	8.4
Mixed fruit and vegetable juice	148	81	55	3.5
Molluscs, excluding cephalopods	5 153	300	6	219
Mushroom-based meals	1	1	100	5.0
Mushrooms and truffles	5 718	2 003	35	64.8
Mustard greens	3	2	67	17.2
Non-alcoholic beverage NES	1119	898	80	51.2
Nutmeg, mace and cardamoms	127	0	0	97.9
Oats	955	76	8	24.4
Offal of cattle, edible	7 430	250	3	765
Offal of pigs, edible	20 298	716	4	383
Offal of sheep, edible	1 277	167	13	138
Oilseeds	971	171	18	108
Okra	56	1	2	13.7
Olive	55	40	73	5.4
Olive oil, refined	225	212	94	3.6
Olive oil, virgin	204	202	99	5.1
Olive, processed	16	7	44	1.4
Onion	1 520	545	36	10.5
Orange juice	804	782	97	1.5
Orange juice, concentrated	20	17	85	4.3
Orange, sweet, sour + orange-like hybrid	838	774	92	2.7
Other foods (foods which cannot be included in any other group)	470	282	60	35.4
Palm hearts	4	2	50	1.6
Palm kernels	2	1	50	0.6
Palm oil, edible	56	56	100	4.1
Papaya	21	20	95	1.3
Parsley	444	85	19	24.7
Parsley, turnip-rooted	166	33	20	17.9
Parsnip	225	35	16	48.7
Passion fruit	4	0	0	37.5
Peaches (including nectarines)	983	790	80	2.9
Peanut	339	12	4	77.4
Peanut butter	131	1	1	55.1
Peanut oil, edible	101	100	99	4.9
Pear	686	481	70	3.6
Peas (dry)	366	134	37	11.9
Peas (green pods and immature seeds)	94	56	60	3.0
Peas, shelled (immature seeds)	456	289	63	4.8
Pepper (black, white)	269	64	24	15.3

Table (continued)

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Peppermint	46	9	20	47.1
Peppers and chillies, dried	283	19	7	76.4
Peppers and chillies, green	1246	566	45	13.1
Persimmon	31	31	100	1.7
Pig meat	13 566	11 605	86	6.8
Pineapple	497	406	82	2.0
Pineapple juice	441	316	72	1.3
Pistachio nut	93	40	43	10.8
Plum (incl. dried)	546	464	85	2.8
Pome fruits	3	3	100	4.2
Poppy seed	516	2	0	501
Potato	5 508	774	14	33.7
Potato-based dishes	65	2	3	18.1
Poultry meat	841	652	78	19.9
Poultry offal	915	157	17	39.0
Poultry, fats	5	2	40	17.5
Prepared salads	132	20	15	28.1
Prickly pear	3	3	100	3.3
Products for special nutritional use NES	492	302	61	23.0
Pumpkins, squashes and gourds	624	319	51	4.0
Quince	24	15	63	2.5
Rabbit meat	749	651	87	4.7
Raisins	120	72	60	2.7
Rape seed oil, edible	208	187	90	3.6
Raspberries, red, black	273	95	35	9.8
Ready-to-eat soups	153	4	3	6.5
Ready-to-eat meals for infants and young children	2 391	1 209	51	7.5
Rhubarb	126	14	11	9.4
Rice	3 606	819	23	24.2
Rice bran oil	3	3	100	2.5
Rice flour	230	17	7	38.3
Rice, polished	5	2	40	4.9
Rice-based meals	172	38	22	26.3
Root and tuber vegetables	1 080	183	17	62.8
Rosemary	79	32	41	7.4
Rye	1 133	284	25	13.2
Safflower seed oil, edible	1	1	100	5.0
Sage and related *Salvia* species	5	1	20	40.8
Sauce and condiments	501	188	38	16.2
Sausages and related products	1 052	636	60	3.0
Sesame seed	394	61	15	23.9
Sesame seed oil, edible	56	55	98	4.7
Shaddock or pomelo and shaddock-like hybrid	5	5	100	2.5

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Sheep meat	2 508	2 331	93	3.5
Sheep milk	214	157	73	2.0
Snack food	272	51	19	27.3
Snack or dessert NES	30	17	57	35.8
Sorghum	9	7	78	0.9
Soya bean (dry)	1 771	74	4	83.3
Soya bean oil, refined	61	61	100	4.5
Soya curd	292	55	19	18.5
Soya sauce	115	24	21	7.8
Spices	454	52	11	98.8
Spinach	1 219	58	5	85.3
Stalk and stem vegetables	10	4	40	32.6
Stone fruits	73	55	75	2.5
Strawberry	1 166	530	45	6.8
String beans	5	0	0	0.9
Sugar and confectionery NES	126	77	61	12.2
Sugar crops	20	0	0	18.7
Sunflower seed	456	5	1	219
Sunflower seed oil, edible	181	176	97	3.2
Swede	61	5	8	20.8
Sweet corn	340	209	61	3.5
Sweet potato	648	26	4	17.0
Taro	33	2	6	25.7
Tarragon	6	0	0	113
Tea, green, black (black, fermented and dried)	6 591	2 360	36	37.4
Herbal teas (solid)	7 025	1 777	25	213
Thyme	51	2	4	53.0
Tomato	1 748	886	51	9.3
Tomato juice	223	56	25	9.8
Tomato paste	2	0	0	45.1
Tree nuts	202	41	20	70.7
Tree tomato	5	0	0	1.9
Turkey meat	1 271	1 166	92	3.8
Turkey, edible offal of	791	18	2	129
Turmeric, root	115	14	12	38.3
Vanilla, beans	5	0	0	0.3
Vegetable juice	197	26	13	16.7
Vegetable-based meals	251	71	28	9.9
Vegetables and vegetable products NES	1 762	236	13	502
Walnut	173	134	77	3.2
Watercress	2	0	0	28.5
Watermelon	335	282	84	2.9
Wheat	5 961	809	14	37.1
Wheat flour	2 735	161	6	23.0
Whey and whey products	31	25	81	9.8

Table (continued)

Food	*N*	*N*<LOD	*N*<LOD (%)	MB mean
Wine	1 229	904	74	1.7
Wine-like drinks (e.g. cider, perry)	135	131	97	3.0
Yams	10	0	0	49.3
Yoghurt	221	159	72	4.0
Total	**277 292**	**128 146**	**46**	

N, number of samples; *N*<LOD, number of samples with cadmium concentrations less than the limit of detection; MB mean, medium bound mean; NES, not elsewhere specified.

Ergot alkaloids

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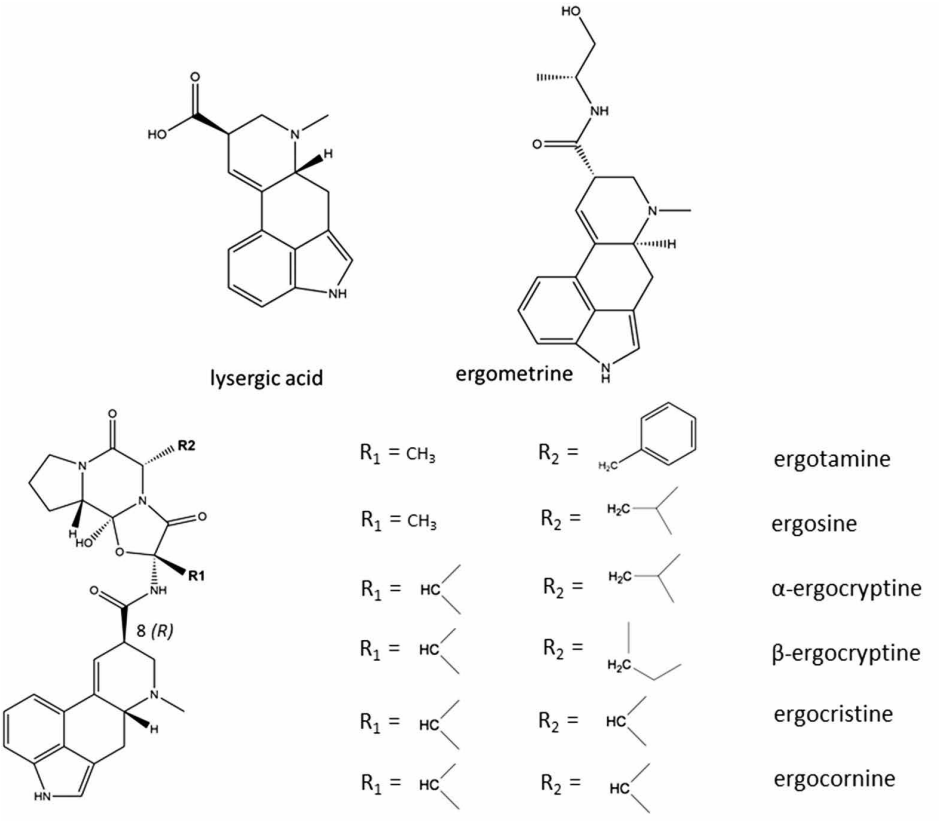
1. Explanation

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).

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



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 (also known as ergonovine), ergotamine, ergosine, ergocristine, ergocryptine (a mixture of alpha (α)- and beta (β)-isomers), ergocornine and the corresponding (*S*)-inine epimers. Ergotamine and ergometrine have been used in human medicine for the treatment of migraine headache, 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 Codex Committee on Contaminants in Foods (CCCF) in 2016.

The search for biological data followed JECFA guidance on conducting a comprehensive literature review (WHO JECFA Secretariat, 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 from 2010 to December 2020 to identify studies of the adverse effects in humans associated with the main EAs used in medicine. Additional studies were identified from previous assessments or reports and drug references (for example, European Food Safety Authority (EFSA), 2010 and Martindale, 2010). 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 January 2000 to December 2020.

2. Biological data

Kinetic studies for the majority of the naturally occurring EAs (for example, ergosine, ergocristine, ergocryptine or ergocornine) are not available. However, for ergotamine and ergometrine, some human and limited animal data were available.

Most of the available data on the biochemical aspects in humans are based on studies with semi-synthetic derivatives of EAs. A brief description of these data has been included; however, as their kinetics may be different to those of natural EAs present in food, these data should be interpreted only as supportive and not definitive evidence.

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

Absorption is a measure of the disappearance of a compound from the gastrointestinal tract and entrance into the systemic circulation, either as unchanged compound or as metabolite(s). The extent of bioavailability, however, is a measure of the amount of unchanged compound that reaches the systemic circulation following an oral dose (Nimmerfall & Rosenthaler, 1976).

Most of the available data are from studies conducted with salts of EAs. It is assumed that the salts dissociate fully in the gastrointestinal tract. When the compound is radiolabelled, the radioactivity has been measured with regard to the base which is the site of the radiolabel.

Animal data

Nimmerfall & Rosenthaler (1976) reported that, following oral administration of 1 mg/kg bw [^{3}H]-dihydroergocristine mesylate to seven Wistar rats, the mean absorption from the gastrointestinal tract was 38.8%. By contrast, the absorption of orally administered [^{3}H]-dihydroergotamine mesylate or [^{14}C]-ergotamine tartrate (both 0.25 mg/kg bw) to six Rhesus monkeys was 31.5 ± 5.2% and 10.3 ± 1.5%, respectively.

After oral administration of [^{3}H]-ergotamine tartrate, dihydro-(9,10-[^{3}H])ergotamine mesylate or dihydro-(9,10-[^{3}H])ergocristine mesylate to groups of six or seven rats at a dose of 1 mg/kg bw, intestinal absorption was estimated at 41.4 ± 9.5%, 12.3 ± 3.3% and 15.0 ± 2.5%, respectively (Eckert et al., 1978).

Human data

Plasma and urinary radioactivity were measured in six healthy male volunteers after oral intake of [^{3}H]-labelled ergotamine tartrate (2 mg), either alone or combined 1:100 with caffeine (Schmidt & Fanchamps, 1974). Their mean age was 41.5 years (39–49 years) and their mean body weight was 73.3 kg (64–76 kg). Volunteers fasted overnight and for 4 hours after dosing. The interval between the two treatments was 10 days. The plasma levels of radioactivity were low; the maximum plasma concentrations corresponded to drug levels of 1–2 ng/mL. No radioactivity could be detected at 15 and 30 minutes after administration of [^{3}H]-ergotamine alone, whereas after administration of the combination with 200 mg caffeine, radioactivity was found after 30 minutes. Peak plasma concentration was reached 2 to 4 hours after both treatments. Plasma level was higher after taking the combination than after ergotamine alone and the difference was significant at certain times. A detectable plasma level was reached 30 minutes after taking the combination but only 1 hour after ergotamine alone. These results indicate faster and more complete intestinal absorption of ergotamine administered in combination with caffeine.

After oral administration of 0.2 to 2 mg of [^{3}H]-labelled ergotamine (in a capsule) or [^{3}H]-labelled dihydroergotamine (in a solution) to different groups of six healthy volunteers, Meier & Schreier (1976) reported an absorption half-life of 0.38 hours and 0.29 hours, respectively.

Aellig & Nüesch (1977) carried out pharmacokinetic studies in six men of nine tritium-labelled EAs: dihydroergotamine, dihydroergotoxine, dihydroergostine, dihydroergocornine, dihydroergovaline, dihydroergonine, ergotamine, 1-methyl-ergotamine and bromocriptine. Each compound was administered to six subjects in a randomized cross-over design as single oral and intravenous doses, with an interval of at least 2 weeks between the administrations. The mean age of the subjects was between 58 and 71 years (range: 37–85 years)

and the mean body weight was between 55 and 71 kg (range: 36–89 kg). Oral doses ranged from 0.2 to 3.0 mg and intravenous doses ranged from 0.1 to 1 mg depending on the compound tested. The compounds were administered in the morning after a 12-hour overnight fast. For ergotamine, the doses tested were 0.2 mg (intravenous) and 1.0 mg (oral).

All compounds showed the highest plasma concentration about 1.8 hours after oral administration (range 1.0–2.7 hours). The mean maximum plasma concentration in ng-equivalents per mL, standardized to a 1-mg oral dose, was 0.56 (range: 0.42–0.77) for hydrogenated and about 2.0 (range: 1.5–2.3) for nonhydrogenated EAs. For ergotamine, the T_{max} was 2.1 ± 0.8 hours and the C_{max} was 1.52 ± 0.09 ng eq/mL.

Cumulative urinary excretion data after oral and after intravenous administration were used to calculate a quotient of absorption. Values between 25 and 30% were found for most dihydrogenated EAs (dihydroergotamine, dihydroergotoxine, dihydroergostine and dihydroergocornine), the only exceptions being dihydroergovaline and dihydroergonine, which were less well absorbed. For ergotamine, the quotient of absorption was 62 ± 3%. Aellig & Nüesch (1977) concluded that all the EAs investigated were rapidly absorbed with an absorption half-life of around 0.5 hours, the peak plasma concentration being reached approximately 2 hours after oral administration.

Plasma ergotamine levels were measured by radioimmunoassay in 11 volunteers (five females and six males) after a single oral dose of ergotamine tartrate (2 mg in tablet form, mean dose of 0.031 mg/kg bw) (Ala-Hurula et al., 1979a). The mean age of the volunteers was 40 years (range: 18–56 years) and the mean body weight was 65 kg (range: 49–75 kg). Blood was collected from 30 minutes to 48 hours after administration. Wide variation in plasma ergotamine levels was observed between subjects. A mean peak concentration of 0.36 ± 0.08 ng/mL was found 2 hours after administration. The level decreased below the detection limit (0.1 ng/mL) beyond 6 hours in most of the participants, but in 7/11 of them, a second rise occurred between 10 hours and 48 hours.

After oral administration of ergotamine tartrate to migraine patients, no ergotamine could be detected in plasma by high-performance liquid chromatography (HPLC) with fluorescence detection (detection limit: 0.1 ng/mL) in two studies (Ekbom et al. 1981; Ibraheem, Paalzow & Tfelt-Hansen, 1983). Ekbom et al. (1981) studied nine male patients with cluster headache 15–600 minutes after oral therapeutic doses of ergotamine tartrate (+ caffeine). The mean age of the subjects was 36 years (range: 29–45 years) and the mean body weight was 70 kg (range: 61–84 kg). Five patients received a constant dose of 2–4 mg daily for at least seven days. Four patients were given 1 mg five times on one day and three patients received a single oral dose of 2 mg. Ibraheem, Paalzow & Tfelt-Hansen (1983) studied seven migraine patients (outside attacks) who received

ergotamine tartrate intravenously and, at least 14 days later, 2 mg of ergotamine tartrate in tablet form after fasting from midnight. After 2 hours, a light meal was served. The mean age of the patients was 43 years (range: 37–54 years) and the mean body weight was 57 kg (range: 51–67 kg). Blood was collected from 10 minutes up to 54 hours after administration. The authors estimated that the maximum possible bioavailablity of ergotamine tartrate would be less than 1% (Ekbom et al., 1981) or 2% (Ibraheem, Paalzow & Tfelt-Hansen, 1983) and, according to Ibraheem, Paalzow & Tfelt-Hansen (1983), most of the radioactivity detected in blood by Aellig & Nüesh (1977) would be in the form of metabolites.

Ala-Hurula et al. (1979b) reported a very low bioavailability of ergotamine tartrate (plasma level below the limit of detection (LOD) by radioimmunoassay, 1 hour after oral administration) in 7 of 18 migraine patients who used the drug daily compared with healthy volunteers.

Dihydroergotamine and its metabolites have been measured in plasma after a single oral administration of 3 mg [^{3}H]-labelled dihydroergotamine mesylate (as a tablet) to six healthy male volunteers (Maurer & Frick, 1984). They fasted for 12 hours before dosing and for 4 hours afterwards. Blood samples were taken up to 60 hours after dosing. Absorption was rapid and the peak plasma concentration was reached within 3.2 ± 0.8 hours. The plasma concentration of 8'-hydroxy-dihydroergotamine, the main metabolite, was 5–7 times higher than the concentration of unchanged dihydroergotamine. The concentrations of dihydrolysergic acid, its amide and a non-identified metabolite were comparable to that of the parent drug, present at very low concentration. The plasma level of non-volatile radioactivity declined biphasically with alpa- and beta-phase half-lives of 2.1 ± 0.5 hours and 32.3 ± 6.2 hours, respectively.

Eight healthy male volunteers received single doses of dihydroergotamine of 5 mg, 10 mg and 20 mg orally and 0.1 mg and 0.5 mg intravenously, with an interval of at least 1 week between administrations (Wyss et al., 1991). The mean age was 26 years (range 22–31 years) and mean body weight was 78 kg (range 60–89 kg). Dihydroergotamine in plasma was measured by specific and polyvalent radioimmunoassay (LOD: 2–14 pg/mL). The maximum plasma levels were observed 1 hour after administration and were below the detection limit after 48 hours except for two volunteers in whom the polyvalent assay found detectable amounts for up to 96 hours. After oral administration, rapid absorption was noted, with a half-life of 8.4 minutes after a lag time of about 10 minutes. From the individual area under the plasma drug concentration-time curve (AUC) values (oral and intravenous) a mean absolute bioavailability of 1% ± 0.6% was calculated, whereas the absolute bioavailability derived from the AUCs after oral administration of 20 mg (using the polyvalent radioimmunoassay (RIA)) was 6.2%. The first-pass extraction of dihydroergotamine was calculated to be 97%.

de Groot et al. (1994) assessed the pharmacokinetics and bioavailability of ergometrine in six men. Their mean age was 41 years (range: 33–50 years) and mean body weight was 72 kg (range: 67–79 kg). A single oral dose of ergometrine maleate 0.2 mg (= 0.147 mg ergometrine) was taken after a standard breakfast. One month later, 0.075 mg ergometrine maleate (= 0.055 mg ergometrine) was injected intravenously in the same volunteers after a similar standard breakfast. Ergometrine in plasma was measured using HPLC with fluorescence detection (LOD: 75 pg/mL). After oral administration, the absorption lag time was subject-dependent and ranged between 0.0073 hours (0.4 minutes) and 0.47 hours (28 minutes). A maximum plasma concentration of 1.16 ng/mL was reached after 54 minutes. The absorption half-life was 0.19 ± 0.22 hours. A large variation in bioavailability was observed in the six participants, between 34% and 117% (mean: 76% ± 32%).

The pharmacokinetics of dihydroergotamine in plasma were examined in six healthy men after single doses of dihydroergotamine: 10 µg/kg intravenously and 10, 20 and 30 mg orally (Little et al., 1982). Tracer amounts of [^{3}H]-dihydroergotamine were used. The mean age of the participants was 23.5 years (range: 18–36 years) and mean body weight was 64.7 kg (range: 60–71 kg). Each participant received the four treatments, 2 to 4 days apart. Dihydroergotamine in plasma was measured by radioimmunoassay. Peak concentrations were apparent within 30 minutes to 1 hour. Mean apparent absorption from the 10-mg dose was 26.6 ± 10% and ranged from 8.9 to 60.3%. The oral bioavailability after the 10, 20 and 30 mg doses averaged 0.47 ± 0.07%, 0.59 ± 0.13% and 0.52 ± 0.14% respectively. Inter-patient variability in bioavailability was sixfold. The low bioavailability was attributed to extensive metabolism during the first passage through the liver.

In six healthy male volunteers who were given a single oral dose of 20 mg of [^{3}H]-α-dihydroergocryptine, rapid absorption into the general circulation occurred with an average rate K_{01} of 0.99 ± 0.73/hour. Mean age was 28 years (23–36 years) and mean body weight was 74 kg (59–85 kg). Subjects fasted for 12 hours before and for 4 hours after dosing. Time to maximum concentration (T_{max}) was reached in approximately 3 hours with a mean of the individual maximum concentration (C_{max}) from the six volunteers of 8.78 ± 5.9 ng eq/mL (Ronca et al., 1996).

In vitro data

Shappell & Smith (2005) showed in vitro that ergovaline in a mixture with its naturally occurring epimer ergovalinine (60:40) readily moves across human intestinal cells (Caco-2 cells). After 6 hours of exposure, 25% and 40% of the administered ergovaline/ergovalinine (6.6 and 25 µM, respectively) crossed the intestinal cell layer.

(b) Distribution

Little is known about the distribution of EAs in the tissues and organs. The information available is summarized below.

Animal data

No information is available on administration of EAs by the oral route in laboratory animals. Very limited data are available on intravenous or intraperitoneal administration, and only for ergotamine. Given the extensive pre-systemic metabolism, information from studies of non-oral routes of exposure is considered to be of limited relevance.

After intravenous administration of [^{3}H]-ergotamine (1 mg/kg bw) to rats, higher radioactivity in liver, lungs, kidney and heart was measured 2 hours after the injection, in comparison to blood, whereas a low concentration of radioactivity was observed in the brain (Kalberer, 1970 cited in: Orton & Richardson, 1982; Eckert et al., 1978 and EFSA, 2012).

In groups of eight male C57Bl/6 J mice, doses of ergotamine (98% pure) at 0, 0.025 or 0.05 mg/kg bw in 1% lactic acid were administered by intraperitoneal injection (Reddy et al., 2020). Tissues were collected 50 minutes post-treatment (liver, kidney and brain: dissected into cerebral cortex, thalamus, cerebellum and brainstem). Quantitation by liquid chromatography coupled with mass spectrometry (LC-MS) showed high levels of ergotamine in the kidney and relatively low levels in the liver. Concentrations of ergotamine in the kidney after the high (1.632 ± 0.289 ng/g) and low dose (0.438 ± 0.157 ng/g) treatment were 11 times (0.148 ± 0.121 ng/g) and 4 times (0.108 ± 0.028 ng/g) higher than in the liver, respectively, with a clear dose-dependent effect. Ergotamine was consistently below detectable limits in the cerebral cortex, cerebellum and thalamus (LOD not mentioned). Following treatment of mice with the high dose, ergotamine was identified in the brainstem tissue (6.909 ± 5.596 ng/g; n = 4). Although there was significant variation, levels were 4.2 and 46.5 times higher than in the kidney and liver, respectively.

In pregnant rats, 60 minutes after intravenous injection of [^{3}H]-ergotamine (2.5 mg/kg bw), radioactivity was detected in the blood (0.3 mg eq./kg) and three times greater concentrations were found in the uterus, placenta and yolk-sac (1.0 to 1.2 mg eq./kg) (Leist & Grauwiler, 1973). Very low radioactivity was detected in the amniotic fluid (0.05 mg eq./kg) and fetal tissues (0.07 mg eq./kg). The transplacental passage of ergotamine was estimated to be 2.8%.

Human data

After oral administration of [^{3}H]-dihydroergotamine to six healthy men, peak concentrations were apparent within 30 minutes to 1 hour. The pharmacokinetic

profile was described by a two-compartment model. The mean volume of distribution was 14.5 ± 3.1 L/kg (Little et al., 1982).

After oral administration of 2 mg ergotamine tartrate to 10 healthy male and female volunteers (following an overnight fast), peak plasma concentrations were achieved about 1 hour after administration as a solid tablet (with 100 mg caffeine) and 45 minutes following administration as an effervescent tablet (with 50 mg caffeine) (Orton & Richardson, 1982).

After intravenous administration of ergometrine to six men (0.075 mg ergometrine maleate), the pharmacokinetic profile was described by a two-compartment model (de Groot et al., 1994). The distribution half-life was 0.18 ± 0.20 hours, and the steady-state volume of distribution was 73.4 ± 22.0 L. After oral administration of ergometrine maleate (0.2 mg), the pharmacokinetic profile was described by a one-compartment model.

Following oral administration of 5 or 10 mg of dihydroergotamine to eight healthy men, a very large volume of distribution (33 L/kg) indicated extensive tissue distribution (Wyss et al., 1991). The multi-exponential decline of dihydroergotamine in plasma, with a long terminal half-life, suggested distribution into a deep compartment. Plasma protein binding was about 94%.

In six healthy male volunteers who were given 20 mg of [^{3}H]-α-dihydroergocryptine, distribution from the central compartment to the peripheral compartment occurred with a mean rate constant (K_{12}) of 0.330 ± 0.22/hour. The mean volume of distribution was 33.9 ± 22.3 L/kg. The rate constant (K_{21}) of radioactivity washout from the tissue to the central compartment was 0.250 ± 0.130/hour. Plasma radioactivity declined biexponentially with an overall elimination constant (K_{10}) of 0.029 to 0.146/hour (i.e. half-lives of 23.9–4.75/hour) (Ronca et al., 1996).

Ala-Hurula et al. (1979b) detected ergotamine tartrate in the cerebrospinal fluid of four subjects 1 or 2 hours after oral administration of 2 mg (+ caffeine). The concentrations were comparable to those detected in plasma. However, Hovdal, Syversen & Rosenthaler (1982) failed to detect ergotamine in the cerebrospinal fluid after intramuscular and rectal administration to 18 hospitalized patients, but these results could be explained by the detection limit of the radioimmunoassay method (0.1 ng ergotamine/mL).

The plasma levels in three healthy male and three healthy female volunteers given 0.25 mg ergotamine tartrate intravenously declined rapidly over the first 15 minutes after injection (Orton & Richardson, 1982). Thereafter, ergotamine concentrations fell more slowly and in five of the six subjects no ergotamine tartrate was detected in the plasma at 240 minutes. The data for each subject were analysed by computer using a 2-compartment model with first-order kinetics, and the mean pharmacokinetic parameters were derived. The mean distribution half-life was 2.43 minutes (range: 1.72–4.55). The apparent

volume of distribution was 140.9 L (range: 68–254), greater than the total body water content, indicating that ergotamine tartrate is concentrated in the tissues.

In vitro data

In an in vitro model using primary porcine brain capillary endothelial cells, Mulac et al. (2012) observed that ergometrin(in)e, ergotamin(in)e and ergocristin(in)e (1 or 10 μM) were able to cross the endothelial cell barrier formed to mimic the blood–brain barrier. For ergotamin(in)e and ergocristin(in)e, the permeability was high, comparable with the permeability of the amino acid L-leucine in the same model system. Ergometrin(in)e showed a 10 times lower permeability. Active transport was identifed for ergometrin(in)e as a substrate for the BCRP/ABCG2 transporter. No active transport was observed for ergotamin(in)e and ergocristin(in)e. Furthermore, for the pure 8-(*S*) isomer, ergocristinine, no transcellular diffusion was observed, indicating that the transport could be selective for the 8-(*R*) form (-ine epimers). In addition, ergocristin(in)e decreased the integrity of the barrier (reduction of initial transendothelial electrical resistance). Ergotamin(in)e and ergocristin(in)e had no impact on barrier integrity. The impact on barrier integrity of ergocristin(in)e was also reflected by [^{14}C]-sucrose permeability and visualized after staining of tight junction protein and cell nuclei. Small parts of the cell monolayer were destroyed, resulting in areas without cell nuclei, and disruption of occludin-staining.

(c) Excretion

Animal data

Nimmerfall & Rosenthaler (1976) investigated the excretion of [^{3}H]-dihydroergocristine mesylate (1 mg/kg bw) in seven male Wistar rats and of [^{3}H]-dihydroergotamine mesylate (0.25 mg/kg bw) and [^{14}C]-ergotamine tartrate (0.25 mg/kg bw) in six Rhesus monkeys after oral and intravenous administration. After ingestion, excretion in the bile was the most important route of elimination in the rat (13.8 ± 2.4% of the radioactivity on average) and in the monkey (7.6 ± 1.3% for dihydroergotamine and 24.1 ± 5.7% for ergotamine). In contrast, urinary excretion occurred only to a small extent (mean excretion: 1.2 ± 0.3% in rats, 2.8 ± 0.7% and 7.4 ± 1.6% in monkeys for dihydroergotamine and ergotamine, respectively). Rat faeces contained 88.6 ± 6.4% of the radioactivity. Monkeys' faeces contained 91.9 ± 5.0% of the radioactivity from dihydroergotamine and 68.2 ± 6.4% from ergotamine. After intravenous administration to rats, 91% of the radioactivity was found in the bile and 3.9% in the urine.

After oral administration of [^{3}H]-ergotamine tartrate (1 mg/kg bw) to six or seven male Wistar rats, excretion was 32.8 ± 9.3% in the bile, 8.6 ± 2.1% in urine and 63.9 ± 6.7% in the faeces (Eckert et al., 1978). After oral administration

of [^{3}H]-dihydroergocristine mesylate (1 mg/kg bw) to six male Wistar rats, excretion was 10.4 ± 3.3% in the bile, 1.9 ± 0.2% in urine and 93.2 ± 1.6% in the faeces (Eckert et al., 1978).

In studies in livestock, no EAs were detected in the milk of eight Holstein dairy cows collected after 4 weeks of exposure to contaminated diets (Schumann et al., 2009). The alkaloid exposure varied between 4.1 and 16.3 µg/kg bw per day. Milk was sampled for four consecutive morning and evening milkings in week four and pooled for analysis by HPLC with fluorescence detection (LOD: 5 or 10 µg/kg, depending on the EA).

Milk does not appear to be a major excretion route for unmetabolized ergovaline in goats (Durix et al., 1999). Four lactating goats received ergovaline intravenously at a dose of 32 µg/kg bw. Milk was sampled after 8, 24, 32 and 48 hours and analysed by liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS) (LOD, 0.2 µg/L and limit of quantification (LOQ), 0.7 µg/L). Ergovaline was found only in milk sampled 8 hours after dosing, at a concentration of 0.71 ± 0.17 µg/L.

Human data

Radioactivity measured in the urine of six healthy male volunteers after oral intake of [^{3}H]-labelled ergotamine tartrate (2 mg) showed a low cumulative excretion over a 24-hour period of 1.96% ± 0.50% of the dose administered (Schmidt & Fanchamps, 1974). Meier & Schreier (1976) also reported a low excretion in urine, after oral administration of 0.2 to 2 mg of [^{3}H]-labelled ergotamine (in a capsule) or [^{3}H]-labelled dihydroergotamine (in a solution) to different groups of six volunteers: 4.23% and 3.06% of the administered dose, respectively. The half-lives of elimination were 34.3 and 30.3 hours, respectively.

The mean elimination half-life for nine tritium-labelled EAs (dihydroergotamine, dihydroergotoxine, dihydroergostine, dihydroergocornine, dihydroergovaline, dihydroergonine, ergotamine, 1-methyl-ergotamine and bromocriptine) in six healthy male volunteers was 2.3 hours (range: 1.4–6.2 hours) for the alpha-phase and 23 hours (range: 13–50 hours) for the beta-phase, the longest times being observed with bromocriptine. For ergotamine, the mean elimination half-life was 2.7 ± 0.9 hours for the alpha-phase and 21 ± 4 hours for the beta-phase (Aellig & Nüesch, 1977). The mean cumulative excretions measured during the period of urine collection were 4.1% (range: 2.0–12.7%) and 12.0% (range: 6.2–8.6%) after oral and after intravenous administration, respectively. Extrapolating to infinity, the values are 4.4% (range: 2.0–13.3%) and 12.3% (range: 6.7–19.1%). After intravenous administration, between 80 and 90% of the EAs are therefore excreted in the faeces (Aellig & Nüesch, 1977).

Orton & Richardson (1982) reported a mean elimination half-life of 96.53 minutes (range: 63.8–154.1) in six volunteers (three males and three females) given 0.25 mg ergotamine tartrate intravenously. During the 4 hours of the study, 4.9% of the dose was excreted in the urine, more than 50% of this during the first hour. In a study of 10 volunteers, 2 mg ergotamine tartrate was administered orally as a solid tablet (with 100 mg caffeine) or as an effervescent tablet (with 50 mg caffeine) after an overnight fast. Six hours after taking the solid tablet, 0.11% of the oral dose was excreted in the urine, and 0.08% after taking the effervescent preparation. In both cases, 50% of the total amount excreted at 6 hours was excreted in the first 2 hours of the study (Orton & Richardson, 1982).

After intravenous administration of [^{3}H]-dihydroergotamine (10 μg/kg) to six healthy men, the half-life of the initial alpha-phase of decline averaged 0.23 hours and that of the slower phase 2.37 hours (Little et al., 1982). Total plasma clearance ranged from 488 to 1379 mL/minute (mean 1002 ± 169 mL/minute). Excretion of the tritiated dose was rapid and nearly complete by 6 hours. By 24 hours an average of 11.1 ± 1.0% of the administered [^{3}H]-dihydroergotamine had been excreted in the urine. Following oral administration of a 10-mg dose, urinary excretion of the tritiated dose was nearly complete by 12 hours and by 24 hours 3.0 ± 1.1% of the tritiated dose had been excreted. The fraction of apparently absorbed dose excreted in the urine would correspond to a range of 7.3 to 13.5%.

Dihydroergotamine and its metabolites have been measured in urine after a single oral administration of 3 mg [^{3}H]-labelled dihydroergotamine mesylate to six healthy male volunteers (Maurer & Frick, 1984). The volunteers fasted for 12 hours before dosing and for 4 hours afterwards. Urinary excretion of total non-volatile radioactivity was low, amounting to 1.01 ± 0.36% of the dose in the urine samples collected during the 72 hours afer dosing (70% of this amount was found in the 0–8-hour urine fraction). The parent drug and four metabolites could be quantified in urine and plasma samples. The concentration of 8'-hydroxy-dihydroergotamine, the main metabolite, was several times higher than the concentration of unchanged dihydroergotamine. Dihydroergotamine was intensively metabolized and only 0.01% of the dose was found in the 0–24-hour urine fraction.

Following oral administration of 5 or 10 mg of dihydroergotamine to eight men, the plasma concentrations declined in a bi-exponential manner (Wyss et al., 1991). A short alpha-phase with a half-life of 1.45 hours was followed by the beta-phase with a long terminal half-life of 15 hours for unchanged dihydroergotamine and a terminal half-life of 34.7 hours with the polyvalent assay (considered to be due to metabolites). The beta-phase contributed 70%, 78% and 87% to the total AUC of unchanged dihydroergotamine after oral administration of 5 mg, 10 mg and 20 mg, respectively. Over a 48-hour period,

the mean cumulative urinary excretion amounted to 6.7% and 8.8% of the dose after intravenous administration of 0.5 and 0.1 mg, respectively, whereas, after oral administration of 20 mg, only 0.04% of the dose was excreted as unchanged dihydroergotamine. A similar terminal elimination half-life was also calculated from the urine data alpha-phase of 1 hour and the beta-phase of 11.8 hours. Renal clearance contributed only 1% to the high total plasma clearance. After oral administration of 5 mg and 10 mg, the amount of dihydroergotamine excreted in the urine was too low to be measured reliably.

After oral administration of 0.2 mg of ergometrine maleate to six men, the elimination half-life was 1.90 ± 0.16 hours (de Groot et al., 1994). In the same study, after intravenous administration of ergometrine (0.075 mg ergometrine maleate), the elimination half-life was 2.06 ± 0.90 hours, and the total body clearance was 35.9 ± 13.4 L/hour.

In six healthy male volunteers who were given 20 mg of [^{3}H]-α-dihydroergocryptine, total radioactivity recovery in urine and faeces was good, with 82.78 ± 6.44% of the dose eliminated in faeces and 3.01 ± 0.65% in urine. The latter concentration was too low to detect metabolites or unchanged drug by radioactivity image scanning. However, the liquid scintillation count of silica gel that had been scraped off the thin layer chromatography plates indicated the presence of metabolites in urine. The terminal elimination half-life was 25.8 ± 7.5 hours (Ronca et al., 1996).

There is no information on the likely presence of ergometrine in the milk of lactating women (EMEA, 1999). However, after oral dosing with 0.25 mg/day of the analogue, methylergometrine, up to 1.3 μg/L was present in the milk of lactating women (Erkkola et al., 1978; Vogel et al., 2004). Vogel et al. (2004) measured methylergometrine in the milk of 10 lactating women on postpartum days 3 to 6 at 0.5, 1, 2, 3, 4 and 5 hours postdose. After administration of a single oral dose of 250 μg of methylergometrine, the breast milk C_{max}, determined by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS), was 0.66 μg/L at a T_{max} of 1.8 hours, then it steadily declined to 0.20 ng/mL at 5 hours. The milk/plasma ratio was 0.18. These results are in agreement with an earlier study by Erkkola et al. (1978). After administration of a single oral dose of 250 μg of methylergometrine to eight lactating women, measurable amounts of the drug were found in only 5 of 16 milk samples, as determined by radioimmunoassay 1 and 8 hours after administration (LOD, 0.5 μg/L). The breast milk C_{max} was 1.3 μg/L at a T_{max} of 1 hour, with levels becoming undetectable after 8 hours. The milk/plasma ratio was 0.3.

2.1.2 Biotransformation

There are limited data available relating to 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 (Horowitz, Dart & Gomez, 1996) and ritonavir (Liaudet et al., 1999). Both are reported to be associated with ergotism following co-administration with ergotamine.

The most common biotransformation of the ergopeptine alkaloids occurs by opening the tricyclic amino acid ring structure at the proline moiety (Eckert et al., 1978).

(a) In vivo studies

In their review, Eckert et al. (1978) described the work of a PhD student (Maurer, 1977) on partly [^{3}H]-radiolabelled dihydro-β-ergocryptine administered intraperitoneally to male Wistar rats. Sixteen bilary metabolites were isolated and identified. Oxidation at the C8 position of the proline was a major route of biotransformation since most of the metabolites had either a hydroxyl or a carboxyl group in this position. Two types of dihydroxylated compounds were isolated: metabolites hydroxylated at C8′ and C9′ and metabolites with hydroxyls at C8′ and C10′, the former occurring in greater quantities. Apart from a glutamic acid derivative with the acid group at C8′, hydroxyl-glutamic acid derivatives were also isolated with OH-groups at C9′ and C10′. The glutamic acid derivative and the dihydroxylated derivatives were the metabolites produced in the greatest quantities; administration of low doses favoured production of the dihydroxylated derivatives and administration of high doses favoured production of the glutamic acid derivatives. A metabolite pathway was proposed, with a central role of the stereo-isomeric metabolites hyroxylated at C8′. The glucuronides of the monohydroxy (C8′) and dihydroxy (C8′ and C9′) derivatives were mentioned as minor metabolites, as well as a metabolite hydroxylated at C9′ and conjugated with glutathione. An epoxide may be formed as an intermediate. Eckert et al. (1978) noted that the isolated metabolites did not include any demethylated derivatives or N-oxides.

Ergometrine maleate at a dose of 3 mg/kg bw was administered intravenously to male and female albino rats and the bile was collected for 6 hours (Slaytor & Wright, 1962). The major route of biotransformation was hydroxylation at position 12 of the ring system, leading to the metabolites 12-hydroxy-ergometrine and its isomer 12-hydroxy-ergometrinine, which are then conjugated to glucuronic acid and excreted in bile. Following a larger dose

(45 mg/kg bw), some unchanged parent compound and its isomer ergometrinine as well as glucuronides of ergometrine and ergometrinine (thought to be modified in the propanolamide side-chain) were observed. Trace amounts of two further isomeric pairs of metabolites less polar than ergometrine (not further identified but considered likely to be N-demethylation products) were observed (EMEA, 1999).

Reddy et al. (2020) used electrospray ionization (ESI) + LC-MS quantitation analysis to determine the presence of ergotamine and its metabolites in the brain, liver and kidney of C57Bl/6 J male mice 50 minutes after intraperitoneal administration of ergotamine (98% pure) at 0, 0.025 or 0.05 mg/kg bw in 1% lactic acid. The previously reported metabolic products of ergotamine (denoted as E1 and E2, in vitro by Rudolph et al., 2019) were present in relatively high concentrations in the liver compared to the kidney. E1 (mass divided by charge number (m/z) 598.2670) and E2 (m/z 614.2640) were the result of ergotamine hydroxylation indicated by the +15.9958 Da (+O) and +31.9928 (+2 O) Da mass shift from the parent ion. As for ergotamine, the fragmentation of the metabolic products E1 and E2 resulted in neutral loss of water m/z 580.2569 and 596.2489. The most intense signals produced by E1 (m/z 223.1231 and 208.0759) and E2 (m/z 223.1230 and 208.0758) were consistent with ergotamine fragment ions of the lysergic acid components. Presence of the fragment ion m/z 308 suggests that hydroxylation of E1 occurs in the peptide group and the fragment ion m/z 567 ion suggests that hydroxylation is confined to the tetrahydropyrrole ring. This is further supported by the unique m/z 308 ion, which results in the fragmentation at the aromatic ring and peptide group containing the hydroxy group attached to the tetrahydropyran ring. The fragmentation spectrum of the E2 biotransformation product produced the m/z 524 ion resulting in the opening of the tetrahydropyrrole ring and loss of the dihydroxylated moiety. The m/z 513 ion also indicates loss of the tetrahydropyrrole ring, further confirming the position of the hydroxyl group. Together these data suggest that the tetrahydropryrrole ring is the primary site of metabolism for the ergopeptide alkaloids (Reddy et al., 2020).

(b) Data from studies in humans

Limited data are available from studies in humans and these are only for dihydrogenated forms of EAs (semi-synthetic derivatives that do not occur naturally). They are expected to have a higher bioavailability than the natural compounds, but the information is considered to be of limited relevance.

Maurer & Frick (1984) found that dihydroergotamine was intensively metabolized in humans and only 0.01% of the dose of the parent compound was found in urine collected during 0–24 hours. 8'-Hydroxy-dihydroergotamine was

the main metabolite in humans (plasma and urine). The authors characterized hydroxylation at carbon 8' of the proline structure of dihydroergotamine. According to the nuclear magnetic resonance spectroscopy (NMR)-integrals of H_{α}-C8' and H_{β}-C8', they found that the metabolite was a mixture (30/70) of 8'α- and 8'β-hydroxy-dihydroergotamine. In receptor-binding studies performed with rat brain preparations, 8'-hydroxy-dihydroergotamine had half-maximal inhibitory concentration (IC_{50})-values at six monoaminergic binding sites similar to those of dihydroergotamine. Maurer & Frick (1984) proposed that the biotransformation of dihydroergotamine in humans is essentially an oxidation of its peptide moiety.

This metabolite, 8'-hydroxy-dihydroergotamine, showed about the same potency as dihydroergotamine for venoconstrictor activity. A placebo-controlled study in seven healthy male volunteers measured changes in venous diameter at an occlusion pressure of 45 mmHg, after direct local infusion of 0.08 and 0.4 µg into superficial hand veins (Aellig, 1984).

After a single oral administration of 20 mg dihydroergotamine mesylate to 16 healthy volunteers (no details provided on the volunteers), levels of 8'-OH-dihydroergotamine in human plasma were already several times greater than the parent compound in the first hour after dosing and throughout the 16 hours of the study (Chen et al., 2002). Bicalho et al. (2005) found similar results with dihydroergocristine mesylate after administration of a single oral dose of 18 mg to 12 healthy male volunteers. The mean peak plasma level of the 8'-OH-derivative was 20 times greater than the parent compound (5.63 ± 3.34 µg/L and 0.28 ± 0.22 µg/L, respectively).

After administration of a single oral dose of 27 mg dihydroergotoxine mesylate (a mixture of semi-synthetic EAs: 35% dihydroergocornine, 33% dihydroergocryptine (2:1 alpha/beta isomers) and 32% dihydroergocristine) to a healthy volunteer (male, age 45 years, weight 103 kg) Bicalho et al. (2008) found that mono-hydroxylated metabolites were one order of magnitude higher in concentration than the parent compounds in human plasma. The plasma concentration of the three parent compounds remained around the LOQ (0.02 mg/L) up to 4 hours following dosing. After that they fell below the LOQ where they remained up to the final blood sampling time at 8 hours. In contrast, the OH-metabolites were determined in all samples collected. Their peaks occurred at about 0.5 hours after dosing and were approximately 1 mg/L hydroxy-dihydroergocornine, 0.5 mg/L hydroxy-dihydroergocryptine and 0.3 mg/L hydroxy-dihydroergocristine.

(c) In vitro studies

In human liver microsomal incubates, 8'-hydroxy-dihydroergotamine was shown to be the primary metabolite of dihydroergotamine (Maurer & Frick, 1984). 8'-hydroxy-dihydroergotamine was also isolated from incubates of rat and monkey liver microsome preparations. The biotransformation product of 8'-hydroxy-dihydroergotamine was a metabolite that contains glutamic acid instead of the initial proline group.

When incubated with bovine liver microsomes, ergotamine was hydroxylated to the more hydrophilic metabolites, M1 and M2. Similarly, its isomer was hydroxylated to M1-Iso and M2-Iso (8-hydroxy-derivatives). Further incubation resulted in a second hydroxylation of M1 and M2 to metabolites M3 and M4 (8,9-dihydroxy derivatives). A similar metabolite profile (M1, M2, M1-Iso and M2-Iso) was produced when ergotamine was incubated with liver microsomes of dexamethasone (an inducer of CYP3A) treated rats (Moubarak & Rosenkrans, 2000).

Supernatants of alkaloid incubations (ergocristine, ergocryptine, ergotamine and ergovaline) with equine and human liver S9 fractions were analysed by reversed-phase liquid chromatography coupled to high-resolution tandem mass spectrometry with full scan and MS acquisition (Rudolph et al., 2019). Although various phase I metabolites could be identified, no phase II metabolites were detected. Metabolite structures were postulated based on their MS spectra in comparison to those of the parent alkaloids. All compounds were extensively metabolized yielding nor-, N-oxide, hydroxy and dihydro-diol metabolites with largely overlapping patterns in equine and human liver S9 fractions. However, some metabolic steps, for example, the formation of 8'-hydroxy metabolites were unique for human metabolism, while formation of the 13/14-hydroxy and 13,14-dihydro-diol metabolites were unique for equine metabolism. Incubations with equine whole liver preparations yielded less metabolites than the S9 fractions.

Mulac et al. (2011) investigated the metabolism of ergometrine, ergotamine and ergocristine in human colon and liver cell lines (HT-29, HepG2), as well as in human primary renal cells (RPTEC), using Fourier transformation mass spectrometry. Different mono- and di-hydroxylations at the proline partial structure could be identified.

Dihydroergotoxine (a mixture of semi-synthetic EAs: 35% dihydroergocornine, 33% dihydroergocryptine (2:1 alpha/beta isomers) and 32% dihydroergocristine) at 30 μm was added to incubates of rat liver microsomes, and the resulting major metabolites were identified as mono-hydroxylated derivatives of the parent compounds (Bicalho et al., 2008). The major metabolite,

8'-hydroxy-dihydroergocristine, was produced in incubates of a bovine liver preparation using dihydroergocristine mesylate as substrate (Bicalho et al., 2005).

Dihydroergotamine was submitted to an in vitro metabolism assay using human and rat liver microsomes (Bauermeister et al., 2016). Besides the formation of the known hydroxylated metabolite 8'-OH-dihydroergotamine, two new major hydroxylated metabolites were isolated and characterized by MS/MS and proton NMR analysis as 5-OH-dihydroergotamine and 11-OH-dihydroergotamine.

Owing to the asymmetric carbon at the C8 position, EAs exist in two forms known as the (*R*)- and (*S*)-epimers. The (*R*)-epimers have the suffix "ine" whereas the (*S*)-epimers have the suffix "inine". Merkel et al. (2012) studied the effect of digestion in vitro on the ratio of (*R*)- to (*S*)-isomers of six EAs (ergometrine, ergosine, ergotamine, ergocornine, ergocryptine and ergocristine) in cookies baked for 13 minutes. With respect to epimerization, ergometrine demonstrated minimal effects, with a 4% change in the amount of ergometrine compared to the total ergometrin(in)e content. For the ergotoxine group (ergocornine, α-ergocryptine, β-ergocryptine and ergocristine), an intense shift towards the (*S*)-epimers was observed. In contrast, digestion of the ergotamine group (ergosine and ergotamine) resulted in a shift towards the (*R*)-epimers. The initial percentage of the (*R*)-epimers compared to the total content increased noticeably after digestion (ergosine: 35 to 55%; ergotamine: 32 to 51%). No change in epimerization was observed when cookies were incubated in saliva alone or in saliva followed by gastric juice. For the ergotoxine group, digestion in duodenal juice led to a shift towards the (*S*)-epimers regardless of whether all enzymes (bile, trypsin and pancreatin) or only one enzyme was added. For the ergotamine group, epimerization towards the (*R*)-epimers occurred with each enzyme. The experiments including all enzymes, bile and pancreatin caused a more pronounced effect.

The in vitro metabolism of α-dihydroergocryptine has been studied in human hepatocytes and two sets of metabolically competent cell lines expressing one single human cytochrome P450 (1A1, 1A2, 1B1, 2A6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1 and 3A4). Mono- and dihydroxy- metabolites could only be detected in the culture media of the cell line expressing human cytochrome CYP3A4. The same metabolites were found in the media of cultured human hepatocytes derived from three different donors. After 24 hours of incubation with 1 μM α-dihydroergocryptine, approximately 60% mono- and approximately 20% dihydroxy- metabolites were detected, i.e. approximately 80% of α-dihydroergocryptine was metabolized. The data suggest that α-dihydroergocryptine metabolism in humans is primarily mediated by the CYP3A4 isoform (Althaus et al., 2000).

Duringer et al. (2005) studied the in vitro metabolism of ergotamine in mouse liver microsomes and investigated the impact of sex and previous exposure

of mice to ergovaline (1381 μg/kg diet for 2 weeks). Microsomal incubations produced nine predominant peaks in the HPLC assay. The peaks were confirmed by LC-MS/MS to be ergotamine, ergotamine epimer, monohydroxylated metabolites (M1, M2, M1e and M2e) and dihydroxylated metabolites (M3–5). Hydroxylation of ergotamine took place on the peptide ring structure. A sex difference for metabolite formation was observed in the non-exposed mice, in that females produced a greater amount of M1, M1e and M3–5 than males. When challenged with the ergovaline diet, mice showed differences in concentration of M3 for line (endophyte-resistant > endophyte-susceptible mice) and sex (female > male) and of M4 and M5 for sex (female > male). Sex differences in the metabolism of ergotamine have not been shown before in these lines of mice or other species used to study metabolism of EAs. This adds a potential source of variation in the susceptibility to EA toxicity not explored previously.

2.1.3 Effects on enzymes and other biochemical parameters

α-Dihydroergocryptine showed an inhibitory effect on CYP3A4-mediated testosterone metabolism and additionally could induce CYP3A4 and CYP2E1 mRNA when added at 10 μM to cultured human hepatocytes (Althaus et al., 2000).

A significant increase in gene expression was observed for various CYP isoforms, i.e. CYP1A1, CYP2C9, CYP2E1 and CYP3A1 in the livers of male Wistar rats fed with a diet contaminated with ergovaline (91.5 μg/kg bw per day) compared to a control group ($n = 12$ per group). A significant reduction in hepatic gene expression for CYP3A7 was observed in the rats fed the contaminated diet. Hepatic gene expression of CYP2C13, CYP1A2 and CYP2C6 was not affected. Genes for the CYP-inducing nuclear receptors pregnane X receptor and retinoid X receptor were upregulated in the livers of rats fed the contaminated diet, compared to the controls. The protein expression of the major CYP isoform, CYP3A1, was induced to a greater extent in the livers of the treated rats than in the livers of the controls. Primary hepatocellular cultures from healthy rats exhibited an ergovaline dose-dependent induction of CYP3A1 protein expression compared to hepatocytes not exposed to ergovaline (Settivari et al., 2008).

Moubarak et al. (2012) studied the effect of ergotamine on the induction of CYP3A and the interaction in vivo and in vitro. Five Sprague-Dawley rats were treated intraperitoneally for 4 days with 100 mg/kg bw ergotamine (in corn oil). The liver was collected and liver microsomes were prepared. The results showed that ergotamine and its isomer are substrates for the isozyme CYP3A, but that these compounds have no effect on the induction of CYP3A after 4 days of treatment. An earlier study on dihydroergotamine in rats showed similar

results (no induction of CYP3A4 after 4 days of intraperitoneal administration) (Moubarak, Rosenkrans & Johnson, 2002).

Using an in vitro assay based on luminescence, Rosenkrans & Ezell (2015) showed that CYP3A4 activity was inhibited by ergotamine and dihydroergotamine (20 or 40 μM), but not by ergonovine. The urine of Angus-sired crossbred steers (n = 39) was collected after grazing tall fescue pastures contaminated with EAs for 105 days. Urine inhibition of CYP3A4 activity and total alkaloids (determined using enzyme-linked immunosorbent assay (ELISA)) were correlated. Steers were genotyped at CYP3A4 single nucleotide polymorphism, C994G. Steer genotype affected inhibition of CYP3A4 activity by urine; heterozygous steers had the least CYP3A4 inhibition.

2.1.4 Physiologically based pharmacokinetic (PBPK) modelling

No data available.

2.1.5 Transfer from feed to food

The information regarding a potential carry-over of EAs into products of animal origin is scarce, as reported in the reviews by EFSA (2005 and 2012).

Broiler chickens were fed diets containing up to 2.03 mg EAs/kg feed for 35 days. At the end of the experiment, EA residues in liver and breast meat were lower than 5 ng/g (Dänicke, 2017). Similarly, when ducks received a diet contaminated with up to 16.4 mg EAs/kg feed for 2 weeks, residues in liver and breast meat were below 5 ng/g (Dänicke, 2015). When pigs with a body weight of 30 to 115 kg were administered feed containing EAs (up to 4.66 mg EAs/kg), no residues were detectable in meat and back fat (Mainka et al., 2005).

For dairy cattle (approximately 400 kg bw), the carry-over following exposure to 50 g ergot sclerotia per animal resulted in milk concentrations (total alkaloid content) reaching 0.086 mg/L (Parkheava, 1979). Two studies were performed by Schumann et al. In the first, Holstein Friesian bulls were fed diets with up to 421 μg/kg dry matter (DM) of total alkaloids for a period of approximately 230 days and no carry-over into tissues could be detected (Schumann et al., 2007). In the second study (Schumann et al., 2009), dairy cows were fed for 4 weeks with EA-contaminated diets (resulting in an alkaloid concentration of 504.9 and 619.5 μg/kg DM per day) and no EAs were detected in the milk produced (LOD, 5–10 μg/kg).

In conclusion, the very limited data on tissue distribution and residual concentrations in edible tissues, milk and eggs, provide evidence of negligible accumulation of EAs in edible tissues.

2.2 Toxicological studies

2.2.1 Acute toxicity

LD_{50} values for some natural EAs following oral or intravenous administration are reported in Table 1 (Griffith et al., 1978). Oral LD_{50} values were always higher than intravenous LD_{50} values in the same species, reflecting the low absorption and high pre-systemic metabolism subsequent to oral administration. Moreover, marked differences in sensitivity were observed between species, with the rabbit being the most sensitive, followed by the rat and the mouse as the less sensitive species. 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 the oral LD_{50} values in rabbit (mg/kg bw), the natural EAs can be ordered as follows: ergometrine (27.8) > ergonine (150) > ergotamine (550) > ergostine (~ 1000). Based on the intravenous LD_{50} values in rabbit (mg/kg bw), the natural EAs can be ordered as follows: ergocryptine (both isomers, 0.34) > ergonine (1.1) ~ ergostine (1.2) ~ ergostine (1.23) > ergovaline (1.7) ~ ergocristine (1.9) > ergotamine (3.0) ~ ergometrine (3.2) > ergostinine (5.3).

The clinical signs of acute sublethal poisoning relate to neurotoxicity, including restlessness, miosis or mydriasis, muscular weakness, tremors and rigidity. Piloerection and tachypnoea were described in mice, rats, rabbits and guinea-pigs after treatment with ergotamine and ergobasine and, with higher doses of ergotamine, ergotoxine and ergometrine, convulsions were seen in all three species. Tail gangrene was observed in 20% of 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.

Administration of ergotamine tartrate to Rhesus monkeys in single intravenous doses of up to 1 mg/kg did not cause any marked adverse effects.

A dose of 10 mg/kg bw of ergotamine maleate (purity not indicated) was administered by intraperitoneal injection to a group of six Wistar rats of both sexes weighing 100–200 g. The treatment induced the $5\text{-}HT_{2A}$ receptor-mediated behavioural syndrome, namely head and whole body shakes, reciprocal forepaw treading, lateral head weaving, flat body posture and hind limb abduction (Thorat et al., 2019).

Reddy et al. (2020) investigated the effects of ergovaline and ergotamine on blood pressure, heart rate (using non-invasive tail cuff plethysmography) and motor coordination (latency of falling using accelerating rotarod) after a single intraperitoneal administration of ergotamine (98% pure) at 0, 0.025 or 0.05 mg/kg bw or ergovaline (> 97% pure) at 0, 0.015 or 0.025 mg/kg bw in 1% lactic acid to groups of eight male C57Bl/6 J mice. According to the authors, these doses correspond to subclinical (low) and potent (high) doses calculated based on levels of feed intake of ruminants, concentrations in pasture and pharmacological

Table 1

LD_{50} values for some natural EAs following oral or intravenous administration (Griffith et al., 1978)

Substance	Species	Oral LD_{50} (mg/kg bw)	Intravenous LD_{50} (mg/kg bw)
Ergometrine	Mouse	460	160
	Rat	671	120
	Rabbit	27.8	3.2
Ergotamine	Mouse	3200	265
	Rat	1300	38
	Rabbit	550	3.0
Ergosine	Mouse	ND	33.5
	Rat	ND	30
	Rabbit	ND	1.23
Ergocristine	Mouse	ND	110
	Rat (male)	ND	64
	Rat (female)	ND	150
	Rabbit	ND	1.9
Ergocornine	Mouse	2000	275
	Rat	> 500	95
α-Ergocryptine	Mouse	ND	275
	Rat	ND	140
	Rabbit	ND	0.95
β- Ergocryptine	Mouse	ND	210
	Rat	ND	49
	Rabbit	ND	0.78
Ergocryptine (both isomers)	Mouse	870	300
	Rat	ND	58
	Rabbit	ND	0.34
Ergostine	Mouse	1700	125
	Rat	> 1000	47
	Rabbit	~ 1000	1.2
Ergonine	Rabbit	150	1.1
Ergostinine	Mouse	ND	180
	Rat	ND	180
	Rabbit	ND	5.3
Ergotoxine (mixture of ergocristine, ergocornine and ergocryptine)	Mouse	ND	90
	Rat	ND	76
Ergovaline	Mouse	ND	175
	Rat	ND	1.7

ND: not determined; number of animals tested not reported by Griffith et al. (1978).

aspects such as bioavailability of the toxins. All treatments (ergovaline high and low dose, ergotamine high and low dose) induced bradycardia and elevated systolic and diastolic blood pressures compared to those in the control mice. High and low doses of ergotamine led to sustained increases in blood pressure and reduced heart rate, which did not return to baseline during the 50-minute testing period. No significant impairment in motor coordination by accelerating rotarod test, 50 minutes post-treatment, was observed.

2.2.2 Short-term studies of toxicity

Griffith et al. (1978) identified repeated-dose toxicity studies in animals treated with ergot derivatives published between 1932 and 1976. Most of them reported on intravenous, intramuscular or subcutaneous administration in various species (cat, cock, dog, frog, guinea-pig, minipig, mouse, rabbit, rat, rhesus monkey and sheep). Only one short-term study was conducted on the effects of ergotamine tartrate administered in feed to eight mice (0.25 mg/kg diet) and four rats (1 mg/kg diet) for 2 months (Langecker, 1932). The original paper (in German) only provides a short description of the experiment. No symptoms were reported in the four rats and in six of the mice. One of the eight mice died at day 10 and another mouse showed alopecia. In other trials conducted for up to 90 days in mice, rats and guinea-pigs, typical symptoms of chronic ergot poisoning were observed, such as reduced body weight, gangrene on the atrium, abdominal wall and tail, as well as diarrhoea, incontinence, paresis of the rear extremities and alopecia.

(a) Mice

No other studies were identified.

(b) Rats

(i) Four-week studies

Ergotamine tartrate at concentrations of 0, 4, 20, 100 or 500 mg/kg diet was given to five groups of six Sprague-Dawley rats per group and sex for 4 weeks (Speijers et al., 1992). The ergotamine tartrate used for the experiment was > 98% pure. Its stability in the diet was tested during the study. At the beginning of the experiment, the specific-pathogen-free (SPF) Sprague-Dawley rats weighed 100–120 g. Urine and blood were collected at the end of the third week of exposure and after 4 weeks of exposure the animals were killed.

Clinical observations revealed effects of treatment only in the animals fed the highest concentration (500 mg ergotamine tartrate/kg diet). Redness of the tail tip was seen in all animals in this group, 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 in the groups fed 100 and 500 mg/kg diet. Slight changes in some haematological parameters were also seen in these two groups. Biochemical analyses showed elevated urea concentration (in animals fed 500 mg/kg diet) and decreased T4 (in animals fed 500 mg/kg diet) and thyroid-stimulating hormone (TSH) (100 and 500 mg/kg diet, in males only). A decrease in cholesterol was observed in the females in the high-concentration group. Urine production was increased in males at 100 and 500 mg/kg diet. In the females fed 20, 100 and 500 mg/kg diet, the relative weights of heart, brain and liver were higher than in the control animals. The relative ovary weights were increased in animals that received 100 and 500 mg/kg diet. In males fed 100 and 500 mg/kg diet the relative weights of heart and liver were increased. Macroscopic examination of controls and animals fed the high concentration was performed. In cases of increased histological findings in animals in the high-concentration group it was also performed on animals that received 100 mg/kg. Animals in the groups receiving 4 and 20 mg/kg diet were not examined. Gaseous distension of the duodenum, pale thyroids, red tail tips and enlarged iliac lymph nodes were observed in the two highest concentration groups. The histopathological examination revealed only a slight increase in regenerative and degenerative changes in the kidneys but strong activation of the iliac lymph nodes in the highest concentration group. The changes in the thyroids seen in the macroscopic inspection were not confirmed by microscopic examination. Degenerative changes in the longitudinal skeletal muscle of the tail were reported in animals of both sexes in the group that received the highest concentration of 500 mg/kg diet (four out of five males and three out of six females) and only in males in the group exposed to 100 mg/kg diet (two out of six males). The tails of animals in the 4 and 20 mg/kg diet groups were not examined.

Ergometrine maleate at concentrations of 0, 2, 10, 50 or 250 mg/kg diet was given to six groups of six Sprague-Dawley rats per group and sex for 4 weeks, equal to approximately 0.2 (range 0.13–0.28), 1 (0.25–1.45), 5 (3.15–6.59) and 25 (16.75–32.74) mg ergometrine maleate/kg bw per day (Peters-Volleberg, Beems & Speijers, 1996). Two control groups were included: one received the control diet *ad libitum*, and the other control group was pair-fed with the highest concentration group in terms of amount of feed provided, to determine any effects secondary to a decreased feed intake.

The ergometrine maleate used for the experiment was > 98% pure. Its homogeneity and stability in the diet was tested during the study. At the beginning of the experiment, the SPF Sprague-Dawley rats weighed 70–99 g. At week 4 of exposure, urine and blood were collected. After 4 weeks of exposure, the animals were killed.

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 increase. No treatment-related effects on haematological and kidney function parameters (creatinine or urea clearance) were seen.

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 from a limited number of animals and showed a wide inter-individual variation in all groups. The authors reported that the levels were markedly decreased in animals of both sexes in the 50 and 250 mg/kg diet groups (without statistical analysis). In females that received the high-concentration diet, relative organ weights of heart, liver and ovaries were increased. In male rats, macroscopic examination revealed a slight concentration-related increase in the incidence of enlarged mediastinal lymph nodes. Microscopic examination showed slight reactive hyperplasia in these enlarged lymph nodes. Treatment-related histopathological changes were observed in the liver of males and females fed 250 mg/kg diet, which showed significant evidence of increased glycogen storage.

α-Ergocryptine at concentrations of 0, 4, 20, 100 or 500 mg/kg diet was given to six groups of six Sprague-Dawley rats per group and sex for 28–32 days (Janssen et al., 1998, 2000a, b). Two control groups were included: one received the control diet *ad libitum*, and the other was pair-fed with the highest concentration group in terms of amount of feed provided.

The α-ergocryptine used for the experiment was > 99.9% pure. Its homogeneity in the diet was tested during the study. At the beginning of the experiment, SPF Sprague-Dawley rats were 23–26 days old. Females weighed on average 112–115 g and males 151–154 g.

The authors observed that the animals fed 20, 100 and 500 mg/kg diet were hyperactive (walking constantly in their cages) and had dirty fur. In addition, the animals in the 500 mg/kg diet group showed a hunched posture and were readily irritated and aggressive on handling. At week 4 of exposure, urine and blood were collected. After 4 weeks of exposure, the animals were killed.

Mean body weight, body weight gain, feed intake and feed effciency were decreased in both sexes in a non-monotonic manner (in the order 100 < 20 = 500 < control = 4 mg/kg diet). Significant changes in animals that received concentrations higher than 4 mg/kg diet were observed in some haematological parameters (decreased mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH)), serum enzyme activities (slightly increased/decreased alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and γ-glutamyl transferase (GGT)), serum urea concentrations (increased), glomular filtration (decreased creatinine and urea clearances). U-shaped changes

were observed for some parameters, which might be caused by the U-shaped concentration–response relationship for feed intake, due 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 due to the activation in the forebrain of behaviours incompatible with feeding (Janssen et al., 1998).

Relative organ weights were increased (brain, thyroid, heart, liver, kidney, adrenals, testis and ovary) or decreased (thymus, uterus). For some organs, the changes were significant in the animals fed 20 and/or 100 mg/kg diet but not those given 500 mg/kg diet. Most of the changes are considered as indirect effects of decreased feed intake.

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). In the tail, the muscular degeneration was characterized by vacuolation, increased eosinophilia, thinning of muscle fibres and necrosis of individual muscle fibres (Table 2). There was only a minimal inflammatory reaction. The degenerative muscular changes were restricted to certain muscle bundles, adjacent to the vertebrae, whereas other muscles of the tail appeared to be unaffected. The changes are assumed to be due to the vasoconstrictive properties of ergocryptine.

In a second article (Janssen et al., 2000b), the authors reported the metabolism and hormonal changes observed in the animals (plasma and serum). Ergocryptine influenced carbohydrate metabolism and affected thyroid and pituitary function. Total cholesterol and high-density lipoprotein (HDL)-cholesterol were decreased in females fed 20, 100 and 500 mg/kg diet but the ratio of HDL-cholesterol/total cholesterol was only decreased in animals fed 20 mg/kg diet. In males, only HDL-cholesterol at 20 mg/kg diet was significantly decreased. Triglycerides and glucose concentrations were decreased in animals of both sexes in the highest concentration groups. Insulin and glucagon were not changed at week 4 but had increased in the highest concentration group at the end of the study, when the animals were allowed to eat prior to blood sampling and necropsy. Prolactin was decreased in animals of both sexes in the 20, 100 and 500 mg/kg diet groups (Table 3). T4 was decreased in females fed 500 mg/kg diet and in males fed 20, 100 and 500 mg/kg diet. TSH was decreased in males fed 500 mg/kg diet. Follicle-stimulating hormone (FSH) was concentration-dependently decreased in females but, owing to wide variations, the difference was significant only for animals in the 500 mg/kg diet group. In males, FSH was increased but was significant only in the 20 mg/kg diet group. Luteinizing hormone (LH) was increased in males fed 20, 100 and 500 mg/kg diet.

Table 2

Microscopic findings in the tail of rats exposed to α-ergocryptine for 4 weeks (incidence report) (Janssen et al., 2000a)

	Females						Males					
	0 mg/kg diet	4 mg/kg diet	20 mg/kg diet	100 mg/kg diet	500 mg/kg diet	0pf	0 mg/kg diet	4 mg/kg diet	20 mg/kg diet	100 mg/kg diet	500 mg/kg diet	0pf
Animals examined	6	6	6	5	6	6	6	6	6	6	6	6
Focal muscular degeneration	0	0	0	0	4	1	0	0	0	2	6	0
Minimal	0	0	0	0	1	1	0	0	0	2	1	0
Slight	0	0	0	0	2	0	0	0	0	0	4	0
Moderate	0	0	0	0	1	0	0	0	0	0	1	0

pf: pair-fed.

Table 3

Prolactin and T4 levels of rats exposed to α-ergocryptine for 4 weeks (Janssen et al., 2000b)

	Females						Males					
	0 mg/kg diet	4 mg/kg diet	20 mg/kg diet	100 mg/kg diet	500 mg/kg diet	0pf	0 mg/kg diet	4 mg/kg diet	20 mg/kg diet	100 mg/kg diet	500 mg/kg diet	0pf
Prolactin (µg/L)	33±9	27±15	6±3[a]	11±14[a]	4±2[ab]	20±13	9±3	7±3	5±6[a]	2±1[a]	2±1[ab]	11±4
T4 (nM, week 4)	51±10	50±16	41±5	36±10	27±11[a]	43±13	61±4	62±15	35±7[a]	29±4[a]	35±7[ab]	55±9
T4 (nM, autopsy)	42±8	42±5	37±8	46±11	22±4 [ab]	40±16	59±8	59±4	37±4[a]	35±4[a]	37±8[ab]	56±12
Free T4 (pM, autopsy)	12±2	11±2	9±2	10±4	11±11	12±2	14±1	15±1	9±1[a]	8±1[a]	9±3[ab]	13±2

pf: pair-fed with the highest concentration group.
[a] Groups significantly different from the control group ($P<0.05$) according to Dunnett.
[b] Groups significantly different from the pair-fed control with the highest concentration group ($P<0.05$) according to Dunnett.

Serum T4 concentrations were also found to be reduced in subacute toxicity studies with ergotamine (Speijers et al., 1992) and ergometrine (Peters-Volleberg, Beems & Speijers, 1996).

(ii) 13-week study

Four groups of 10 Sprague-Dawley rats per group and sex were fed ergotamine tartrate (EAT) at concentrations of 0, 5, 20 or 80 mg/kg diet and observed for

13 weeks (Speijers et al., 1993). The concentrations and the parameters were selected based on an earlier subacute toxicity study (Speijers et al., 1992).

The EAT used for the experiment was > 98% pure. Its stability and the homogeneity in the diet were tested during the study. At the beginning of the experiment, the SPF Sprague-Dawley rats weighed 50–70 g (and were approximately 4 weeks old). Feed and water intakes were measured twice a week. Body weight gain was recorded weekly. After 7 weeks and 12 weeks, urine and serum were sampled to measure the kidney function. After 10 weeks, blood was sampled for haematological examination. At the end of the experiment, blood and a small portion of the liver were sampled for biochemical analyses. After necropsy, organ weights were recorded, and tissues were fixed for a complete histopathological examination.

After 13 weeks, both body weight gain and feed intake were significantly decreased in female rats (not in male rats) fed the high concentration of 80 mg EAT/kg diet compared to the controls.

The haematological examinations showed a signifiant increase in packed cell volume and erythrocyte (RBC) count in the animals (both males and females) in the high-concentration group (80 mg EAT/kg diet) only. The other blood parameters were not significantly different from those of the controls (haemoglobin concentration (Hb), white blood cells (WBC), thrombocytes (PLT), MCV and MCH concentration (MCHC)).

Biochemical examination showed increased alkaline phosphatase activity, LDL-cholesterol and decreased glucose concentrations in the serum of female rats in the high-concentration group (but not in male rats) after both 12 and 13 weeks. Urine analyses revealed increased urine volume in the females fed the high (80 mg EAT/kg diet) and low (5 mg EAT/kg diet) concentration diet after 12 weeks. A significant decrease in the urine volume was observed in the males in the high-concentration group. No effects were seen on the other urine parameters (creatinine concentration and clearance, or osmolarity). Relative spleen and brain weights in the females in the group fed the high concentration were significantly higher than in the controls and relative pituitary weight was significantly lower in the high-concentration group (but not in male rats).

Macroscopic examination revealed pale thyroids in animals (9/10 females and 3/10 males) in the high-concentration group and, in males, haemorrhagic changes were seen in the lymph nodes (2/10). No changes in the thyroids were observed in the microscopic examination. The only treatment-related histopathology finding was muscular atrophy in the caudal longitudinal muscles of the tail of animals in the high-concentration group (0/10 females and 1/10 males in the control group and the group exposed to 5 mg EAT/kg diet, 2/10 females and 1/10 males in the group exposed to 20 mg EAT/kg diet, and 7/10 females and 7/10 males the group exposed to 80 mg EAT/kg diet) (Table 4). This

Table 4
Microscopic findings in the tail of rats exposed to ergotamine tartrate (EAT) in the diet for 13 weeks (incidence report) (Speijers et al., 1993)

	0 mg EAT/kg		5 mg EAT/kg		20 mg EAT/kg		80 mg EAT/kg	
	f	m	f	m	f	m	f	m
Animals examined	10	10	10	10	10	10	10	10
No abnormality detected	10	9	10	9	8	9	3	3
Muscular atrophy: slight	0	0	0	1	2	0	0	0
Muscular atrophy: moderate	0	1	0	0	0	1	4	4
Muscular atrophy: severe	0	0	0	0	0	0	3	3

f, female: m, male.

atrophy consisted of partial or total disappearance of fibres, tinctorial changes and fibrosis. The low incidence in the control and lower concentration group is considered by the authors as a background level. In addition to the atrophy in animals 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.

Ghanem et al. (2005, 2006) exposed groups of three male albino rats to EAT (purity not mentioned) by gavage for 13 weeks. Doses per body weight were stated as being close to the doses used in human treatment (0, 0.03, 0.06, 0.09 or 1.25 mg in 0.5 mL, 1.5 mL or 3 mL of solution, respectively). The rats were 12 weeks old and weighed 250 g at the beginning of the experiment. The strain of rats is not stated. During the study, the rats did not show any marked abnormal behaviour or signs of illness. Slight changes in haematological parameters were observed. Histopathological examination showed damage to the kidney and the liver with a dose–response relationship. In the kidneys of animals that received the low dose, slight changes in the interstitium and glomeruli, and inflammation were observed. At the highest dose, destruction of the glomerular structure and Bowman's capsule was seen. In the liver of animals given the low dose, minor changes and a normal structural pattern was observed. Necrosis of the hepatic cells and degeneration of the cytoplasm were observed at the dose of 0.06 mg. At the highest dose, the changes were severe, and the normal pattern of hepatocytes and architecture of the liver tissue disappeared. The epithelium lining the central vein disappeared completely; necrosis and inflammation of hepatic tissue was obvious. Blood vessels were damaged: infiltration of macrophages and other

inflammatory cells and occlusions of some of the vessels were observed. Sinusoids were dilated. Necrosis was also observed in the vicinity of the portal tract area with disintegration of hepatic cords and hepatocytes. The Committee noted that similar histopathological findings were not observed in other studies with higher doses.

(c) Pigs

Three groups of 24 weaned piglets were exposed for 28 days to control feed or feed contaminated with 1.2 or 2.5 g of sclerotia/kg (i.e. at concentrations close to EU regulatory limits in feed) (Maruo et al., 2018). Based on the mean feed intake (1163 and 962 g/day after 2 and 4 weeks of exposure, respectively) and body weight (17.5 and 27.8 kg after 2 and 4 weeks of exposure, respectively), 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.

After preparation, diets were analysed four times and the mean concentrations of EAs were 2.36 and 5.05 mg/kg, for diets with ergot concentrations of 1.2 or 2.5 g of sclerotia/kg, respectively (Table 5). The most abundant EA was ergotamine, followed by ergosine, ergocristine and their corresponding -inine epimers.

Contamination with other mycotoxins was investigated. Deoxynivalenol (DON) was naturally present in wheat (19 µg/kg) and corn (171 µg/kg) but the amounts were insignificant. The amounts of other mycotoxins, including deoxynivalenol (DON) acetylated, nivalenol, T-2, HT-2, zearalenone and fumonisin were below the detection limit.

Seventy-two castrated, 21-day-old crossbreed (P76 X Naïma) piglets (mean weight 10.7 ± 0.9 kg) were housed in the facility with free access to control starter feed and water. When they were 34 days old, they were housed in individual pens and assigned to one of three groups of 12 males and 12 females. The first group was fed the control diet, the second the ergot concentration 1 diet and the third the ergot concentration 2 diet. Animals received the experimental diets for 28 days. Weight and feed consumption were measured on the first day of the experiment, 14 days later and on the last day of the experiment. At the end of the experiment (when the piglets were 62 days old) the mean weights were 27.9 ± 3.8 kg, 27.8 ± 2.6 kg, 25.6 ± 3.1 kg for animals in the control group, ergot concentration 1 and ergot concentration 2 diet groups, respectively. The animals were observed daily to detect possible signs of ergot intoxication such as balance disorders or necrosis of the extremities. At the end of the experiment, blood samples were taken from the external jugular vein of all the animals for biochemical and haematological analyses. In addition, six male animals from

Table 5
Content of EAs in experimental feed in a study of pigs by Maruo et al. (2018)

	Contamination of the diet (g of sclerotia/kg diet)	
Ergot alkaloids (mg/kg diet)	**1.2**	**2.5**
Ergotamine	0.52	1.03
Ergotaminine	0.24	0.58
Ergosine	0.29	0.58
Ergosinine	0.16	0.34
Ergocristine	0.26	0.47
Ergocristinine	0.18	0.40
Ergometrine	0.17	0.44
Ergometrinine	0.06	0.12
Ergocornine	0.15	0.30
Ergocorninine	0.11	0.29
Ergocryptine	0.13	0.30
Ergocryptinine	0.09	0.21
Total alkaloids	**2.36**	**5.05**

each group were euthanized and liver, jejunum and jejunum with Peyer's patches were sampled for histopathological analysis.

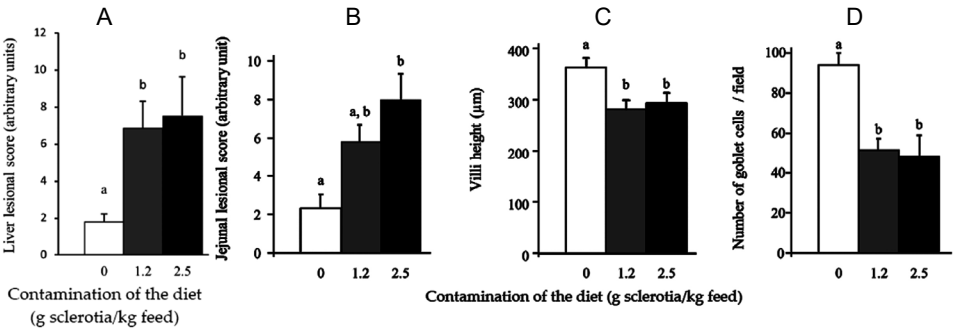
Assessment of clinical signs revealed no typical symptoms of acute toxicity, such as convulsions or muscle spasms, or necrosis of the extremities. Similarly, no vomiting or fever was observed in animals exposed to EAs.

The effects of EAs on feed intake were detected during the second week in animals exposed to the highest concentration. For animals exposed to the lower concentration of ergot, this reduction only appeared in the last 14 days of treatment during the experimental period. The daily feed intake of animals exposed to the higher concentration of EAs was reduced by about 18% in comparison with the control group. The reduction in feed ingestion led to a decrease of animal weight gain, which was significant in the group exposed to the higher concentration of EAs.

Haematology and serum biochemical analysis performed at the end of the experiment found that only a few parameters were affected. The percentage of neutrophils was reduced and the decrease was significant in the group exposed to the lower concentration, but not in the group exposed to the higher concentration. Animals exposed to EAs showed an increase in the percentage of lymphocytes and the difference was again significant in animals exposed to the lower concentration, but not in the group exposed to the higher concentration. The percentage of haematocrit was significantly increased in animals in the high-

Fig. 2

(A) Liver lesion score. Values are means with the standard errors of the mean represented by vertical bars ($n = 6$ animals). Mean values with different letters are significantly different ($P < 0.05$). (B) Jejunal lesion score; (C) villi height; (D) number of goblet cells.[a]



[a] Values are means of the score, villi height and number of goblet cells/field respectively, with standard errors of the mean represented by vertical bars ($n = 6$ animals).
Source: Maruo et al. (2018).

concentration group. Biochemical analysis showed a significant concentration-dependent reduction in the levels of creatine kinase in animals exposed to EAs, but the values were within the normal range for this species. In addition, the level of cholesterol decreased, but the difference was significant only in the group exposed to the lower concentration, and glucose levels increased upon exposure to EAs, but the difference was significant only in the group exposed to the higher concentration. The level of bilirubin was decreased in the group exposed to the higher concentration.

The effects of EAs on the liver and the intestine were investigated through histological analyses. Exposure to EAs 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. Furthermore, animals fed the contaminated diets exhibited a significant increase in the liver lesion score (Fig. 2).

The main histological changes observed in the jejunum were atrophy of the villi, oedema of the lamina propria and cytoplasmic vacuolation of enterocytes. Animals exposed to the higher concentration of EAs displayed a significant increase of the lesion score in the jejunum compared to control animals (3.4-fold increase, $P < 0.005$). Morphometrical analysis revealed a significant decrease in height of the villi (1.2-fold decrease at both concentrations). The number of goblet cells decreased significantly in the jejunum (mean 1.6-fold decrease,

$P < 0.001$) of piglets exposed to EAs. Similar effects were observed in jejunum areas with Peyer's patches (Maruo et al., 2018).

The effects of EAs on mRNA expression in the jejunum were also studied. The expression of 34 genes coding for junctional proteins, inflammatory and immunological mediators was evaluated by real-time quantitative polymerase chain reaction (RT-qPCR). The profile of mRNA expression of different toll-like receptors and cytokines (TLR4, nuclear factor kappa B (NFkB), interleukin (IL)-6, IL-8 and tumour necrosis factor-α) showed a tendency to downregulation. In animals exposed to the higher concentration of ergot, analysis of mRNA expression of junctional proteins revealed a significant increase in claudin-3 (CLDN3), claudin-4 (CLDN4), occludin (OCLN), zonula occludens-1 (ZO-1), junctional adhesion molecule (JAM-A) and E-cadherin (ECAD). The expression of genes coding for mucin-1 (MUC1), alkaline phosphatase (ALP) and proliferating cell nuclear antigen was also significantly increased. The overexpression of the genes involved in maintaining the structure of the intestinal mucosa may reflect an attempt by the tissue to re-establish its function.

Oresanya et al. (2003) investigated the effect of EAs on growth performance and clinical symptoms in weaned pigs. Wheat ergot sclerotia (1880 mg alkaloid/kg – ergocristine (755 mg/kg), ergotamine (680 mg/kg), ergosine (205 mg/kg), ergocryptine (130 mg/kg), and ergocornine (110 mg/kg)) were added to a basal diet at 0, 0.05, 0.10, 0.25, 0.50 and 1.00% (corresponding to dietary concentrations of total alkaloids of 0, 1.04, 2.07, 5.21, 10.41 and 20.82 mg/kg respectively) and fed to 192 weaned pigs (20.4 ± 3.4 days; 6.9 ± 1.3 kg), 32 pigs per concentration, for 28 days, beginning 7 days post-weaning. Pigs fed the 1.00% diet gained 82 and 38% less weight than the controls at 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 first 14 days. EAs significantly decreased serum prolactin and urea nitrogen concentrations measured on day 28. The Committee noted that there was no concentration–response relationship in the prolactin reduction. The authors identified maximum tolerable ergot levels in the diet of 0.10 and 0.05% based on average daily weight gain and average daily feed intake, respectively, corresponding to 2.07 mg and 1.04 mg EAs/kg diet.

Ten weaned 28-day-old Lacombe × Yorkshire × Landrace pigs were arbitrarily paired, one male with one female, and fed one of five experimental diets (0.0, 0.225, 0.45, 0.9 or 1.8 g ergot sclerotia/kg diet) *ad libitum* for 50 days (Dignean, Schiefer & Blair, 1986). Ergot sclerotia from barley contained 2.27 g alkaloid/kg sclerotia: ergocristine 48%, ergotamine 18%, ergocristinine 9.5%, ergocryptine 8%, ergometrine 6%, ergosine 4% and ergocornine 3%. Blood was collected every 14 days. At the end of the trial, the pigs were necropsied. Tissue samples were taken from liver, kidney, heart, adrenal gland, spleen, lung, thymus,

brain, thyroid, duodenum, ileum, mesenteric lymph nodes, pancreas, ovaries and peripheral skin of the ear margin and coronary band.

Pigs fed the highest concentration of ergot were less efficient at conversion of feed to weight gain in spite of greater feed consumption, and animals on the higher levels of ergot were observed to be much more excitable and difficult to restrain than control animals after 3 weeks of being fed the experimental diet. However, no other clinical signs were observed. Haematological evaluation revealed only occasional leukopenia and monocytosis. Hepatic enzymes and blood urea nitrogen values varied considerably within the normal range but showed no correlation with ergot concentration. No gross lesions were present in any pigs. Histopathological changes were confined to the liver, kidney and spleen. Hepatic changes were most obvious: in ergot-fed animals, periportal hepatocytes were swollen with only a little stained cytoplasm evident peripherally. Nuclei varied in size and in staining intensity. As many as four rows of cells on each side of the portal area were affected. The remaining cells had granular cytoplasm, and there was moderate centrilobular (or periacinar) vacuolation identified by oil-red-O staining as lipid deposition. In animals fed the higher concentrations of ergot, bile ducts were prominent and ductules were more numerous, suggesting hyperplasia. The extent of these alterations reflected the ergot concentration. Cellular vacuolation and cytoplasmic disruption were also evident in renal tubular epithelium. There was also some attenuation of tubular epithelium associated with tubule dilation. Hyaline droplets were evident in Bowman's space, and there was more lipid within tubules than in control sections. Again, the severity was associated with the level of ergot in the diet. Splenic differences were not as readily quantitated, but pigs fed high ergot concentrations had proportionally less white pulp than red pulp and fewer distinct follicles with mature lymphocytes.

2.2.3 Long-term studies of toxicity and carcinogenicity

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 (Fitzhugh, Nelson & Calvery, 1944), three series of experiments were conducted with Osborne-Mendel rats (3 weeks of age) fed powdered crude ergot (composition not known) in a high protein diet composed of cornstarch 72%, casein 6%, corn oil 6%, brewer's yeast 5%, whole liver powder 5%, salt mixture 4% and cod liver oil 2%.

In the first experiment, groups of 20 female rats received a diet with 0, 1, 2 or 5% crude ergot for 6 months. In the second experiment, groups of nine male and nine female rats received a low protein diet with 0, 1, 2 or 5% crude ergot for 6 months. Since it had been learned from the first experiment that some weanling

rats refused to eat the feed containing 5% ergot, this group was started at a lower dosage (2%) and the level was increased to 5% after 2 weeks.

In these two experiments, the body weight gain was significantly reduced at week 15 in the groups fed 5% ergot compared to the controls. In the low protein experiment, the reduction was more severe in males than in females. For the late growth gain, no significant difference was observed. A few animals died (exact number not known). The authors only mentioned that nine rats survived in the groups fed 5% ergot.

In the third experiment, groups of eight male and eight female rats received a low protein diet that included 0 or 5% crude ergot, 5% defatted ergot, 5% ergot oil or ergotoxine ethanesulfonate at a dose equivalent to 5% ergot for at least 1 year and up to 2 years. After 6 months, the diet was changed – the whole liver powder was eliminated and the starch and casein were increased to 2% and 3%, respectively. The body weight gain was significantly reduced at week 15 in the groups fed 5% crude and defatted ergot compared to the controls. In both groups, the males grew more slowly than the females. Males in the ergotoxine group were also significantly smaller than the controls. A large number of the rats from the groups on diets containing whole ergot and defatted ergot died (exact number not known). At the end of the year, animals in the groups fed diets containing ergotoxine were not significantly different from the controls.

The pathological changes observed were classified into three categories: i) those observed only in the treated animals and not present in the controls, ii) those present to some extent in the controls but to a greater extent in the treated animals, and iii) those caused by inadequacies in certain of the diets and present equally in control and treated animals.

The authors observed three types of lesions in the first category: 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 second category of lesions included calcified renal tubular walls and tubular casts, most numerous around the corticomedullary junction, and focal hyperplasia of the squamous epithelium of the proventriculus (forestomach).

The tumours of the ears were all neurofibromas of a uniform gross and microscopic appearance. They were usually multiple, with individual tumours as large as 1.2 cm in diameter, although the usual diameter was from 4 to 6 mm. In two animals, these tumours were also observed in the tail. 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 restarted.

Table 6
Number of rats with ear tumours (Fitzhugh, Nelson & Calvery, 1944)

Groups	Number of rats in the group	Number of rats with ear tumours
Long-term experiment – low protein diet plus apparent vitamin E deficiency		
5% crude ergot	16	6
5% defatted ergot	16	3
5% ergot oil	16	0
5% ergotoxine	16	1
Control	16	0
6 months – low protein diet		
5% crude ergot	18	17
2% crude ergot	18	8
1% crude ergot	18	2
Control	18	0
6 months – high protein diet		
5% crude ergot	20	6[a]
2% crude ergot	20	3
1% crude ergot	20	0
Control	20	0

[a] Only six rats from this group were studied microscopically; the small size of this group is also due to the death of many rats before the end of the experiment.

2.2.4 Genotoxicity

Data from in vitro and in vivo genotoxicity studies are available only for a limited number of naturally occurring EAs or their salts (Tables 7 and 8). Ergometrine tartrate showed no mutagenic potential in vitro in *Salmonella* Typhimurium strains (Zeiger et al., 1987). α-Ergocryptine did not induce sister chromatid exchange in Chinese hamster ovary cells (Dighe & Vaidya, 1988). Agroclavine (a precursor compound) showed a weak mutagenic response when activated with rat liver S9 in *S.* Typhimurium strains (Glatt et al., 1987). However, ergometrine maleate and ergotamine tartrate induced chromosomal damage in human leukocytes in vitro (Jarvik & Kato, 1968; Roberts & Rand, 1977a; Dighe & Vaidya, 1988). Ergotamine tartrate and ergometrine maleate induced sister chromatid exchange in Chinese hamster ovary cells (Dighe & Vaidya, 1988). Semi-synthetic dihydrogenated derivatives (dihydroergocristine and α-dihydroergocryptine) also provided negative results in *S.* Typhimurium strains (Dubini et al., 1990; Adams et al., 1993).

In vivo, ergotamine tartrate showed no genotoxic potential in the micronucleus test after intraperitoneal injection in mice and Chinese hamster (Matter, 1976) and provided negative results in the dominant lethal test after intraperitoneal injection in mice (Roberts & Rand, 1978; Matter, 1982). Semi-synthetic dihydrogenated derivatives (dihydroergocristine and α-dihydroergocryptine)

Table 7
Results of in vitro assays for genotoxicity with EAs

Test system	Test object	Concentration and test substance	Results	Reference
Reverse mutation	*Salmonella* Typhimurium TA98, TA100 and TA1537	Agroclavine, up to 2 µM per plate (in DMSO)	−S9: negative +S9: weak positive	Glatt et al. (1987)
Reverse mutation	*Escherichia coli* (WP2 uvrA)	Agroclavine, up to 2 µM per plate (in DMSO)	Effects were extremely weak and quantification was not possible	Glatt et al. (1987)
Reverse mutation	*Salmonella* Typhimurium TA98, TA100, TA1535 and TA1537	3.3, 10, 33, 100, 333, 667, 1000 or 2000 µg/plate ergometrine tartrate (in DMSO)	Negative ± S9	Zeiger et al. (1987)
Reverse mutation	*Salmonella* Typhimurium TA98, TA100, TA1535, TA1537 and TA1538	Dihydroergocristine at 10 000 µg/plate on TA98 and TA1538 strains and at 3000 µg/plate on TA1535, TA1537 and TA100	Negative ± S9	Dubini et al. (1990)
Reverse mutation	*Salmonella* Typhimurium TA98, TA100, TA1535, TA1537 and TA1538	α-dihydroergocryptine at 50 to 5000 µg/plate	Negative ± S9	Adams et al. (1993)
		The chromosome aberration assay was conducted with Chinese hamster ovary K1-BH4 cells with and without S9, at 3 to 120 µg/mL. The DNA repair test was conducted in rat hepatocytes, at 0.01 to 100 µg/plate		
Mutation	V79 Chinese hamster cells	Dihydroergocristine between 0.3 and 30 µg/mL	Negative ± S9	Dubini et al. (1990)
Mutation	Mouse lymphoma L5178Y TK+/− cells	α-dihydroergocryptine at 20 to 100 µg/mL	Negative ± S9	Adams et al. (1993)
Sister chromatid exchange	Human lymphocytes	0.1, 0.25 and 0.5 µg/mL of dihydroergotoxine[a] mesylate	Negative	Tsuchimoto, Matter & Deyssenroth (1979)
Sister chromatid exchange	Chinese hamster ovary cells	10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M of ergotamine tartrate	Positive	Dighe & Vaidya (1988)
		10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M of ergonovine maleate	Positive	
		10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M of methylergonovine maleate	Positive	
		Ergocristine only at 10^{-8} M	Positive	
		10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M of α-ergocryptine	Negative	
Chromosomal aberration	Human leukocytes	0.1 µg/mL of ergometrine maleate	Positive	Jarvik & Kato (1968)
Chromosomal aberration	Human lymphocytes	0.1, 0.25 and 0.5 µg/mL of ergotamine tartrate	Positive	Roberts & Rand (1977a)
		0.1, 0.25 and 0.5 µg/mL of dihydroergotoxine[a] mesylate	Positive	
Chromosomal aberration	Human lymphocytes	0.1, 0.25 and 0.5 µg/mL of dihydroergotoxine[a] mesylate	Negative	Tsuchimoto, Matter & Deyssenroth (1979)

Table 7 (continued)

Test system	Test object	Concentration and test substance	Results	Reference
Chromosomal aberration	Human lymphocytes	1, 3 and 10 µg/mL of dihydroergocristine	Negative	Dubini et al. (1990)
Chromosomal aberration	Chinese hamster ovary K1-BH4 cells	α-dihydroergocryptine at 3 to 120 µg/mL. The DNA repair test was conducted in rat hepatocytes, at 0.01 to 100 µg/plate	Negative ± S9	Adams et al. (1993)
Unscheduled DNA synthesis	Rat hepatocytes	α-dihydroergocryptine at 0.01 to 100 µg/plate	Negative	Adams et al. (1993)
Reporter assay	Human lymphoblastoid TK6 cells	up to 1 mM of dihydroergotamine	Negative	Hastwell et al. (2009)

DMSO, dimethyl sulfoxide.
[a] Hydergine, an ergot derivative consisting of equal amounts of dihydroergocornine, dihydroergocristine and dihydroergocryptine.

Table 8
Results of in vivo assays for genotoxicity with EAs

Test system	Test object	Concentration and test substance	Results	Reference
Chromosomal aberration	Mouse bone marrow	25, 50 or 100 mg/kg bw of dihydroergotoxine[a] mesylate intraperitoneally	Positive	Roberts & Rand (1977b)
		25, 50 or 100 mg/kg bw of ergotamine tartrate intraperitoneally	Positive	
Germinal mutations	Dominant lethal test in mice	25, 50 or 100 mg/kg bw of dihydroergotoxine[a] mesylate intraperitoneally	Positive	Roberts & Rand (1978)
		25, 50 or 100 mg/kg bw of ergotamine tartrate intraperitoneally	Negative	
Micronucleus formation	Mouse bone marrow	Dihydroergotoxine[a] mesylate given twice intraperitoneally with an interval of 24 hours at various doses up to 200 mg/kg bw	Negative	Matter & Grauwiler (1975)
Micronucleus formation	Chinese hamster bone marrow	Dihydroergotoxine[a] mesylate given twice intraperitoneally with an interval of 24 hours at various doses up to 200 mg/kg bw	Negative	Matter & Grauwiler (1975)
Micronucleus formation	Mouse bone marrow	Ergotamine tartrate given twice intraperitoneally with an interval of 24 hours at various doses up to 300 mg/kg bw	Negative	Matter (1976)
Micronucleus formation	Chinese hamster bone marrow	Ergotamine tartrate given twice intraperitoneally with an interval of 24 hours at various doses up to 300 mg/kg bw	Negative	Matter (1976)
Micronucleus formation	Mouse bone marrow	Dihydroergocristine at two doses (50% and 16% of LD_{50}) orally	Negative	Dubini et al. (1990)
Micronucleus formation	Mouse bone marrow	α-dihydroergocryptine at 2.8 g/kg (the maximum tolerable dose in a preliminary study) orally by gavage	Negative	Adams et al. (1993)
Chromosome damage	Dominant lethal test in mice	Ergotamine tartrate at 125 mg/kg bw intraperitoneally	Negative	Matter (1982)
Chromosome damage	Human lymphocytes	8 and 12 weeks after oral intake by 12 healthy adult male volunteers three times per day of 1.5 mg of dihydroergotoxine[a] mesylate	Negative	Tsuchimoto & Stalder (1976)

[a] Hydergine, an ergot derivative consisting of equal amounts of dihydroergocornine, dihydroergocristine and dihydroergocryptine.

also gave negative results in the in vivo mouse micronucleus assay after oral administration (Dubini et al., 1990; Adams et al., 1993). However, ergotamine tartrate was reported to induce a significant number of chromosomal aberrations in bone marrow cells after intraperitoneal injection in mice (Roberts & Rand, 1977b). The semi-synthetic dihydrogenated derivative (dihydroergotoxine mesylate) was also positive in this assay (Roberts & Rand, 1977b) but negative in the micronucleus test after intraperitoneal injection in mice and Chinese hamster (Matter & Grauwiler, 1975; Matter, 1976).

Using a combination of quantitative structure activity relationship (QSAR) platforms (i.e. VEGA, LAZAR, T.E.S.T. and OECD QSAR Toolbox), the mutagenic and carcinogenic potential for various EAs were estimated (Glück et al., 2018). Ergotamine, ergotaminine, ergocristine, ergocristinine, ergosine, ergosinine, ergocornine, ergocorninine, α- and β-ergocryptine, and α- and β-ergocryptinine would be classified as non-mutagenic substances (low probability for mutagenicity). Ergometrinine would be classified as mutagenic with low reliability.

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

2.2.5 Reproductive and developmental toxicity

In animals, EAs induce effects on ovulation, implantation, early pregnancy, embryonic and fetal development resulting in abortion, high neonatal mortality, fetal malformations and growth retardation (Carlsen, Zeilmaker & Shelesnyak, 1961; Deanesly, 1968; Carpent & Desclin, 1969; Mantle, 1969; Floss, Cassady & Robbers, 1973; Grauwiler & Schön, 1973; Schön, Leist & Grauwiler, 1975; Griffith et al., 1978; Holstege & Traven, 2014). The effects vary according to the EA (Griffith et al., 1978).

One of the ways in which EAs can affect reproduction in animals is by reducing the secretion of prolactin from the pituitary. The release of prolactin from the pituitary is normally inhibited by dopamine from neurons in the hypothalamus and the activity of these dopaminergic neurons, in turn, is regulated by prolactin itself, operating in a short-loop negative feedback system. EAs reduce prolactin secretion from the pituitary through their activity as dopamine agonists.

In rodents, such as the mouse, rat and hamster, prolactin originates almost exclusively in the pituitary. In these species, the maintenance of pregnancy after mating is critically dependent on release of pituitary prolactin, which maintains the activity of the corpora lutea that secrete progesterone, allowing implantation to occur. EAs, by inhibiting prolactin release, can cause loss of implants and termination of pregnancy in rodents. However, in species such as humans, other

primates and guinea-pigs, prolactin is not involved in maintenance of the corpus luteum in early pregnancy. Rather, the corpus luteum is maintained initially by LH from the pituitary, then, from the time of implantation, by human chorionic gonadotrophin (hCG) produced by the early trophoblast. In humans, prolactin levels increase steadily from about week 20 of gestation onwards and prolactin is clearly involved in promoting growth of the mammary glands and in release of breast milk during suckling. With respect to lactation, the function of prolactin in humans is similar to that in rodents. Unlike rodents, however, humans also have numerous extrapituitary sites of production of prolactin, where it is regulated locally by transcriptional mechanisms rather than by dopamine (Ben-Jonathan, LaPensee & LaPensee, 2008).

(a) Mice

Carlsen, Zeilmaker & Shelesnyak (1961) studied the effect of a single subcutaneous injection of ergocornine methanesulfonate on early pregnancy in mice. The animals studied were 3- to 4-month-old hybrid mice (♀C57B1 × ♂CBA)F1, weighing 22 to 24 g. In the first experiment, 16 pregnant mice were divided into groups of four and given a single injection of 0.05, 0.1, 0.25 or 0.5 mg ergocornine methanesulfonate in ethanol. A group of eight mice served as untreated controls to confirm the fertility index. Ergocornine methanesulfonate had no effect at 0.05 mg, but terminated pregnancy in 50% of the mice at a dose of 0.1 mg and 100% of the mice given doses of 0.25 and 0.5 mg. In the second experiment, 32 mice in groups of four were given a single injection of 0.25 mg ergocornine methanesulfonate from day 0 to day 7 of gestation. Pregnancy was terminated in all the mice treated on days 3 or 4. Following treatment on all other days, the animals delivered normal litters. No evidence of any abnormalities among the offspring was observed.

Mantle (1969) conducted a series of experiments with female BS/VS mice fed with 2% sclerotia of *Claviceps fusiformis* in the diet or with agroclavine added to the diet at 0.5 mg/kg. The total alkaloid content of the sclerotia was 0.3% expressed as agroclavine (the major alkaloidal components were agroclavine and elymoclavine and the minor components were penniclavine, setoclavine and chanoclavine). At the end of a 20-day period of dietary exposure to the sclerotia, none of the five female mice in the treated group became pregnant whereas all the female mice in the control group did. In another experiment with mice fed 0.5 mg ergoclavine/kg diet, the author observed that the most effective period of treatment by the oral route was during the 2 to 3 days before implantation. A daily intake of 250 µg agroclavine on days 3 and 4 was found to terminate pregnancy without inducing any signs of toxicity. Interruption of pregnancy was also observed after a daily intake of 250 µg ergosine:ergosinine (60:40) on days 3,

4 and 5. Other experiments with female mice exposed to agroclavine in the diet for a few days or 8 weeks showed that they were able to have a normal pregnancy when they returned to a normal diet. These results suggest that ergoclavine prevents implantation and that the effects do not last beyond the end of the exposure period. Agroclavine had no effects on conception.

Fertility

Matter, Tsuchimoto & Deyssenroth (1978) found no effects of dihydroergotoxine mesylate in the total reproductive capacity test in female mice. Forty-nine 8-week-old virgin female CD mice were injected intraperitoneally with 250 mg/kg bw of dihydroergotoxine mesylate and another 49 with the solvent only. Six hours after treatment, each female was caged permanently for 347 days with an untreated male of the same strain, aged 8 weeks at first mating. Breeding pens were checked 5 days a week for the presence of newborns. These were culled immediately after being scored for litter size to maximize the number of births for each mother. No effects of dihydroergotoxine mesylate on fertility were found when assessed by the following criteria: average number of newborns per female and per litter, average number of litters per female, and average length of the fertile period.

Ergotamine tartrate (98.3% pure) had no effect on male fertility in CD-1 mice (aged 8–14 weeks) after a single intraperitoneal injection of 125 mg/kg bw (Matter, 1982). The males were mated with untreated virgin females on days 8–14 post-treatment. This period corresponded to the late spermatid stage, which, on the basis of the dominant lethal test, was suspected of being the stage most sensitive to both ergotamine and triethylenemelamine (TEM), used as positive control. All F1 males resulting from conceptions during this interval – i.e. 220 males in the ergotamine group and 144 males in the TEM group – as well as 233 control males whose fathers were not treated, underwent individual fertility tests. Each F1 male was allowed to mate with two virgin females per week for 4 weeks (a total of eight females/male). Matings were detected by daily examination for vaginal plugs; pregnant females were thereafter kept separately. These females were killed 13–16 days after plug formation for uterine analysis according to the standard protocol of the dominant lethal test. The classification of F1 males as to their fertility pattern was made on the basis of dead implantations only. Thus, F1 females were classified as (i) fertile, i.e. not more than 20% dead implantations, (ii) partially sterile, i.e. 50% or more dead implantations, (iii) sterile, and (iv) questionable, i.e. 21–49% dead implantations. The fertility patterns in the control and ergotamine groups were similar, whereas in the TEM group there was a slight reduction in the number of total and living implantations, and an increase in dead implantations.

(b) **Rat**

Ergocornine methanesulfonate was given by subcutaneous injection to pregnant rats (Carpent & Desclin, 1969). When administered on the fifth day at low doses (0.18–0.3 mg), ergocornine methanesulfonate did not interfere with implantation. Doses from 0.35 to 1.0 mg, although they did not always inhibit implantation, were very deleterious for the maintenance of pregnancy but did not significantly increase the incidence of embryopathies. The administration of 1 mg of ergocornine on the eighth day seriously disturbed pregnancy and induced a very significant increase in fetal malformations. When the same treatment was given on the twelfth day, it had no detrimental effect on pregnancy. Treatment with progesterone (5 mg daily), beginning on the day when ergocornine was given, allowed pregnancy to be almost perfectly maintained. Fetal malformations decreased when the supplementary progesterone treatment was started on the eighth day. It was concluded that the teratogenic activity of ergocornine seems to be mediated by the progesterone deficiency that it induces.

In a short communication, Schön, Leist & Grauwiler (1975) reported that a single oral administration of ergotamine (10 mg/kg bw by gavage) to pregnant rats led to death in the fetuses. In early pregnancy, from days 4 to 10, mortality was only 0–11%, and on days 14 and 15, it was 62% and 59%, respectively. Exposure on days 13–16 resulted in fetal anomalies; no anomalies occurred before day 12 (i.e. during organogenesis). The effects of ergotamine were totally antagonized by α-adrenergic blockade with phenoxybenzamine (10 mg/kg subcutaneously). The authors suggested that the effects of ergotamine were attributable to its action on maternal cardiovascular function and not to direct embryotoxicity.

In their review, Griffith et al. (1978) summarized the studies conducted in the 1950s by Shelesnyak and collaborators on ergotoxine, a natural EA with three components: ergocornine, ergocryptine and ergocristine. A single subcutaneous injection of ergotoxine ethanesulfonate to rats during early gestation (up to day 7 *post coitum*) terminated pregnancy within 1–3 days; threshold doses ranged between 0.1 and 1.0 mg/rat. Rats given ergotoxine after implantation was established (day 8 *post coitum*), delivered normal litters. Of the three components, ergocristine was only weakly active, the other two were equally potent. Termination of pregnancy was associated with suppressed deciduoma formation and with the occurrence of proestrus and estrus within 48–98 hours after administration. Further investigations showed that:

- Ergotoxine had no inherent estrogenic or gonadotrophic activity.
- Ergotoxine in doses effective in terminating pregnancy did not modify the gonadotrophin content of the pituitary.
- The presence of the adrenal glands was not essential for this action of ergotoxine.

- Exogenous prolactin was effective in stimulating the ovary to produce enough additional progesterone to reverse the ergotoxine action.
- Reserpine was effective in overcoming the action of ergotoxine.

These findings suggested that, after ergotoxine treatment, the uterus could still respond to progesterone, the ovary to prolactin and the pituitary to reserpine, i.e. a direct toxic action on these target organs could be excluded.

Griffith et al. (1978) also compared the potency of some EAs to inhibit implantation in rats after subcutaneous administration. Ergocryptine was the most potent, followed by ergocornine, DH-ergocornine, ergonine and DH-ergocryptine. The least potent were ergometrine, ergotamine, DH-ergotamine and DH-ergonine. Based on the oral median effective dose (ED_{50}) for implantation inhibition in rats, the order was as follows (in mg/kg per day on days 6–15 *post coitum*): ergocornine (1.4), ergonine (5.0), DH-ergocornine (14.2), DH-ergotamine (>30), DH-ergocryptine (>30), DH-ergonine (59), ergotamine (133) and ergometrine (209). Ergocryptine was not tested (Griffith et al., 1978).

Griffith et al. (1978) reported unpublished results on doses of various EAs (oral dose in mg/kg daily from days 6–15 *post coitum*) needed to produce 50% embryonic mortality when administered to female rats. The order was as follows: ergonine (4.5), ergovaline (9.7), ergostine (11.8), DH-ergovaline (21.4), ergotamine (27.3), ergometrine (86.9), DH-ergonine (104), DH-ergotamine (617) and DH-ergostine (1095).

Fertility

In their review, Floss, Cassady & Robbers (1973) reported the results of a study conducted in the 1950s by Shelesnyak and collaborators on the effectiveness of ergotoxine and ergocornine as fertility-control agents in rats. In these trials, only 23 pregnancies occurred as a result of 1303 coital contacts. The fertility index was decreased to between 1.5 and 4% from a normal 96.5% in the same colony. It was assumed that ergotoxine terminated early pregnancy by preventing nidation.

(c) Mice, rats and rabbits

OF-1 mice (20–30 g), OFA rats (180–200 g) and Silver Fawn rabbits (2.5–3.5 kg) were treated orally with ergotamine during mid-gestation following the procedure of teratological drug testing recommended in the FDA guidelines of 1966 (Grauwiler & Schön, 1973). Ergotamine tartrate (purity not mentioned) was administered by gavage (in 2% gelatin) at doses of 0, 30, 100 or 300 mg/kg bw in mice; 1, 3, 10, 30 or 100 mg/kg bw in rats, and 0, 1, 3, 10 or 30 mg/kg bw in rabbits. Groups of 23–29 mice and 12–28 rats received daily doses of ergotamine on days 6–15, and groups of 11–17 rabbits on days 6–18. The control animals received 2% gelatin solution. A significant reduction in maternal weight gain was observed in

mice at all doses, in rats at all doses (except 3 mg/kg bw) and in rabbits only at the highest dose. Prenatal resorption was significantly increased at the doses of 100 and 300 mg/kg bw in mice, at 10, 30 and 100 mg/kg bw in rats and at all doses in rabbits. The mean weight of live fetuses was significantly reduced in mice and rats, but not in rabbits. At the same dose levels, fetal anomalies (retardation of skeletal ossification) were observed in mice and rats. In rabbits, type B anomalies (presumably occurring during the early fetal phase of organ differentiation) were more frequent at a dose of 30 mg/kg bw. In rats, litter size decreased in parallel with the increase in prenatal mortality. No teratogenic activity was detected in any of the three species. The authors explained the effects observed in the study as a result of an impairment of blood supply to the uterus and placenta, mainly as a consequence of ergotamine-induced vasoconstriction. A uterotonic effect may also be involved in the mechanism of action (Grauwiler & Schön, 1973).

(d) Rabbit

Morris et al. (1967) investigated the effect of a single subcutaneous injection of ergocornine methanesulfonate given on day 3–4 of gestation on ovum implantation and development in five white New Zealand rabbits (body weight 3.5–4.5 kg). No effects on the number of implantations and no lost or abnormal fetuses were observed at the dose tested (1.5–2.5 mg/kg bw). At higher doses (4–6 mg/kg bw) death of rabbits occurred.

(e) Guinea-pig

Unmated female guinea-pigs received subcutaneous injections of ergocornine methane sulfate 3 to 5 days after estrus; two animals received 1 mg and three animals received 2 mg (in two successive daily doses). The cycle length was not affected and there was no premature return of estrus. Two other females had three daily injections each of 2 mg, 6, 7 and 8 days after estrus; one was autopsied 1 day after the last injection and the ovary sectioned serially. The corpora lutea were normal in size and appearance. In the other female, estrus returned at the normal time, 9 days after the last injection (Deanesly, 1968).

In the guinea-pig, enlargement of the corpora lutea occurs at the time when ovarian progesterone is most essential for embryonic growth and differentiation, between days 15 and 20. This is, therefore, the best time at which to test substances, such as ergocornine, which may be expected to interfere with luteal cell secretion.

Six mated guinea-pigs were injected subcutaneously with ergocornine methane sulfate. One female exposed to 2 mg on day 6 was normally pregnant when killed on day 15. Of the two females exposed to 2 mg on day 10, one was pregnant when killed on day 14, and the other ovulated before being killed on

day 16. Two more females had doses of 2 mg on days 13 and 14 and were killed on day 18; again one was normally pregnant, while the other showed a fresh ovulation. The last female was given 4 mg on day 13 and was pregnant on day 20. None of the six females showed signs of pregnancy regression. All pregnant animals had normal corpora lutea and there was no indication that ergocornine had effectively checked progesterone secretion at a critical developmental period (Deanesly, 1968).

(f) **Pig**

Twenty-five Landrace × Yorkshire sows were selected when they had weaned their last litter (Dignean, Schiefer & Blair, 1986). The sows had all reared at least two previous litters and were randomly assigned to receive one of five diets (0.0, 0.25, 0.5, 1.0 or 2.0 g ergot sclerotia/kg diet) at the time of breeding and maintained on this diet until they weaned their litter. Ergot from barley contained 2.27 g alkaloid/kg: ergocristine 48%, ergotamine 18%, ergocristinine 9.5%, ergocryptine 8%, ergometrine 6%, ergosine 4% and ergocornine 3%. They were fed 2.0 kg of the diet per day during gestation and *ad libitum* during lactation. Feed consumption was recorded daily and sows were monitored for abortion throughout pregnancy. The number of piglets born live or dead, as well as individual birth weights, were recorded. Number of piglets weaned and individual weaning weights (at 21 days of age) were tabulated. These parameters were compared between groups of sows. All pigs that died were necropsied and the cause of death determined. Blood was collected every 2 weeks during pregnancy, then 1 week prior to farrowing, on the day of farrowing and 4 days afterwards. Collected serum was frozen and prolactin levels were determined by radioimmunoassay. The mean of the first five post-breeding serum values was considered as the prolactin baseline. The peak height was the difference between the highest value, measured on the day of farrowing, and the baseline value.

Sows on diets with higher ergot concentrations took longer to consume their daily allotment and were observed to be more irritable and active than control animals. Abortion was not seen in any of the sows. Farrowings were uneventful and piglets were vigorous. Two sows on the 0.25 g/kg diet produced abnormal piglets. The first had two mummified fetuses and one with hydrocephalus; the second had one piglet with atresia ani. Neonatal mortalities were due to crushing and there were no intergroup differences in the number of piglets lost. There were no significant differences between groups with respect to any factor examined (reproductive performance and piglet performance). All sows had complete mammary development and piglets grew at rates consistent with established herd levels.

Serum prolactin peaks showed marked variation between individual sows regardless of ergot concentration group, and no statistically significant differences between the groups were found. Four sows, however, failed to exhibit a prolactin peak at parturition, with levels below 50 ng/mL. All these sows were on contaminated diets, one on 0.5, two on 1.0 and one on 2.0 g/kg ergot. This failure to peak was not associated with clinical agalactia. Peripheral gangrene was not observed, but one sow on the 1.0 g/kg ergot diet experienced transitory swelling of a rear limb. One sow on the diet with the highest ergot concentration became recumbent after farrowing and was killed when her condition deteriorated. She had peripelvic abscesses and fractures of the pelvis, but no evidence of cutaneous gangrenous lesions.

(g) Other species

Ford & Yoshinaga (1975) observed that ergocryptine mesylate terminated pregnancy in hamsters when administered (subcutaneously) on day 5 at 1 mg (no females were pregnant on day 11 in a group of seven females treated) and 0.5 mg (only 2/7 females were pregnant on day 11). When ergocryptine was given on day 6, the response was diminished (3/6 females given 0.5 mg and 3/8 females given 1 mg were pregnant on day 11), and pregnancy continued after treatment on day 7. The abortifacient action of ergocryptine in hamsters on day 5 of pregnancy was overridden by exogenous prolactin but not FSH and LH.

Sharma et al. (2002) studied the reproductive toxicity of EAs in mink. Four groups of 12 females were fed diets containing EAs at concentrations of 0, 3, 6 or 12 mg/kg diet from 2 weeks prior to the breeding season until the kits were approximately 33 days old (133 days in total). The contaminated oats used in the diets contained 110 mg/kg total EAs (6.8% ergosine, 12.9% ergotamine, 14.7% ergocornine, 16.0% ergocryptine and 49.6% ergocristine). Females were mated with untreated males. EAs caused a transient decrease in feed consumption, but body weights and organ weights were unaffected. The number of mink whelping was significantly reduced: 9 mink whelping in the control and 3 mg/kg groups, compared to four mink in the 6 mg/kg group and one in the 12 mg/kg group. The gestation period of mink in the 6 mg/kg group was longer than that of controls (61 versus 47 days). In the group that received 12 mg/kg, only one female whelped, after a gestation period of 62 days. EAs had a significant effect on kit survival: 75% in the control group survived, 33% in the 3 mg/kg group, 6% in the 6 mg/kg and none in the 12 mg/kg group. However, average litter size was not affected. During necropsy, one female in the 3 mg/kg group had four live fetuses in her uterus at 78 days of gestation, and two females in the 6 mg/kg group were discovered to have live fetuses. The female in the 3 mg/kg group and the two females in the 6 mg/kg group had five and six live fetuses at 68 and 69 days of gestation, respectively. Besides the

females that had live fetuses in the uterus, there were three females in the 12 mg/kg group that had eight, six and two fetuses in the process of being resorbed at 65, 64 and 56 days of gestation, respectively. Serum prolactin (at the end of the trial) was significantly depressed in the groups fed with EAs compared to the control group. Histopathological examination of the tissues showed no alterations attributable to EAs. This study indicated that ingestion of EAs at concentrations of 3 mg/kg or higher resulted in reproductive toxicity in mink. The authors postulated that these effects could be due to induction of uterine motor activity, impairment of the blood supply to the uterus or the placenta, and reduced prolactin concentration.

Poole & Poole (2019) reviewed the effects of EAs on female reproduction in grazing livestock species, including 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 circulating steroid hormone concentrations, leading to reduced pregnancy rates. Conversely, EA exposure has minimal impact during reproductive cyclicity and a greater impact during pregnancy and postpartum in sheep and mares.

2.2.6 Special studies

(a) Mode of action

The pharmacological mechanisms associated with ergot toxicity are complex and have not been fully delineated (Holstege & Traven, 2014). They include peripheral vasoconstriction, peripheral adrenergic blockade, reduced secretion of prolactin and stimulation of uterine smooth muscle (Peters-Volleberg, Beems & Speijers, 1996).

EAs are structurally related to biogenic amines such as norepinephrine, dopamine and serotonin. This structural feature allows the EAs to interact with evolutionary related G-protein coupled receptors – GPCR (for example, 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 (Zajdel et al., 2015). Ergotamine displayed high affinity for adrenergic (α1, α2), dopaminergic (D1, D2) and serotoninergic (5-hydroxytryptamine) (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{5A}, 5-HT_{5B} and 5-HT_{6}) receptors. Ergotamine behaves as an antagonist at adrenergic receptors, partially inhibits the 5-HT_{1} receptor, modulates neurotransmitter release presynaptically, and excites the 5-HT_{2}, 5-HT_{3}, 5-HT_{4}, 5-HT_{6} and 5-HT_{7} 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 (Mantegani, Brambilla & Varasi, 1999). Serotonin (5-HT, 5-hydroxytryptamine) is an important neurotransmitter that mediates many central and peripheral physiological functions including food intake, sleep, sexual behaviour, memory and blood pressure. 5-HT achieves this wide variety of functions by acting on distinct receptor types (Mantegani, Brambilla & Varasi, 1999).

Ergotamine, ergosine, ergocornine, α-ergocryptine and ergocristine generally have a higher affinity for α-adrenergic receptors than the lower molecular weight alkaloids such as ergometrine. However, ergometrine is a more selective antagonist of 5-HT receptors in smooth muscle. Alkaloids of the ergotoxine group (ergocornine, α-ergocryptine and ergocristine) have the greatest α-adrenergic blocking activity as well as having the highest prolactin suppression potency through their agonistic actions on dopaminergic receptors. On the other hand, ergotamine is the most potent vasoconstrictor of the natural EAs. It also has high emetic potency through dopaminergic receptors and produces strong stimulation of the uterus and other smooth muscles through 5-HT receptors (Goodman & Gilman, 1990).

Ergotamine and dihydroergotamine have small structural variations but these lead to clinically important differences in their pharmacological profiles. For example, dihydroergotamine is about 10 times less potent than ergotamine at binding to the 5-HT_{1B} receptor. Sullivan et al. (2020) investigated the binding interactions of ergotamine and dihydroergotamine to the 5-HT_{1B} receptor using molecular dynamics simulations and dynamic network analysis. They reported that ergotamine bound more tightly to 5-HT_{1B} with fewer fluctuations and that the 5-HT_{1B}–ergotamine complex was in a more active conformation state than the 5-HT_{1B}–dihydroergotamine complex.

Previous in silico modelling also enabled a deep understanding of the binding sites and receptor–ligand contacts of ergotamine with 5-HT_{1B} and 5-HT_{2B} receptors (Wacker et al., 2013; Wang et al., 2013; Marti-Solano et al., 2014; Rodríguez, Ranganathan & Carlsson, 2014; Rodriguez et al., 2014) and of dihydroergotamine with 5-HT_{1B} and 5-HT_{2B} receptors (Wang et al., 2013).

Using in silico modelling, Dellafiora, Dall'Asta & Cozzini (2015) investigated the interaction between 5HT_{2A} and 5HT_{2B} receptors with ergotamine metabolites, both experimentally detected molecules (n = 10) and predicted phase I derivatives (n = 22). Six mono- and di-hydroxylated metabolites were predicted to interact with 5HT_{2A}, and only one hydroxylated metabolite was predicted to interact with 5HT_{2B}. None of the putative conjugated metabolites

were predicted as able to interact with the receptors. As more than two-thirds of the metabolites studied were predicted as active, Dellafiora, Dall'Asta & Cozzini (2015) highlighted the need for further data on serotonergic activity of ergotamine's metabolites.

(b) Vascular effects

The vascular effects of EAs have been known for centuries. The vasoconstrictor effect is related mainly to the partial agonistic properties at α-adrenoreceptors and is secondary to the partial agonistic effects at 5-HT receptors (Goodman & Gilman, 1990). EAs have an agonist effect on the α-adrenergic receptors of the peripheral vascularization. Blood vessels with α-adrenergic receptors are present in the skin, the sphincters of the gastrointestinal system, kidney (renal artery) and brain. α-Adrenergic receptors primarily mediate smooth muscle contraction. In the smooth muscle cells of blood vessels, the principal effect of activation of these receptors is vasoconstriction, which can lead to ischaemia, and has been associated with the development of gangrene of the extremities in poisoned individuals (Tran, Montastruc & Montastruc, 1983 in Cornière, 2014). The occurrence of gangrene in various animal species correlates closely with the vasoconstrictor potential of the EAs (Griffith et al., 1978). Subacute and subchronic toxicity experiments with ergotamine in rats showed necrosis and fibrosis in the tail tips of animals exposed to high concentrations, which is explained by the vasoconstrictive properties of ergotamine (Speijers et al., 1992, 1993).

Decreased blood flow to the reproductive and digestive systems and to the central nervous system (CNS) affects hormonal control of these systems, nutrient delivery, metabolism and excretion. Stimulation of the vascular smooth muscle causes constriction of the blood vessels in the vascular bed, and the resulting constriction of intracranial arteries is useful in the treatment of migraine. The sensitivity of the smooth muscle of the uterus to stimulation by ergot increases along with the stage of gestation, so the resulting contractions at the end of the third trimester may induce labour. EAs have been administered postpartum to prevent haemorrhage (Floss, Cassady & Robbers, 1973).

Janssen et al. (1998) reported that ergocryptine and ergocornine have vasoconstrictor potencies similar to that of ergotamine, the most potent vasoconstrictor of the natural EAs.

In isolated human mesenteric and crural veins, ergotamine tartrate induced long-lasting contractions (Mikkelsen et al., 1981). These contractions were resistant to repeated washout and were not affected by α-adrenoceptor blockade but could be abolished by removal of extracellular calcium or by the presence of the calcium-blocker nifedipine. In contrast to its effect on human

mesenteric and crural veins, ergotamine had no contractile effect, but a marked relaxant effect, on mesenteric arteries mediated via blockade of α-receptors. The ergotamine-induced contraction was not affected by indomethacin (0.28–2.8 μM) nor was it influenced by serotonin (5-HT). In both mesenteric and crural veins, the ergotamine-induced contraction was diminished by the 5-HT blocking agent, methysergide. In veins, development of tachyphylaxis to 5-HT was demonstrated. The authors concluded that ergotamine has a direct contractile effect on isolated human mesenteric and crural veins. These effects are dependent on unhindered influx of extracellular calcium and are at least partly mediated via 5-HT receptors. In mesenteric arteries, ergotamine acted as an α-adrenoceptor blocker, and had no contractant effect.

In isolated human superficial temporal artery, ergotamine tartrate (7.6×10^{-9} M) also induced long-lasting contractions refractive to additional stimulations and resistant to repeated washout. When tested against 5-HT, ergotamine acted as a non-competitive antagonist (Ostergaard, Mikkelsen & Voldby, 1981). The maximum response to ergotamine was only 20% of the maximum response to serotonin.

The maximal contractions induced in vitro by dihydroergotamine were higher in the human saphenous vein, followed by the meningeal artery and the proximal coronary artery, and were smaller in the distal coronary artery, confirming its venous contractile properties (Labruijere et al., 2015).

In isolated perfused middle cerebral artery of rats, ergotamine and dihydroergotamine induced similar dose-dependent contractions (Tfelt-Hansen, Nilsson & Edvinsson, 2007). Smooth muscle cells of the rat middle cerebral artery contain primarily 5-HT_{2A} receptors and only a minor proportion of 5-HT_{1B} receptors. The artery returned to baseline only after repeated washing, illustrating the long on and off effects, probably due to a slow dissociation from the receptor.

In rabbit, ergonovine maleate was used to provoke coronary spasm by intravenous injection (0.45 μmol/kg) during the infusion of norepinephrine (12 nmol/kg per minute) through a marginal ear vein (Koike et al., 2016).

In male Wistar pithed rats, the vasopressor (systemic vasoconstriction) responses to intravenous bolus injections of ergotamine tartrate (up to 310 μg/kg given cumulatively) were determined after administration of vehicle (saline), or several α1 or α2-adrenoceptor antagonists, and compared with baseline heart rate and diastolic blood pressure. The results suggested that the vasopressor responses to ergotamine were mainly mediated by α1A-, α1B-, α1D-, α2A- and α2C-adrenoceptors. The vasopressor responses to 310 μg/kg ergotamine were long-lasting, with no recovery (Villamil-Hernández et al., 2013). Further investigations in male Wistar pithed rats suggested that ergotamine tartrate induced inhibition of the vasopressor sympathetic outflow by activation of prejunctional 5-HT_{1A}, 5-$HT_{1B/1D}$, α2-adrenoceptors and D2-like receptors (Villamil-Hernández et

al., 2014). González-Hernández et al. (2019) also reported that prejunctional 5-$HT_{1B/1D}$, D2-like and α2-adrenergic receptors mediate the sensory-inhibition induced by ergotamine (intravenous injection, 0.31 μg/kg per minute) in pithed Wistar rats, whereas higher doses may involve other receptors. Rivera-Mancilla et al. (2017) also used male Wistar pithed rats to show that the vasopressor responses to intravenous bolus injections of dihydroergotamine mesylate (up to 3100 μg/kg given cumulatively) were mediated by α_{1-} (probably α_{1A}, α_{1B} and α_{1D}) and α_{2-} (probably α_{2A}, α_{2B} and α_{2C}) adrenoceptors.

A vasoconstrictive response (reduced diameter) was observed in sheep (ewes') uterine and umbilical arteries after exposure to EAs in the diet (4.14 mg ergovaline + ergovalinine per kg) during pregnancy, which would explain the reduced fetal weights (Britt et al., 2019; Klotz et al., 2019). Empty uterine and total placentome weights were lower in ewes exposed to EAs during late gestation. Britt et al. (2019) suggested that EAs impaired vascular development of the placenta.

In vitro, ergotamine and ergovaline induced a contractile response in uterine and umbilical arteries collected from pregnant ewes (Klotz et al., 2019). Like the responses to serotonin, both ergotamine and ergovaline were 100-fold more vasoactive in the umbilical artery than in the uterine artery. This contractile response is driven by serotonin receptor $5HT_{2A}$ mediating vasoconstriction in the umbilical artery. In nonpregnant horses, ergotamine and ergonovine were not vasoactive in uterine artery but produced a contractile response in the palmar artery and vein, as did ergocryptine, ergocristine, ergocornine and ergonovine (Klotz & McDowell, 2017). Ergovaline was the most vasoactive EA in both the palmar artery and the palmar vein, followed by ergonovine, whereas ergocristine induced the lowest contractile response. In lateral saphenous vein biopsies from Angus steers, ergotamine and ergovaline altered the contractile response to serotonergic and adrenergic agonists (Klotz et al., 2016). These effects were reversible after a minimum of 35 days free of EAs (Klotz et al., 2016).

Ergometrine, ergotamine, ergocristine, ergocryptine, ergocornine and ergovaline produced contractile responses in lateral saphenous veins collected from Holstein steers (Pesqueira et al., 2014). The various classes of alkaloids differed in the type of contractile response elicited since ergonovine (an ergoline alkaloid) did not display the sustained contractile response observed with ergopeptine alkaloids (Pesqueira et al., 2014).

In isolated rat tail artery, ergovaline also induced a sustained contractile response (very slow dissociation from the 5-HT_{2A} receptor) (Schöning, Flieger & Pertz, 2001). Ergotamine was equipotent with ergovaline in eliciting contractile responses. In rat thoracic aorta, ergovaline showed low efficacy in the activation of α1-adrenoceptors. It was concluded that the constrictor effect of ergovaline was mediated by activation of vascular 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors.

An in vitro study on bovine vascular smooth muscle cells from the dorsal metatarsal artery showed that ergonovine and α-ergocryptine stimulated cell growth, supporting the hypothesis that EAs exert their vascular effects through hyperplasia of the intima (Strickland et al., 1996). Ergovaline exhibited a dual action on the cell growth, stimulating growth of quiescent cells but inhibiting growth of growing cultures.

Owing to the asymmetric carbon at the C8 position, EAs exist in two forms known as the (*R*)- and (*S*)-epimers. The (*R*)-epimers have the suffix "ine", whereas the (*S*)-epimers have the suffix "inine". The (*S*)-epimers of EAs are thought to be biologically inactive and, therefore, harmless. A major mechanism by which the (*R*)-epimers of EAs produce their toxic effect is through vasoconstriction. Therefore, Cherewyk et al. (2020) sought to examine the vasoactivity potential (contractile response) of four (*S*)-epimers, namely ergocryptinine, ergocristinine, ergocorninine and ergotaminine utilizing an in vitro arterial tissue bath system. Bovine metatarsal arteries were collected from healthy mixed-breed beef steers. To assess the contractile response of each (*S*)-epimer, a cumulative contractile dose–response curve was constructed by incubating arteries with increasing concentrations (1×10^{-11} to 1×10^{-6} M) of that (*S*)-epimer. Contractile responses were recorded as grams of tension and were normalized to an initial contraction of phenylephrine. Contrary to the widespread belief, all (*S*)-epimers tested were found to be 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 (Müller-Schweinitzer, 1984). The effects of dihydroergotamine mesylate and five of its main metabolites, namely 8′-hydroxy-dihydroergotamine, 8′,10′-dihydroxy-dihydroergotamine, 2,3seco,N(1)formyl,3-keto,8′-hydroxy-dihydroergotamine, dihydrolysergic acid amide and dihydrolysergic acid were investigated on human and canine veins in vitro, on canine veins in situ, and in the ganglion-blocked rat in vivo (Müller-Schweinitzer, 1984). Like dihydroergotamine, the metabolites 8′-hydroxy-dihydroergotamine, 8′,10′-dihydroxy-dihydroergotamine and dihydrolysergic acid amide caused contriction of human varicose veins and only weak α-adrenoceptor blockade. On canine femoral vein strips, the same compounds produced predominantly α-adrenoceptor blockade and only negligible stimulation. 8′-OH,N(1)formyl-dihydroergotamine and dihydrolysergic acid were inactive. The same compounds, which were agonists on human vein strips in vitro, induced dose-dependent reduction of venous compliance when infused locally into the dog saphenous vein in situ. In the ganglion-blocked rat, besides the parent compound, only 8′-hydroxy-dihydroergotamine and 8′,10′-dihydroxy-

dihydroergotamine produced an increase in diastolic blood pressure when injected intravenously.

According to Holstege & Traven (2014) ergocryptine and ergocornine have vasoconstrictor potencies similar to that of ergotamine, the most potent vasoconstrictor of the natural EAs.

(c) Uterotonic activity and effects during gestation

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 (Tran, Montastruc & Montastruc, 1983; in Cornière, 2014). Janssen et al. (1998) reported that ergocryptine, ergocornine and ergocristine display oxytocic activity, cause vascular spasms, and inhibit ovulation and implantation. Of the EAs, ergometrine has the most oxytocic activity.

In their review, Griffith et al. (1978) suggested that embryotoxicity in rats given ergotamine is caused by α-adrenoceptor stimulation producing hypoxia. This was based on studies showing that the ergotamine effect can be totally antagonized by α-adrenoceptor blockade (phenoxybenzamine 10 mg/kg, administered subcutaneously) (Grauwiler & Leist, 1973; Schön, Leist & Grauwiler, 1975).

Grauwiler & Leist (1973) showed that ergotamine (2.5 mg/kg intravenously) reduced the transplacental passage of [^{3}H]-L-leucine, used as a marker for uteroplacental blood supply, for at least 3 hours, and that this effect could be antagonized by phenoxybenzamine. However, ergometrine (10 mg/kg administered intravenously on day 14 of gestation) produced no inhibition of uteroplacental blood circulation and had no adverse effect on the fetuses. Ergometrine has a uterotonic activity comparable to that of ergotamine but only very slight vasoconstrictor potential (Leist & Grauwiler, 1974).

In Sprague-Dawley rats, ergometrine given orally (1 mg/rat) induced a significant increase in maximal intrauterine pressure (denoting uterotonic effects) in postpartum females but not in pregnant females (Monji et al., 2018).

In vitro, ergonovine maleate (10^{-10} to 10^{-5} M) induced contractility in myometrial samples obtained from women undergoing caesarean delivery. The combination of oxytocin with ergonovine produced a superior response to that of oxytocin alone (Balki et al., 2015). Ergometrine also induced uteronic effects (on amplitude and frequency of contractions) in human myometrium in vitro (Morrison, Crosby & Crankshaw, 2016; Crankshaw, Crosby & Morrison, 2017; Fanning et al., 2017; Ryan, Crankshaw & Morrison, 2019). The α-adrenergic antagonist phentolamine and the more selective α1-adrenergic antagonist

prazosin inhibited the ergometrine-mediated increase in motility index, amplitude and frequency (Fanning et al., 2017).

(d) Lactation

Mice

A few days before parturition, groups of 6 to 18 female BS/VS mice were fed with a diet containing sclerotia of *Claviceps fusiformis* at 0, 0.5, 1.0, 2.0, 3.0 and 5.0%, equivalent to an intake of EAs of approximately 0, 100, 210, 420, 630 and 500 µg (Mantle, 1968). Chromatographic analysis showed that the major components were agroclavine and elymoclavine; the minor components were penniclavine, setoclavine and chanoclavine. In the groups fed the two highest concentrations, the diet was not well eaten and one and three females, respectively, died before parturition. Mantle (1968) observed that the females fed on concentrations of ergot sclerotia higher than 1% failed to raise their litters, and the condition of the pups suggested impaired lactation.

In another experiment, 10 pregnant BS/VS mice were studied. Three days after the birth of litters, when pups were growing vigorously and white milk-filled stomachs were clearly visible through the abdominal wall, the maternal diet was changed to 2% ergot (equivalent to 350 µg/day of EAs). Within 1 or 2 days the extent of the abdominal milk lines in all replicates had decreased noticeably and within 4 to 26 days of commencing the 2% ergot diet all the pups had died. At postmortem examination the stomachs were almost empty. Similar effects on pup mortality were observed when seven lactating mice were fed with 70 mg agroclavine/kg diet (Mantle, 1968).

Pregnant BS/VS mice were fed untreated meal or a meal containing 50 mg/kg agroclavine from 10 to 12 days before parturition. Within a few hours of birth, five treated and five control mice were dissected to remove mammary tissue (Mantle, 1968). The weight of mammary tissues was decreased in treated females compared to the controls, being in the same range as for virgin untreated mice.

Rat

Zeilmakla & Carlsen (1962) showed that intraperitoneal injection of 1 mg ergocornine methane sulfonate to lactating rats temporarily inhibited milk production, the effect being prevented by treatment with prolactin.

Griffith et al. (1978) compared the potency of some EAs to inhibit prolactin secretion in rats after subcutaneous or intraperitoneal administration: ergocornine was the most potent followed by ergocryptine, dihydro-ergocryptine and dihydro-ergocornine. Ergotamine and ergometrine were the least potent.

Flint & Ensor (1979) administered α-ergocryptine subcutaneously (2 mg/kg bw) on day 4 of pregnancy to inbred Wistar rats ($n = 6$) experiencing concurrent lactation and pregnancy. The α-ergocryptine induced a marked reduction of serum prolactin concentration (collected from the tail vein) compared to a group control ($n = 6$). To study long-term effects, nine treated females had a subsequent pregnancy. The growth rate of their young was significantly reduced on days 5, 6, 7 and 8 of lactation. Some deaths of young occurred in five litters, and only four out of the nine rats maintained their pregnancy compared with all nine in the group control.

Shaar & Clemens (1972) correlated the effect of a number of EAs on serum prolactin levels with parameters such as litter weight gain, body weight gain of mothers and weight of mammary tissue. Ergocornine hydrogenmalienate, 0.5 mg and 1.0 mg; ergonovine maleate, 4.0 mg; dihydroergocornine, 1.0 mg; ergotamine tartrate, 4.0 mg; and ergocryptine mesylate, 0.5 mg; were administered subcutaneously once a day to lactating female rats from day 4 postpartum (delivery = day 0) to day 8 postpartum. Serum prolactin levels as measured by radioimmunoassay were depressed significantly as a result of treatment with each EA when compared with corn oil-treated control rats. These compounds significantly depressed lactation as assessed by litter weight gain. The total mammary tissue weight determined in each animal in an ergocornine hydrogenmalienate-treated group was reduced compared to the weight in animals in a control group receiving corn oil. Hypophysial homografts equivalent to two pituitary glands from donor litter mates were transplanted beneath the kidney capsules of mature hypophysectomized female rats 3 days after hypophysectomy. The rats were treated for 2 days with 0.5 mg ergocornine hydrogenmalienate beginning 10 days after transplantation. Serum prolactin levels from ergocornine-treated rats were lower than levels in hypophysectomized, pituitary-grafted rats receiving corn oil alone. These results indicate that EAs have a direct action on the pituitary, thus preventing prolactin secretion, and that the inhibition of lactation occurs partly as a result of this deficiency in prolactin secretion. Floss, Cassady & Robbers (1973) commented that action on the pituitary might also be mediated through the hypothalamus.

Comparisons of the ability of various EAs to suppress lactation through their potential to inhibit nidation showed good correlation for ergotoxine, ergocornine, ergocryptine and dihydroergocornine but not for ergometrine and dihydroergotamine (ineffective for both activities). Ergotamine showed adverse effects on lactation but little activity in inhibiting implantation. Another possibility is that differences in the pharmacokinetic properties could account for the differences in the quantitative effectiveness of various EAs in inhibition of lactation and nidation (Floss, Cassady & Robbers, 1973; Griffith et al., 1978).

Pig

Fifty-one pregnant sows were sequentially inducted into an experiment each week in groups of four to seven, as they approached within 14 days of farrowing (Kopinski et al., 2007). Diets containing sorghum ergot sclerotia (*Claviceps africana*) at 0 (control), 0.3%, 0.6%, 0.9%, 1.2% or 1.5% w/w (equivalent to 0, 1.4, 2.8, 4.2, 5.6 and 7 mg EAs/kg, 86% as dihydroergosine/kg) were randomly allocated and individually fed to sows. Diets with ergot were replaced with control diets after farrowing. Postfarrowing milk production was assessed by direct palpation and observation of udders, and by piglet responses and growth. Blood samples were taken from sows on three days each week for prolactin estimation.

The three sows fed 1.5% ergot for 6–10 days preceding farrowing produced no milk, and 87% of their piglets died as a result of agalactia. One sow in a group of 13 fed 0.6% ergot for 7–14 days produced no milk. No effects were observed on milk production in the other 12 sows in this group. In the other groups, reduced milk production was observed in 3/3 sows fed 1.2% ergot for 8 days, 5/9 sows fed 0.9% for 6–10 days and 1/9 sows fed 0.3% for 5–9 days. No neonatal piglet mortality was observed in these groups. EAs caused pronounced reductions in blood prolactin, and first-litter sows had lower plasma prolactin than multiparous sows, increasing their susceptibility to ergot (Kopinski et al., 2007).

Diets containing 3% sorghum ergot sclerotia (*Claviceps africana*) (16 mg alkaloids/kg, including 14 mg dihydroergosine/kg) were fed to 12 sows from 14 days post-farrowing (mid-lactation) until weaning 14 days later, and their performance was compared with that of 10 control sows (Kopinski et al., 2008). Ergot-fed sows displayed a smaller weight loss during lactation (24 kg/animal versus 29 kg/animal in control sows) despite feed consumption being less (61 kg/head total feed intake versus 73 kg/head by control sows). The litters of ergot-fed sows had poorer weight gain over the 14-day period (16.6 kg/litter versus 28.3 kg/litter for controls) despite an increase in consumption of creep feed by the piglets from the ergot-fed sows (1.9 kg/litter compared with 1.1 kg/litter by the controls). After 7 days, in sows fed ergot, plasma prolactin was reduced to 4.8 µg/L compared with 15.1 µg/L in the control sows, and then, at weaning, prolactin was 4.9 µg/L compared with 8.0 µg/L in the control sows. Two sows fed ergot ceased lactation early (after 10 days of ergot feeding), and their feed intakes, body weight losses with litter weight gains and creep consumption indirectly indicated an effect of ergot on milk production (Kopinski et al., 2008).

Milk production was not affected in sows fed up to 0.2% ergot from barley (containing 2.27 g alkaloid/kg; ergocristine 48%, ergotamine 18%, ergocristinine 9.5%, ergocryptine 8%, ergometrine 6%, ergosine 4% and ergocornine 3%; equivalent to 4.5 mg alkaloid/kg diet) from mating until 21 days of lactation.

However, the authors noted that their result was at variance with other reports showing agalactia produced by lower ergot levels, and it was suggested that ergocristine might have lower toxicity than other rye EAs such as ergotamine and ergocryptine (Dignean, Schiefer & Blair, 1986; Kopinski et al., 2007).

(e) Prolactin

The dopaminergic activity of EAs (dopamine agonist) reproduces the effects of dopamine at the level of the hypothalamic–pituitary pathways and inhibits the secretion of prolactin. Intoxication with EAs (or their medical use) therefore decreases lactation or may even lead to agalactia in sensitive species. Ergometrine, ergocornine and ergocryptine show this activity (Tran, Montastruc & Montastruc, 1983 in Cornière, 2014). Janssen et al. (1998) reported that ergocryptine, ergocornine and ergocristine have the greatest prolactin suppression potency and the largest α-adrenergic blocking potency.

Prolactin is a hormone that is essential for mammogenesis and lactogenesis in mammalian species (Farmer, 2001). It is secreted by the anterior pituitary, also known as the adenohypophysis. Prolactin plays a dominant role in several aspects of the breast, including growth and development of the mammary gland (mammogenesis), synthesis of milk (lactogenesis) and maintenance of milk secretion (galactopoiesis) (Binart, 2017). Shaar & Clemens (1972) suggested that EAs have a direct action on the pituitary thus preventing prolactin secretion, and that the inhibition of lactation occurs partly as a result of this deficiency. Floss, Cassady & Robbers (1973) commented that the action on the pituitary is also mediated through the hypothalamus.

EAs significantly decreased serum prolactin in weaned pigs fed with 0, 0.05, 0.10, 0.25, 0.50 and 1.00% wheat ergot sclerotia in the diet (1880 mg alkaloid/kg; ergocristine, ergotamine, ergosine, ergocryptine and ergocornine constituting 40, 36, 11, 7 and 6% of the total, respectively) for 28 days, beginning 7 days post-weaning (Oresanya et al., 2003).

In pregnant sows fed diets containing sorghum ergot sclerotia (*Claviceps africana*) at 0 (control), 0.3%, 0.6%, 0.9%, 1.2% or 1.5% w/w (equivalent to 0, 1.4, 2.8, 4.2, 5.6 and 7 mg EAs/kg, 86% as dihydroergosine/kg) for 5–14 days, a pronounced reduction in blood prolactin was observed, and first-litter sows had lower plasma prolactin than multiparous sows (Kopinski et al., 2007). Sow plasma prolactin was reduced in animals fed a diet containing 3% sorghum ergot (16 mg alkaloids/kg, including 14 mg dihydroergosine/kg) after 7 days of exposure postfarrowing (4.8 μg/L compared with 15.1 μg/L in the control sows). And, at weaning, plasma prolactin was 4.9 μg/L compared with 8.0 μg/L in the control sows (Kopinski et al., 2008).

(f) Satiety

EAs have an agonist effect on the α-adrenergic receptors. Activation of α1-adrenergic receptors produces anorexia. Reduced appetite was observed in rat studies (Speijers et al., 1992,1993; Janssen et al., 2000a,b). and in rabbit studies (Canty et al., 2014; Solano-Baez et al., 2018) and, subsidiary to this, decreased body weight was reported. Janssen et al. (1998) suggested the U-shaped pattern of changes for some parameters observed in the subacute rat toxicity study with α-ergocryptine in the diet might be caused by the U-shaped concentration–response relationship for feed intake. This may be explained by the dopaminergic properties of α-ergocryptine, leading 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.

Ergotoxines (ergocryptine, ergocristine and ergocornine) are dopamine agonists and may inhibit feed intake by activating the satiety mechanisms in the lateral hypothalamic area and/or produce effects in the forebrain incompatible with feeding behaviour (Opara et al., 1996; Oresanya et al., 2003).

(g) Immunotoxicity

Filipov et al. (1999) investigated whether major splenocyte-derived cytokines (interleukin 2 (IL-2), interleukin 4 (IL-4), interferon gamma (IFN-γ)) and macrophage-derived cytokines (interleukin 1b, (IL-1β), interleukin 6 (IL-6), and tumour necrosis factor alpha (TNF-α)) were affected by ergotamine. Two groups of male BALB/c mice (n = 5/treatment) were treated with ergotamine tartrate (subcutaneous injection) for 10 days at doses of 0, 0.4, 2, 10 or 50 mg/kg bw. Twenty-four hours after the last treatment, splenocytes (S) were isolated from one group of animals and macrophages (MO) from the other group for determination of IL-2, IL-4 and IFN-γ, and IL-1β, IL-6 and TNF-α, respectively. Following activation with 5 µg/mL concanavalin A (S) and 10 µg/mL lipopolysaccharide (MO), cells were incubated for 48 and 24 hours, respectively, and supernatants were collected and assayed for cytokines by ELISA. Additionally, differential white blood cell (WBC) counts were performed and the neutrophil (N):lymphocyte (L) ratio calculated. Ergotamine treatment significantly increased IL-6 levels at doses of 2 mg/kg and greater and TNF-α at the highest dose. There was no treatment effect on IL-1β, IL-2, IL-4 and IFN-γ. Also, no effect was observed upon total and differential WBC counts or N:L ratio. Filipov et al. (1999) concluded that the IL-6 increased by ergotamine treatment would result in an increased inflammatory response.

A high alkaloid content in the diet fed to rabbits for 4 weeks adversely affected the immune system. Rabbits that consumed the diets with 5 and 15% sclerotia developed respiratory problems (Solano-Báez et al., 2018).

(h) Other effects

Janssen et al. (1998) reported a concentration-related decrease in body temperature in rats exposed to α-ergocryptine in the diet at fluctuating concentrations from 100 to 750 mg/kg for several days. Hyperthermia in rabbits but hypothermia in rats and mice was reported after ergotoxine exposure. These effects were reported to be related to a direct action of ergotoxine on the central "heat regulating centre" (Loew et al., 1978 in Janssen et al., 1998).

Administration of ergotamine tartrate (40 µg/kg bw per day) for 224 days increased rectal temperature in bulls (Schuenemann et al., 2005a). British-Continental steers grazing endophyte-infected tall fescue had raised rectal temperatures in spring but not in autumn (Parish et al., 2013). The vasoconstrictive effects of EAs on bovine vasculature could result in impaired ability to dissipate heat.

No effect on body temperature was observed in sows fed for 5–14 days with a diet containing sorghum ergot sclerotia (*Claviceps africana*) up to 1.5% w/w (equivalent to 7 mg EAs/kg, 6 mg as dihydroergosine/kg) (Kopinski et al., 2007). Nor was any effect seen in ewes exposed to endophyte-infected fescue seed during gestation (4.14 mg ergovaline + ergovalinine per kg, equivalent to 17.9–21.2 µg ergovaline and ergovalinine/kg bw) (Britt et al., 2019) or exposed to ergovaline in the diet (497 ± 52 µg/kg, equivalent to 6.8 µg/kg bw) for 28 days during lactation (Zbib et al., 2014).

Intrathecal and intraplantar injection of ergotamine tartrate (15 nmol) to female Wistar rats blocked formalin-induced nociception (measured by the mean number of flinches of the injected paw). By use of antagonist compounds, it was shown that this antinociceptive action took place via $5HT_{5A}$ and $5HT_{1B/1D}$ receptors located at both spinal and peripheral sites (Vidal-Cantu et al., 2016).

After intrapenile injection to groups of 30 male Wistar rats, dihydroergotoxine mesylate (1 mg/0.1 mL) showed an erectogenic effect and ergotamine tartrate showed a detumescent effect compared with saline solution (Radosavljevic et al., 2012).

Ergotamine tartrate (1 µM) tested in vitro on isolated rat distal colon increased the contractile tension and the frequency of contraction (Dalziel, Dunstan & Finch, 2013). This result is consistent with effects of serotonin agonist drugs that increase gastrointestinal motility via specific serotonin (5HT) receptors.

(i) Cytotoxicity

Ergotamine and ergocornine showed moderate cytotoxicity in V79 cells (lung fibroblasts from male Chinese hamster) with IC_{50} of 20 and 32 μM, respectively (Behm, Föllmann & Degen, 2012) compared to IC_{50} of 3 and 14 nM for the most cytotoxic mycotoxins tested in the assay, i.e. T-2 and HT-2.

Ergotamine showed no cytotoxic potential in studies on two renal cell lines in vitro when tested up to 46 μM (HK62, a human immortalized kidney cell line and SA7K, a renal cell model using pseudo-immortalized human primary renal proximal tubule epithelial cells) (Li et al., 2017).

Mrusek et al. (2015) investigated the cytotoxicity of six EAs (agroclavine, ergosterol, ergocornine, ergotamine, dihydroergocristine and 1-propylagroclavine tartrate) in a panel of cell lines of different tumour origin (ovarian carcinoma, brain tumour, prostate cancer, lung cancer, melanoma, colon cancer, renal carcinoma, breast cancer or leukaemia). 1-Propylagroclavine tartrate showed the strongest inhibitory activity on tumour cells. Ergocornine, ergotamine and dihydroergocristine showed similar activity (IC_{50} around $10^{-4.5}$ M). Leukaemia cell lines were more sensitive to EAs.

Dihydroergotamine tartrate (10 μM) reduced survival of human lung cancer cells (A549, NCI-H226 or NCI-H460) by the induction of apoptosis and mitophagy suggesting that dihydroergotamine tartrate could be developed as a therapeutic agent against lung cancer (Chang et al., 2016).

(j) Effects on tumours

In rodent studies, ergocornine and ergocryptine induced regression of carcinogen-induced mammary adenocarcinomas (Floss, Cassady & Robbers, 1973).

The effect of ergocornine on transplanted D2-mammary tumour growth was investigated in 28 BALB/c mice, which were divided into two groups and administered subcutaneous injections for 5 weeks (Singh et al., 1972). The control group received saline-ethanol. The experimental group received 20 μg ergocornine/day for the first week, 50 μg/day from the second to the fourth week, and 100 μg/day for the fifth week. Throughout treatment, tumours in control mice grew more rapidly than tumours in the ergocornine-treated animals. Mammary tumour growth was suppressed by ergocornine, but the tumours did not regress significantly below their initial size. When ergocornine treatment was terminated at the end of 5 weeks, a prompt renewal of mammary tumour growth was observed, and the rate of growth paralleled that of tumours in the controls. Pituitary prolactin levels were decreased about 70% by ergocornine treatment as compared to the controls. These results indicate that growth of transplantable D2-mammary tumours in BALB/c agent-free mice is decreased by ergocornine, and that this is associated with reduced pituitary prolactin levels.

Nagasawa & Meites (1970) found that ergocornine inhibited the growth of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumours in rats injected daily for 15 days with a dose of 0.6 mg ergocornine/day. Cassell, Meites & Welsch (1971) reported a study involving a longer treatment period (28 days). Sixty-eight female Sprague-Dawley rats with tumours were divided into five groups, and three groups were given subcutaneous injections of ergocornine or ergocryptine every day for 4 weeks; one intact and one ovariectomized group served as controls. The intact controls showed a significant increase in number and size of tumours throughout the treatment period, whereas ergocornine produced a significant reduction in number and size of tumours, paralleling the effects seen in the ovariectomized controls. Ergocryptine suppressed mammary tumour growth throughout the treatment period but produced no significant decrease in number and size of tumours. When ergocornine treatment was terminated at the end of 4 weeks, prompt renewal of mammary tumour growth was observed. Inhibition of mammary tumour growth in rats by these compounds is believed to be due to their demonstrated suppression of pituitary prolactin secretion.

In the study by Clemens & Shaar (1972), administration of ergocornine at 0.4 mg/rat daily 11 days before and 5 days after treatment with DMBA significantly inhibited the induction of mammary tumours in rats. Ergocornine caused rapid and complete (to palpation) regression of 62% of established, DMBA-induced, mammary tumours. Examination of sites where tumours had completely regressed to palpation 2 months after ergocornine treatment was discontinued, revealed that 64% of the tumours did not recur. The effectiveness of ergocornine in regressing tumours was found to be related to initial tumour size. Tumours with volumes up to 1.8 cm^3 regressed rapidly after administration of ergocornine; whereas tumours approximately 14.1 cm^3 and larger did not respond to treatment. Tumours of intermediate size showed growth inhibition, and many showed regression to varying degrees. Therefore, mammary tumours start to lose their hormonal dependence as they become larger and may eventually lose it completely.

2.3 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, 2015). 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. The effects of EAs

on lactation vary with the livestock species. Ingestion of EAs reduces milk yield in cattle, horses and sheep (Poole & Poole, 2019).

(a) Pigs

In piglets (n = 8 castrated males and 8 females), ergot mixed into cereal–soyabean meal based diets at concentrations of 0, 0.5, 1, 2 and 4 g/kg for 35 days induced a significant decrease in feed intake and live weight gain at the highest concentration tested (Mainka et al., 2005). The total alkaloid content of the ergot was 2775 mg/kg (ergocristine 14.9%, ergometrine 8.1%, ergotamine 5.4%, ergocornine 3.2% and α-ergocryptine 1.9%; the remaining 66.5% alkaloid content was unidentified). No effects on performance were observed in chickens (n = 28 males) exposed for 21 days; however, the heart weights showed a significant linear decrease.

On a farrow-to-finish pig farm, the exposure of sows to 3.49 mg EAs/kg diet (sum of ergotamine, ergocristine, ergosine, ergocryptine, ergocornine and ergometrine) for 10 to 15 days before the end of gestation and to 8.06 mg EAs/kg diet over 3 to 4 days at the beginning of lactation led to agalactia in 13 of 20 sows in a batch and to high neonatal mortality rates for all litters (79% on average). No clinical signs associated with vasoconstrictive effects were observed (Waret-Szkuta et al., 2019).

(b) Cattle

Angus heifers (n = 36) received either endophyte-infected fescue seed (E+) or noninfected fescue seed (E–; control) in a total mixed ration for 63 days (Poole et al., 2018). Infected fescue seeds (E+) contained ergovaline 3.9 mg/kg, ergocryptine 1.9 mg/kg, ergotamine 1.4 mg/kg, ergosine 1.4 mg/kg, ergocornine 0.96 mg/kg and ergocristine 0.67 mg/kg. The authors used a dietary concentration of 500 µg ergovaline + ergotamine per kg. The heifers were synchronized at the same stage of estrous cycle by hormone treatment. Average daily weight gain was decreased in animals in the E+ group (0.8 kg/day) compared to control heifers (1.0 kg/day) although daily intake was not affected. Heart rate, rectal temperature, respiration rate and blood pressure did not differ between treatment groups. Using Doppler ultrasonography, vasoconstriction was observed in the caudal artery, but not the caudal vein in heifers consuming the E+ diet. No differences were observed in corpus luteum area or circulating progesterone and prolactin concentrations in heifers on the E+ diet compared to controls. There was a significant decrease in the number of medium-sized follicles and in the diameter of arteries and veins servicing the ovary and uterus on days 10 and 17 of the estrous cycle. Reduction in blood flow to the reproductive organs during critical times in the estrous cycle may contribute to the reductions in ovarian function and pregnancy rates associated with fescue toxicosis.

Lactating Hereford cows (n = 4 per group) received a control (<15 μg total EAs/kg dry matter), low (132 μg/kg diet equal to 0.12 μg/kg bw), medium (529 μg/kg equal to 0.58 μg/kg bw), or high (2115 μg/kg equal to 2.43 μg/kg bw) diet (Cowan et al., 2018). Ergotized barley used for the ration contained ergocristine 18 mg/kg, ergotamine 12 mg/kg, α-ergocryptine 7 mg/kg, ergocornine 3.4 mg/kg, ergometrine 3.2 mg/kg and ergosine 2.7 mg/kg). The study included three experimental periods: pretreatment (4 days), treatment (7 days) and post-treatment (4 days). The caudal, median sacral and internal iliac arteries were examined daily using ultrasonography in B-mode and Doppler (colour and spectral) mode. Caudal artery diameter decreased in the medium- (10%, −0.3 mm) and high-dose (19%, −0.5 mm) groups compared to pretreatment values. During the post-treatment period, the diameter returned to the pretreatment values for animals in both groups. The pulsatility index was increased in animals given all the ergot treatments during the post-exposure period compared to the control group. Blood volume per pulse (mL) and blood flow (mL/minute) through the caudal artery during the treatment period were reduced in the medium- (−1.0 mL) and high-dose (−1.1 mL) groups compared to pretreatment values but did not differ when compared to the control group. The median sacral artery diameter decreased in animals given the medium- and high-dose treatments compared to the control group. No differences were detected in any haemodynamic end-points for the internal iliac artery except changes in pulse rate. Prolactin levels, body weight and rectal temperatures were not affected by the treatment.

A similar study was conducted in periparturient Hereford cows with an exposure period extended to 9 weeks (Cowan et al., 2019). Beginning before parturition, cows were fed mixed rations containing <15 μg EAs/kg of dry matter (control, n = 9), 48 μg/kg (low, n = 9), 201 μg/kg (medium, n = 8), or 822 μg/kg (high, n = 6). The study included three experimental periods: pretreatment (2 weeks), treatment (9 weeks), and post-treatment (3 weeks). Caudal artery diameter decreased by 14% (−0.6 mm) in animals in the high-concentration group during the treatment period compared to pretreatment values. During the post-treatment period, the diameter returned to the pretreatment value. Reductions in caudal artery blood flow (37%, 29%) and blood volume per pulse (29%, 11%) were recorded during the treatment period in animals in the high- and medium-concentration groups, respectively, compared to pretreatment values (and they had returned towards pretreatment values by the post-treatment period). Internal iliac artery diameter and blood flow decreased by 13% (−1.0 mm) and 40%, respectively, during the treatment period in animals in the medium-concentration group but were not significantly reduced in those in the high-concentration group. Animals in the medium- and high-concentration groups showed moderate reductions (12–25%) in the mean blood velocity

during the treatment and post-treatment periods, and decreases (12–17%) in the peak systolic velocity of both arteries during the post-treatment period were also detected. Prolactin levels and rectal temperatures were not affected by the treatment. The study documented moderate vasoconstriction in the caudal artery and the internal iliac artery in cows fed 201–822 μg EAs/kg of dry matter for a 9-week period around the time of parturition. The Committee noted that the authors did not make any distinction between measurements taken during the prepartum period and the postpartum period and that the data only allow a qualitative conclusion on the existence of a concentration–response relationship.

Angus cows were fed daily with either 40 μg/kg bw of ergotamine tartrate (n = 37) or a control diet (n = 38) for up to 65 days (Schuenemann et al., 2005b). Compared to the controls, prolactin concentration and the percentage of embryos that developed to compacted morula or greater were decreased in cows exposed to ergotamine. Pregnancy rates of transferred embryos did not differ between treatment groups. Thus, administration of ergotamine altered the developmental potential of embryos, but did not affect uterine competency to establish pregnancy.

Semen parameters, fertilization and endocrinology were studied in Angus bulls exposed to ergotamine (Schuenemann et al., 2005a). Bulls were allotted to a control diet (n = 8) or a diet supplemented daily with 40 μg/kg bw of ergotamine tartrate (n = 8). Administration of ergotamine tartrate increased rectal temperature and resulted in lower scrotal temperatures (a sign of vasoconstriction) compared to control bulls. However, average daily weight gain, prolactin, scrotal circumference, testosterone, and semen motility and morphology did not differ between groups throughout the experimental period (224 days). Cleavage rates of embryos derived from in vitro fertilization with semen of bulls fed ergotamine tartrate were reduced compared to controls; however, development of cleaved embryos to blastocyst did not differ between treatment groups. In conclusion, extended exposure of bulls to ergotamine tartrate appeared to reduce fertilization potential of sperm.

To study the effects of EAs on male reproduction, Pratt et al. (2015) exposed Angus bulls to endophyte-infected tall fescue (E+, n = 5, no quantification) or noninfected tall fescue (E−, n = 7, control) for 155 days. Bulls fed E+ showed decreased total gain, average daily gain and body weight compared with control bulls (E−). Sperm concentration and velocity were lower, and the number of abnormal sperm was higher. In addition, spermatozoa motility and progressive motility were decreased on thawing in semen samples. No differences were observed for serum testosterone concentrations.

Pregnant Hereford cross beef cows (n = 10 per group except for the highest concentration, n = 6) were fed a diet containing 5 (control), 48, 201 or 822 μg/kg of EAs (sum of six EAs: ergosine, ergocornine, ergocristine, ergocryptine,

ergotamine and ergometrine) for 9 weeks (Grusie et al., 2018). Concentrations of EAs up to 822 µg/kg did not alter the weight of peripartum and postpartum beef cows or nursing calves, rectal temperature or plasma prolactin concentrations. Ergot did not influence the time or the progesterone concentration at the time of first postpartum rise, or the size of the first and second follicles to ovulate. The maximum size of the first postpartum corpus luteum was 4 mm larger in the group fed 822 µg/kg ergot compared with the controls for the first ovulation postpartum, but not for the second. There was no effect of ergot exposure on the number of days until the appearance of the first or second corpus luteum postpartum. EA concentrations up to 822 µg/kg did not affect pregnancy rates. In conclusion, exposure to EAs for 9 weeks at concentrations up to 822 µg/kg did not alter performance in pregnant and postpartum beef cattle at moderate ambient temperatures.

Holstein Friesian bulls were fed with a diet contaminated with EAs (ergotamine 25%, ergocristine 15% and ergosine 13% as the main alkaloids) for 230 days. No carry-over of alkaloids was found in serum, abdominal fat, muscle, liver, kidney, bile or urine (Schumann et al., 2007). No EAs were detected in the milk of eight Holstein dairy cows collected after 4 weeks of exposure to contaminated diets (Schumann et al., 2009). The alkaloid exposure varied between 4.1 and 16.3 µg/kg bw per day. Milk was sampled through four consecutive morning and evening milkings at week 4 and pooled for analysis by HPLC with fluorescence detection (LOD: 5 or 10 µg/kg).

Angus beef steers grazed a low (LE; 23 µg/kg dry matter EAs mainly as ergovaline + ergovalinine, $n = 9$) or a high toxic endophyte (HE; 746 µg/kg of EAs, $n = 10$) tall fescue pasture (Brown et al., 2009). After 85 days of grazing, serum concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, lactate dehydrogenase (LDH) and prolactin were significantly lower for steers in the HE than in the LE group. At slaughter, hepatic content of cytosolic phosphoenolpyruvate carboxykinase was significantly greater in steers that grazed the HE than LE pastures. Hepatic content of AST was also greater, whereas renal and LM content were not. No differences were observed for hepatic, renal and LM content of AST, glutamate dehydrogenase, glutamine synthetase and three glutamate transport proteins. Longer hair, mud accumulation on hair coats, and decreased average daily weight gain were observed in steers grazing the HE forage for 85 days. They exhibited symptoms of the classic summer slump phenomenon associated with tall fescue toxicosis.

(c) Sheep

Suffolk ewes (n = 36) were exposed to endophyte-infected fescue seed (E+) or noninfected fescue seed (E–, control) during two stages of gestation (MID, days 35–85 and LATE, days 86–133) (Britt et al., 2019). Exposure to EAs (4.14 mg ergovaline + ergovalinine per kg, equivalent to 17.9–21.2 µg/kg bw ergovaline and ergovalinine) reduced prolactin levels. Exposure during LATE (days 86–133) gestation had the greatest impact on placental development by reducing uterine and placentome weights. Total fetal weight per ewe was significantly reduced for ewes fed E+ during LATE gestation compared with E–, but not in ewes exposed during MID gestation.

In lactating ewes (n = 8) exposed to ergovaline in the diet (497 ± 52 µg/kg, equivalent to 6.8 µg/kg bw) for 28 days, serum prolactin concentration was reduced but no consequences were observed on milk quantity or quality (Zbib et al., 2014). No ergovaline residues were found in milk collected on day 22 (LOD 0.15 µg/L) or in organs collected at the end of the study (LOD 0.15 µg/kg).

Canadian Arcott ram lambs (n = 8 per group) received experimental diets for 42 to 91 days. They were fed 34 (control), 930, 1402 or 2447 µg alkaloids/kg, based on total *R* and *S* epimers (ergocristine was the dominant *R* epimer, followed by ergometrine, with ergocristinine the dominant *S* epimer). Initial and final body weight, dry matter intake and feed efficiency did not differ among animals given the different treatments. Increasing alkaloid concentration caused a linear decrease in average daily weight gain. Compared to control lambs, rectal temperatures were 0.33 °C higher in lambs fed diets with added alkaloids. Serum prolactin concentrations declined linearly with increasing alkaloid concentration (Coufal-Majewski et al., 2017).

Vasoconstrictive activity of ergovaline and ergovalinine has also been reported in ovine uterine and umbilical arteries (Klotz et al., 2019).

(d) Horse

Gravid mares grazing endophyte-infested (E+) tall fescue exhibited increased gestation lengths, agalactia, foal and mare mortality, tough and thickened placentas, weak and dysmature foals, increased sweating during warm weather, reduced serum prolactin and progesterone, and increased serum estradiol-17β levels (Cross, Redmond & Strickland, 1995). Unlike many other species, horses consuming E+ tall fescue did not exhibit increased body temperature. Young horses consuming only E+ pasture did not gain as much weight as those consuming E– pasture.

(e) Rabbit

Korn et al. (2014) reported that EAs may have been the cause of tail necrosis observed in 14 out of 103 rabbits kept in outdoor group housing, fed with hay and a commercial pelleted feed. Feed analysis for EAs found a mean content of EAs of 410 ± 250 µg/kg and and a maximum content of 1700 µg/kg. Faeces of affected rabbits contained EAs at levels up to 200 µg/kg. The mean and maximum dietary intake of total EAs were 17 and 71 µg/kg bw, respectively. Fusarium toxins (trichothecenes, zearalenone and fumonisins) were also found in the feed, but at levels that did not explain the observed effects.

(f) Poultry

Pekin ducks (day 0–49, n = 54/group) received a diet with 0, 0.6, 7.0, 11.4 or 16.4 mg/kg diet of EAs (sum of 12 compounds dominated by ergotamine 26%, ergocristine 18%, ergocornine 5%, ergocryptine 5% and ergonovine (=ergometrine 5%) (Dänicke, 2015). At the beginning of the experiment, feed intake decreased significantly by 9%, 28%, 41% and 47% in treated groups compared to the control group. The experiment was terminated after 2 weeks for ducks exposed to the two higher concentrations owing to significant growth retardation. Residues of EAs in liver, breast meat and serum were below the LOQ (5 µg/kg). Ergonovine was the only EA detected in the bile, with a mean concentration of 40 ng/g in animals fed 7 mg/kg EAs. Monocyte proportions were significantly lower in all treatment groups compared to the controls, which might hint at an effect of EAs in modulation of immune responses. The author identified a lowest-observed-adverse-effect level (LOAEL) of 0.6 mg/kg diet.

Chickens (male broilers of the strain Lohmann Meat) received a diet with four levels of EAs up to 6.76 mg/kg (sum of 12 compounds, dominated by ergotamine 25%, ergocristine 22% and ergosine 10%) for 2 weeks (n = 80 per group) or 5 weeks (n = 72 per group) (Dänicke, 2017). Feed intake significantly decreased with increasing dietary ergot content. Feed intake was identified as the most sensitive end-point suitable for deducing both LOAEL and no-observed-adverse-effect level (NOAEL). Animals fed diets with the highest ergot level were partly unable to stand and displayed uncoordinated movements. Residues of EAs in liver, breast meat and serum were below the LOQ (5 µg/kg). No carry-over of EAs into egg yolk and albumen, blood, liver and breast muscle was found in laying hens fed with EAs up to 14.56 mg/kg diet (Dänicke, 2016). Most of the laying performance and reproductive traits were significantly compromised during the test period between 22 and 42 weeks of age when the diet with the highest EA content was fed.

Table 9
Biomarkers of exposure to EAs

Country, year	Population	Analytical method	EAs	LOD/LOQ (ng/mL)	Results detection (%) serum	Results detection (%) urine
EFCOVAL, 2006–2010; Belgium, Czech Republic, France, the Netherlands, Norway	600 adults (45–65 years); 188 with serum sample and 268 with urine sample	LC-MS/MS	Ergocornine	99/296	14 (5.2)	16 (8.5)
			Ergocorninine	99/296	38 (14.1)	22 (11.7)
			Ergocristine	99/296	19 (7.1)	14 (7.4)
			Ergocristinine	99/296	23 (8.6)	18 (9.6)
			Ergocryptine	99/296	4 (1.5)	9 (4.8)
			Ergocryptinine	50/99	22 (8.2)	16 (8.5)
			Ergometrine	60/99	12 (4.5)	6 (3.2)
			Ergometrinine	83/99	19 (17.1)	10 (5.3)
			Ergosine	99/209	32 (11.9)	10 (5.3)
			Ergosinine	99/889	30 (11.1)	17 (9.0)
			Ergotamine	99/296	14 (4.2)	9 (4.8)
			Ergotaminine	99/296	1 (0.4)	10 (5.3)

EFCOVAL, European Food Consumption Validation project; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation.
Source: De Ruyck et al. (2020).

2.4 Observations in humans

2.4.1 Biomarkers of exposure

Few studies of biomarkers of exposure to EAs were available. De Ruyck et al. (2020) measured EAs in serum and urine of 600 men and women participating in the European Food Consumption Validation (EFCOVAL) project (De Ruyck et al., 2020). In addition, 24-hour dietary recall interviews were conducted twice, 1 month apart, and matched against the EFSA database on mycotoxin contamination to estimate exposure to EAs. Participants provided a non-fasting blood sample approximately 1 week before the first 24-hour dietary recall interview. Urine samples were collected during the 24-hour period following the 2 days on which participants had been asked for a 24-hour dietary recall. Six EAs and their -inine epimers were included in the study. Overall, EAs were detected in 116 (out of 268) serum samples and 106 (out of 188) urine samples (Table 9).

Participants in the EFCOVAL project had a median daily exposure (ng per kg bw) of 41.4 for EAs, overall. The median exposure ranged from 1.44 for ergometrinine to 24.0 (ergocryptine plus ergocryptinine). Of 188 participants with both urine and dietary exposure estimates, the agreement between the two measurements was 53% for EAs overall, and ranged from 5% (ergocryptine) to 72% (ergometrine). Spearman correlations were generally not statistically significant, and most were < 0.1. Of the 268 participants with serum measurements, agreement between the two measurements was 51% for EAs overall, ranging from 1%

(ergocryptine) to 74% (ergometrinine and ergocryptinine). Spearman correlation coefficients were generally < 0.1 with the exception of a significant correlation reported for ergotamine ($\rho = 0.15$). Across all mycotoxins evaluated (58 analytes, including parent compounds and metabolites), Cohen's κ statistic indicated only slight ($\kappa < 0.1$) agreement between exposure estimates and concentrations in serum or urine (De Ruyck et al., 2020). These findings suggest that biological measurements were generally not sufficient for describing chronic dietary exposure, but they may provide information on single exposures.

In a study describing technical issues pertaining to the use of LC-MS to measure ergotamine (Favretto et al., 2007), the method was applied to a 40-year-old woman with a 7-day history of clinical ergotism. The woman had taken an oral preparation containing 1 mg ergotamine tartrate and 100 mg caffeine per tablet for prophylaxis and as a rescue treatment. Measured levels of ergotamine in urine, blood and hair were as follows: urine 100 pg/mL, blood 320 pg/mL, proximal hair 24 pg/mg and distal hair 15 pg/mg. Although the exact dose and time of oral treatment in relation to sample collection was not reported, it can be assumed that the patient had been using ergotamine chronically. The authors concluded that the presence of ergotamine in both proximal and distal hair samples was consistent with prolonged use of ergotamine (roughly 2 years, considering an average hair growth rate of 0.6 to 1.4 cm/month), which is consistent with the patient's reported clinical history.

2.4.2 Biomarkers of effect

No studies of biomarkers of effect were identified.

2.4.3 Clinical observations

Data pertaining to the use, dose and precautions for the medicinal use of ergotamine and ergometrine are summarized in Table 10, based on information in *Martindale's complete drug reference* (Martindale, 2010). Ergotoxine esilate and ergotoxine phosphate were formerly used as an oxytocic and in the treatment of migraine. Ergotoxine is a mixture of naturally occurring EAs including ergocornine, ergocristine and ergocryptine. Although semi-synthetic EAs were screened and considered for inclusion in this monograph, the Committee concluded that they were not relevant because their kinetic properties are different from those of the naturally occurring EAs that may be present in food and feed.

2.4.3.1 Ergotamine

Adverse effects

Ergotamine is recommended for treating patients with migraine who experience infrequent attacks, long duration headaches, and are likely to adhere to the

Table 10
Summary of use, dose, and precautions for the medicinal use of ergotamine tartrate and ergometrine maleate

EA	Uses	Oral dose	Precautions
Ergotamine tartrate	Migraine and cluster headache	1 to 2 mg, repeated 30 minutes later; no more than 4–6 mg in 24 hours, no more than 8 mg per attack, no more than 12 mg per week, no more than two courses per month.	Contraindicated in people with hypertension, shock, severe or persistent sepsis, PVD, IHD, temporal arteritis, hyperthyroidism, hepatic or renal impairment, basilar or hemiplegic migraine, and pregnant and breastfeeding women. Increased risk of peripheral vasoconstriction if used with beta-blockers and CYP3A4 substrates (azole antifungals, macrolide antibiotics, HIV-protease inhibitors).
Ergometrine maleate	Induction of uterine contraction and prevention of postpartum haemorrhage in the third stage of labour	Oral dosage 0.2 mg initially, and up to 0.4 mg 2–4 times daily. Dose may be administered for 2–7 days. Also administered by intravenous and intramuscular routes.	Contraindicated in people with hypertension, heart disease venoatrial shunts, mitral valve stenosis or obliterative vascular disease, sepsis, hepatic or renal impairment. Contraindicated in the first stage of labour and in patients with pre-eclampsia, eclampsia, or at risk of preterm birth.

CYP3A4, cytochrome P450 3A4; HIV, human immunodeficiency virus; IHD, ischaemic heart disease; mg, milligram; PVD, peripheral vascular disease.
Source: *The complete drug reference, ergotamine tartrate, ergometrine maleate* (Martindale, 2010).

dosing restrictions (Silberstein & McCrory, 2003; Tfelt-Hansen & Diener, 2014). The adverse effects of ergotamine are related to its effects on the CNS and its vasoconstrictive properties (Schiff, 2006; Martindale, 2010; Tfelt-Hansen & Diener, 2014). At therapeutic doses, nausea and vomiting are common (Silberstein & McCrory, 2003). Weakness, muscle pain in the extremities, and numbness and tingling of the fingers and toes may also occur.

At greater than therapeutic doses, vasoconstriction, cardiovascular effects, gangrene, confusion and convulsions have been reported. As summarized by Martindale (2010), "symptoms of acute overdosage include nausea, vomiting, diarrhoea, extreme thirst, coldness, tingling and itching of the skin, a rapid and weak pulse, hypertension or hypotension, shock, confusion, convulsions and unconsciousness; fatalities have been reported". Side-effects typically occur as a result of prolonged use for migraine headaches rather than following acute single doses. Prolonged use or overuse of ergotamine can result in "severe circulatory disturbances". Symptoms of ergotism associated with overdosage or poisoning include numbness, cold, tingling, pale or cyanotic extremities accompanied by muscle pain, with the possibility that gangrene may develop (Martindale, 2010). Cardiovascular symptoms including angina, tachycardia or bradycardia, hypertension or hypotension, and myocardial infarction have been reported and vasoconstriction of blood vessels in the brain, eye, intestines and kidneys may also occur (Fisher et al., 1985; Martindale, 2010; Deviere, Reuse & Askenasi, 1987; Galer et al., 1991; Lazarides et al., 1992; Redfield et al., 1992). Symptoms of

Table 11
Case reports of adverse effects associated with ergotamine

Reference	Dose and duration	Adverse effects
(Sran & Vathsala, 2016)	Ergotamine tartrate (1 mg/100 mg caffeine tablets) intermittently over 20 years. Recent use of daily tablet with three tablets in 24 hours before admission	A 54-year-old male presented with severe sudden onset left loin pain resulting from renal infarction
(Pérez Baztarrica et al., 2019)	Ergotamine use for migraine, 8 mg within 12 hours before the hospital admission (formulation not reported)	A 48-year-old female presented with acute coronary syndrome; i.e. inferior and posterior ST-segment elevation myocardial infarction
(Pakfetrat et al., 2013)	Ergotamine tartrate (2–4 mg) daily as needed for 4 years, for migraine treatment	A 22-year-old female presented with hypertension, renal failure and tubulo-interstitial nephritis on biopsy. Neurological examination was normal
(Maréchaux et al., 2015)	1–3 mg ergotamine for 30 years	Mitral regurgitation during exercise echocardiography in a 67-year-old female with a history of hypertension and migraine
(Murad, Miller & Glockner, 2011)	Ergotamine–caffeine (dose not reported) suppositories used on average three times per week for 12 years	A 43-year-old woman presented with increasing shortness of breath and bilateral leg oedema of 3 months duration. Multi-valvular heart disease and retroperitoneal fibrosis was demonstrated
(Patel et al., 2013)	Extensive use of ergotamine (10 years), dose not reported	A 47-year-old female presented signs and symptoms of decompensated heart failure; echocardiogram showed aortic and mitral valvulopathy
(Bois et al., 2012)	High-dose ergotamine suppositories over 34 years (dose not reported)	Valvulopathy (mitral stenosis and recurrent chylous pleural effusion) in a 55-year-old woman
(Küçükalp, Durak & Bilgen, 2013)	4–5 ergotamine tartrate (0.75 mg) tablets daily for 10 years	Vasospasm affecting arteries in the lower limb 10 days post-trauma in a 46-year-old male

peripheral vasoconstriction or of cardiovascular disturbances, as seen in chronic ergotamine poisoning, may also occur following acute overdosage (Martindale, 2010). Other adverse effects include confusion and convulsions (Martindale, 2010). In rare cases, pleural and peritoneal fibrosis and fibrosis of the cardiac valves have been reported (Lepage-Savary & Vallières, 1982; Robert et al., 1984; Damstrup, 1986; Martindale, 2010).

Several case reports involving patients who developed ergotism following prolonged oral administration of ergotamine tartrate (i.e. 1–6 mg/day for 2 weeks up to 21 years) were described by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) (EFSA, 2012). Cases of ergotism following rectal and sublingual administration of ergotamine tartrate were also described (EFSA, 2012). Seminerio et al. (2014) described the potential for ergotamine to induce localized ischaemia leading to ischaemic colitis. Additional reports of adverse effects among patients using ergotamine are listed in Table 11.

Drug interactions

Because ergotamine is metabolized by CYP3A4, its effects can be altered by co-administration of other agents that are also metabolized by CYP3A4 such as azole antifungals, macrolide antibiotics, and HIV-protease inhibitors (Martindale, 2010; EFSA, 2012). Information relevant to interactions of EAs with drugs used for antiviral therapy in patients with HIV and interactions with stimulants such as cocaine is discussed by Ayarragaray (2014). Srisuma, Lavonas & Wananukul (2014) studied a retrospective case series using data from all patients referred to a poison centre in Bangkok, Thailand due to ergotism from January 2006 to August 2013. Nine of the 12 cases identified involved interactions between ergotamine (1 mg ergotamine/100 mg caffeine combination tablets) and CYP3A4 inhibitors (Srisuma, Lavonas & Wananukul, 2014). Most patients presented with symptoms of vascular insufficiency, such as cooling, numbness, pain and pulse deficits in distal limbs but recovered following treatment. A 49-year-old female with HIV in this case series developed acute kidney injury and rhabdomyolysis and died from cardiac arrest. Additional recent case reports of interactions between ergotamine and drugs used to treat HIV and macrolide antibiotics are summarized in Table 12.

Pregnancy

Ergotamine is contraindicated during pregnancy because it can cause premature labour and vasoconstriction (Martindale, 2010). EFSA (2012) reviewed reports of accidental exposure to ergotamine during pregnancy that resulted in uterine contraction, fetal tachycardia, arrested cerebral maturation and Moebius syndrome (EFSA, 2012). Most of the women affected were inadvertently given suppositories containing 2 mg ergotamine and 100 mg caffeine. In addition, one case of fatal jejunal atresia was reported in an infant born preterm (35 weeks gestation) to a mother who had taken ergotamine tartrate (6–8 mg/day) in tablet form throughout her entire pregnancy (Graham, Marin-Padilla & Hoefnagel, 1983). A more recent case report describes a neonate delivered by caesarean section at 32 weeks gestation, with unilateral renal agenesis, urethral atresia and pulmonary hypoplasia, whose mother took oral ergotamine tartrate (0.75 mg plus 80 mg caffeine tablets, 1.5 mg daily) during her first trimester of pregnancy (Demirel et al., 2012). The infant died due to cardiopulmonary arrest after 13 hours.

2.4.3.2 **Ergometrine**

Adverse effects

Ergometrine maleate is used to induce uterine contractions and prevent haemorrhage in the third stage of labour and for prevention and treatment of

Table 12
Case reports of drug interactions with ergotamine

Reference	Dose and duration	Adverse effects
Navarro et al. (2017)	1 mg ergotamine plus 100 mg caffeine for 5 days in combination with antiretroviral therapy	Acute leg ischaemia in a 33-year-old male patient with HIV
Ferry et al. (2014)	Two tablets each containing 1 mg ergotamine tartrate plus 100 mg caffeine six times daily for 3 days in combination with highly active antiretroviral therapy (after discontinuing treatment 2 years previously)	Ankle pain resulting in amputation in a 32-year-old female with HIV
Fröhlich, Kaplan & Amann-Vesti (2010)	1 mg ergotamine tartrate tablet per day for 2 weeks in combination with antiretroviral therapy	Paraesthesiae and coldness of the left upper extremity consistent with ergotism in a 29-year-old male positive for HIV
Iardino et al. (2018)	Ergotamine-containing medication (dose not reported) in combination with antiretroviral therapy	A 34-year-old male positive for HIV presented with severe vasospasm resulting in left foot pain and swelling (i.e. a cold, purple, left foot, and absent pedal and tibial pulses, and no arterial flow from the popliteal artery)
Reghukumar & Benson (2020)	Ergotamine twice daily (dose not reported) 4 days before presentation in combination with antiretroviral therapy	A 24-year-old female presented with severe, burning pain in the legs. Diffuse, symmetric narrowing of the arteries in both legs was observed. Perfusion improved 2 weeks after initial presentation
Tseng et al. (2010)	Three tablets each containing 1 mg ergotamine plus 100 mg caffeine in combination with macrolide antibiotic (erythromycin)	A 35-year-old woman presented with acute paraesthesia, diffuse vasospasm of the femoral arteries and the right brachial artery visible on angiogram
Demir et al. (2010)	Ergotamine tartrate for 3 years (dose not reported) in combination with erythromycin for 3 days	Pain and mild cyanosis resulting from arterial vasospasm in an 18-year-old female
Ozpelit et al. (2016)	Ergotamine tartrate (1.5 mg/day for the past 5 days) in combination with a 14-day course of clarithromycin	A 53-year-old female with Takotsubo cardiomyopathy
Adam et al. (2014)	Ergotamine (long-term use, dose not reported) with macrolide antibiotic for 4 days	Vasospastic ischaemia with acute arterial embolism

postpartum haemorrhage. 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 (Sanders-Bush & Mayer, 2006). Uterotonic effects can be observed in postpartum women within 10 minutes after oral administration of 0.2 mg of ergometrine; however, there may be a wide variation in patient response (Sanders-Bush & Mayer, 2006).

Nausea and vomiting are common side-effects of ergometrine maleate when used at normal therapeutic doses (Martindale, 2010). Less common effects include abdominal pain, diarrhoea, headache, dizziness, tinnitus, chest pain, palpitations, bradycardia and other cardiac arrhythmias, coronary artery vasospasm, myocardial infarction, dyspnoea and pulmonary oedema (Martindale, 2010; EFSA, 2012). Intravenous administration has been associated with rapid increases in blood pressure (Martindale, 2010; EFSA, 2012). Overdosages may cause seizures and gangrene as well as gastrointestinal symptoms, dizziness,

Table 13
Case reports of adverse effects associated with ergometrine use

Reference	Dose and duration	Adverse effects
Wang, Liu & Chen (2017)	Oral ergometrine (0.2 mg), four doses	Transient sick sinus syndrome with complete atrioventricular block with junctional escape rhythm in 38-year-old woman treated with oral ergometrine to induce uterine contractions
Birch & Lu (2019)	Syntocinon followed by 250 IU ergometrine administered intravenously	Atrial fibrillation in 36-year-old woman undergoing caesarean section
Johnston & Hughes (2013)	Oxytocin (5 IU) and 0.5 mg ergometrine maleate administered intramuscularly postpartum	Bronchospasm in non-asthmatic patient

increased blood pressure, loss of consciousness, numbness in the extremities, chest pain and hypercoagulability (Martindale, 2010; EFSA, 2012).

Recent case reports are summarized in Table 13. Wang, Liu & Chen (2017) reported a case of transient sick sinus syndrome following oral exposure to ergometrine, whereas the other reports listed in Table 13 describe effects following intravenous and intramuscular administration of ergometrine (Johnston & Hughes, 2013; Birch & Lu, 2019).

2.4.3.3 Ergotoxine

Adverse effects

Ergotoxine is a mixture of ergocornine, ergocristine and ergocryptine. Ergotoxine esilate and ergotoxine phosphate were formerly used as oxytocics and in the treatment of migraine (Martindale, 2010). The effect of ergocornine (2 mg during the post-ovulatory phase) on progesterone metabolism and its potential as an oral inhibitor of implantation (at a dose of 2–20 mg) have been investigated, but the results from the studies identified were inconclusive (Shelesnyak et al., 1963; Koi et al., 1966; Lindner et al., 1967; Floss, Cassady & Robbers, 1973; EFSA, 2012). No new studies of ergotoxine or its components (for example, ergocornine) were identified.

2.4.4 Epidemiological studies

2.4.4.1 Ergotamine

As reported in a review by Tfelt-Hansen et al. (2000), ergotamine induces bradycardia and can produce coronary vasoconstriction, ischaemia and angina in coronary heart disease (Tfelt-Hansen et al., 2000). A more recent review identified two observational studies (Velentgas et al., 2004; Wammes-van der Heijden et al., 2006) that reported serious cardiovascular effects (i.e. myocardial infarction, stroke, ventricular arrhythmia and transient ischaemic attack) in association with intensity, but not recency, of ergotamine use. A pooled risk estimate of 2.28 (95%

confidence interval (CI): 1.18–4.41) for the association of ergotamine intensity with serious cardiovascular effects was determined (Roberto et al., 2015). Velentgas et al. (2004) defined current, recent and non-use categories for EAs. The exposure intensity metric was defined as the total days that the medication was dispensed within the previous 6 or 7–12 months. Roberto et al. (2015) used the effect estimate from the study by Velentgas et al. (2004) for the highest intensity category (≥61 days supplied) in the previous 6 months to derive the pooled estimate (odds ratio (OR): CI: 1.47, 0.34–6.27). Velentgas et al. (2004) did not observe a dose–response relationship across the intensity categories (i.e. the OR for the association between EAs dispensed for 11–26 days in the previous 6 months was 4.54 (95% CI, 2.26–9.10) and no associations were observed in some higher and lower categories). Wammes-van der Heijden et al. (2006) defined intensity as the number of prescribed doses within the year before the index event (i.e. hospitalization for ischaemic complications), including all prescribed ergotamines. The highest intensity category (≥ 90 daily doses), indicating the overuse of ergotamine, was associated with ischaemic complications in this study (OR, 2.55; 95% CI, 1.22–5.36). Risk estimates for a specific agent or formulation were not presented in either study (i.e. ergotamine, dihydroergotamine and possibly methysergide, alone or in combination with other drugs were included in the analyses).

Poisonings

Several retrospective studies of ergotamine poisoning were identified. Robblee et al. (2020) analysed intentional overdoses of ergotamines as a single agent or in combination with other drugs reported to the United States National Poison Data System from 2014 to 2018. Of 48 reports of poisonings involving EAs, 16 involved oral exposure to ergotamines as a single agent and were included in the authors' main analysis. Major effects or death were not reported in association with these EA overdoses. Common symptoms included abdominal pain, vomiting, numbness, nausea, diarrhoea and vertigo. Although exposures were characterized as intentional overdoses, no dose estimates were provided.

Srisuma, Lavonas & Wananukul (2014) conducted a retrospective study of ergotism cases referred to the poison centre in Bangkok, Thailand (January 2006–2013). A total of 378 EA exposures were identified with 12 subjects showing signs and symptoms of ergotism. All of the symptomatic cases and most (318 of 366) of the asymptomatic cases involved the ingestion of 1 mg ergotamine (plus 100 mg caffeine). Other asymptomatic cases involved ingestion of tablets containing 0.1 mg ergotamine/20 mg phenobarbital (n = 29) or 1 mg ergotamine as a single agent (n = 17). Of the 12 exposures that resulted in symptoms, all involved 1 mg ergotamine/100 mg caffeine tablets and most (n = 9) were the result

of interactions with CYP3A4 inhibitors. The remaining cases included suicide attempts ($n = 2$) and unsupervised ingestion (unknown dose) by a 15-month-old boy who presented in a continuous state of seizure.

Exposures of children to EAs reported to the California Poison Control System from 1997 to 2008 were analysed to determine the risk of toxicity due to oral exposure in children less than 7 years old (Armenian & Kearney, 2014). Median doses and ranges reported in this study were based on estimated maximum possible doses that were determined using reported pill counts and observer accounts. Only dose estimates coded as "certain" were included in the analysis and doses were missing for 97 of 256 patients with symptoms. Of 56 reports of ergotamine exposure, seven (13%) resulted in gastrointestinal symptoms (nausea, vomiting, abdominal pain and/or diarrhoea), whereas eight (14%) resulted in other (CNS or respiratory) symptoms. None of the cases were characterized as serious; the median dose among the children with clinical symptoms was 1 mg (range: 0.2–11 mg). Although there were 61 reports of co-exposure to caffeine, the authors noted that this information was incomplete. There were also 15 reports of exposure to dihydroergotamine resulting in one case involving gastrointestinal symptoms and two cases involving other CNS or respiratory symptoms (median dose among the children with clinical symptoms: 1 mg (range: 0.25–3 mg)).

Pregnancy

As discussed previously, ergotamine is contraindicated in pregnancy. Amundsen et al. (2015) reviewed studies of ergotamine exposure among pregnant and lactating women and identified only one eligible study. In this case–control study, Bánhidy et al. (2007) found an association of ergotamine with low birth weight (OR, 2.8; 95% CI, 1.2–6.5) and preterm birth (OR, 1.9; 95% CI, 1.0–4.0), but the authors did not control for maternal smoking, a known cause of low birth weight (Bánhidy et al., 2007). The women in this study received ergotamine drops or tablets. Mean daily dose of ergotamine ranged from 0.3 mg (tablets) to 1.5 mg (drops) for 1 day to 7 months.

No increase in the overall incidence of congenital abnormalities was found in a study of 924 children of women who had migraine headaches, 71% of whom were said to have taken ergotamine at some time during pregnancy (month and trimester not stated); the number of children with abnormalities in the cohort was 31 (Wainscott, 1978). Two analyses of the Hungarian Case-Control Surveillance of Congenital Abnormalities dataset reported associations of ergotamine use during pregnancy with neural tube defects. In the first analysis of 1202 infants with neural tube defects, there was an association with maternal use of ergotamine during pregnancy (OR, 3.3; CI, 1.4–7.7), based on six cases

(Medveczky & Czeizel, 2004). A later analysis, which was based on three cases, found an association of ergotamine drops (mean dose 1.5 mg/day during the second month of pregnancy) with neural tube defects (OR, 6.9; CI, 2.0–24.2) (Ács et al., 2006).

2.4.4.2 **Ergometrine**

Liabsuetrakul et al. (2018) sought to compare the use of ergometrine (or methylergometrine) by any route with no intervention to determine the efficacy and side-effect profile. Of the eight studies identified one examined oral ergometrine (De Groot et al., 1996). De Groot et al. (1996) conducted a randomized trial to determine whether oral ergometrine reduced postpartum blood loss by 30% and found that blood loss was reduced by a smaller amount. The ergometrine group received 0.4 mg (two tablets of 0.2 mg). Among women receiving oral ergometrine whose blood pressure was measured, no significant elevation of blood pressure was observed (no other end-points were measured). Another systematic review (Gallos et al., 2018) was designed to identify the most effective drugs to prevent postpartum haemorrhage and examine their side-effect profiles. The study reviewed 140 trials overall, including 21 studies of ergometrine. The typical dosage was 500 μg ergometrine plus 5 IU oxytocin administered intramuscularly. The side-effect profile for ergometrine was relatively poor compared to other drugs. An association with hypertension was reported (Gallos et al., 2018).

Poisoning

Reports of ergometrine maleate poisonings following accidental administration to neonates were reviewed by the EFSA CONTAM Panel (EFSA, 2012). Effects reported included peripheral vasoconstriction, encephalopathy, convulsions, respiratory failure, acute renal failure and temporary lactose intolerance (EFSA, 2012). Over the long term most infants recovered, with the exception of one infant who died after receiving an oral dose of 0.2 mg ergometrine maleate (AHFS, 1995; EFSA, 2012). More recently, exposures of children to EAs reported to the California Poison Control System from 1997 to 2008 were analysed to determine the risk of toxicity due to oral exposure in children less than 7 years old (Armenian & Kearney, 2014). The maximum possible dose was estimated from information provided regarding pill counts and observer accounts. A total of 353 ergot exposures were included in the analysis. Most (351 of 353) exposures were due to ingestion by the oral route. As shown in Table 14, there were reports involving exposure to ergometrine ($n = 37$), methylergometrine ($n = 231$) or methysergide ($n = 3$) totalling 271 reports. Forty-two (42) of the 271 reported exposures (15.5%) resulted in gastrointestinal symptoms, whereas 15

Table 14
Clinical effects and doses of EAs reported to the California Poison Control System (*n* = 271 children < 7 years old, 1997–2008)

EA (*n*)	Gastrointestinal symptoms N	Other symptoms[a] N	Median dose (range) (mg)
Ergometrine (37)	4	1	0.4 (0.2–2)
Methylergometrine (231)	37	13	0.4 (0.1–3)
Methysergide (3)	1	1	2 (2)

[a] Other symptoms include central nervous system and respiratory effects.
Source: adapted from Table 3 of Armenian & Kearney (2014).

(5.5%) resulted in CNS or respiratory symptoms. Two patients presented with significant vascular and CNS symptoms that eventually resolved:

1) a 2-year-old child who ingested an unknown quantity of 0.2 mg methylergonovine tablets was lethargic with cool, pale extremities and prolonged capillary refill time when she or he was admitted to the hospital (temperature 36.9 °C, pulse 90 beats per minute, blood pressure 94/60 mmHg, respirations 20 per minute, oxygen saturation 82%); and
2) a 3.3-kg newborn who received 0.2 mg of intramuscular methergine (methylergonovine maleate) experienced respiratory depression with oxygen saturation of 80% (temperature 36.7 °C, pulse 120 to 140 beats per minute, blood pressure 73/43 mm Hg, and a normal respiratory rate).

In another study, Davanzo et al. (2015) examined cases of inadvertent oral administration of methylergometrine maleate to children up to 5 years old which were reported to the national poison centre in Milan, Italy. A total of 642 cases of unintentional poisoning were reported during the study period. The most common symptoms included vomiting and abdominal pain. Also reported were hyperactivity, tachycardia and cyanosis. The severity of clinical symptoms reported for most cases was minor (i.e. mild, transient, spontaneously resolved (n = 43)). Infants aged 1 year or younger with minor symptoms included those exposed to doses ranging from 0.003 to 0.050 mg/kg bw. Older children (1–5 years old) with minor symptoms were exposed to doses ranging from 0.003–0.089 mg/kg bw. Moderate symptoms (pronounced or prolonged) were reported in infants (<1 year old) who were exposed to doses ranging from 0.11 to 0.86 mg/kg bw, including three who experienced repeated exposures. Severe poisoning (i.e. cyanosis, apnoea, coma and cardiac arrest) was reported in one child <1 week old exposed to 0.100 mg/kg bw for 4 days. Sepsis-like symptoms and encephalopathy

were reported in a case-series study of 12 newborns exposed to intramuscular methylergonovine in a hospital in Türkiye (Bas et al., 2011).

2.4.4.3 **Ergotoxine**

Poisoning

Exposures of children to EAs, reported to the California Poison Control System from 1997 to 2008, were analysed to determine the risk of toxicity following oral exposure in children less than 7 years old (Armenian & Kearney, 2014). There was one report of dihydroergotoxine exposure (dose 1 mg), which did not result in symptoms.

2.4.4.4 **Sclerotia of *Claviceps* spp.**

Two forms of ergotism can occur concurrently, namely, vasospastic gangrenous and convulsive forms (EFSA, 2012). Lactation inhibition has also been reported in association with outbreaks of ergotism. Preparations of sclerotia of *C. purpurea* in tablet or liquid form have been used to accelerate labour. Single doses ranged from 0.2–3 mg and daily doses from 6–7.5 mg (EFSA, 2012). The practice of oral administration of ergot by midwives in Europe was discontinued because it was associated with an increased risk of stillbirth.

Acute poisoning events are rare; the convulsive form of ergotism has not been reported in Europe for more than a century (EFSA, 2012). The last reported outbreak of gangrenous ergotism noted by EFSA occurred in Ethiopia in 2001. EFSA (2012) and WHO-ICPS (1990) described poisonings associated with grain contaminated with *C. purpurea*. Large outbreaks killing thousands of people occurred in Europe between the ninth and eighteenth centuries. The most recent outbreaks in Europe occurred in the United Kingdom and Russia (1926–1928). Overall, ergot concentrations of 0.1% were thought to be harmless, whereas concentrations of 1% were associated with the risk of toxicity, and concentrations of 7–10% were associated with mortality (EFSA, 2012). The types of ergotism associated with these outbreaks were the vasospastic gangrenous and convulsive forms and some outbreaks were associated with high rates of infant mortality due to the inhibition of lactation in mothers (Wirth & Gloxhuber, 1981; WHO-ICPS, 1990; EFSA, 2012).

The most recent outbreaks identified occurred in Ethiopia in 1978 and 2001 (EFSA, 2012; Belser-Ehrlich et al., 2013). The outbreak in 1978 was associated with 2–3 months of exposure to grain with 0.75% ergot content (i.e. ergometrine detected by thin layer chromotography) (Demeke, Kidane & Wuhib, 1979; King, 1979; Belser-Ehrlich, 2012; EFSA, 2012). The 2001 outbreak was associated with concentrations ranging from 2.1–26.6 mg ergotamine/kg and 0.9–12.1 mg ergometrine/kg (Urga et al., 2002; EFSA, 2012; Belser-Ehrlich et al.,

2013). Finally, two case reports have been published. One described exposure of a 13-year-old girl who ate muesli contaminated with 12% sclerotia of *C. purpurea* for several months. The other described a farmer exposed via inhalation to ergotamine-containing millet dust, resulting in high plasma concentrations of ergotamine (Stange et al., 1996; EFSA, 2012).

Outbreaks associated with *C. fusiformis* in contaminated pearl millet have also occurred in India, including outbreaks in the twentieth century. The concentrations reported in unaffected villages ranged from 1–38 g ergot/kg (15–26 mg/kg total EA content) whereas concentrations in affected villages ranged from 15–175 g ergot/kg (15–199 mg/kg total EA content) (Krishnamachari & Bhat, 1976; WHO-ICPS, 1990; EFSA, 2012).

3. Analytical methods

The following sections review analytical methods for EAs in cereals and cereal products, with particular emphasis on the period since 2000. Databases searched for publications since 1980 were Scopus and PubMed with the terms "ergot" and "determination", resulting in 3597 and 963 hits, respectively. The Scopus search was further restricted by limiting it to chemistry and the term "alkaloids" (1131 hits). In addition, information has been drawn from the annual summaries of advances in mycotoxin analytical methods previously published as general referee reports of the Association of Official Analytical Chemists International and subsequently by the *World mycotoxin journal*. Recent reviews have also covered the topic (Komarova & Tolkachev, 2001b; Scott, 2007; Krska & Crews, 2008; EFSA, 2012; Crews, 2015).

3.1 Chemistry

The first EA to be identified was ergotamine in 1920 and since then about 50 more have been discovered (Lorentz, 1979; Flieger, Wurst & Shelby, 1997). They are produced, among others, by species of the genus *Claviceps*, which infect a range of cereal crops, including rye, wheat, barley, millet, oats and sorghum. The EAs (also known as ergolines) are found in the fungal sclerotia in amounts that range between 0.01 and 0.5% by weight. The alkaloid constituents and quantities depend on factors such as fungal strain, geographical region and plant host (Lorentz, 1979).

The EAs are characterized by the ergoline ring system consisting of four fused rings (Flieger, Wurst & Shelby, 1997; Komarova & Tolkachev, 2001a). 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, the major ones being divided into four groups. Firstly, there are those derived from precursors of lysergic acid (see Fig. 1), termed clavine alkaloids, such as agroclavine (8,9-didehydro-6,8-dimethylergoline). The second group are simple lysergic acid derivatives, such as ergometrine (D-lysergic acid-L-propanolamide, also known as ergonovine). The third group are the peptide alkaloids or ergopeptines (ergopeptides, cyclol EAs), in which the substituent at C8 is a cyclized tripeptide containing a variable α-hydroxyl amino acid, a second variable L-amino acid and L-proline. The fourth group are the ergopeptames (noncyclol lactam EAs), which also contain a tripeptide substituent, but lack the α-hydroxy group on the first amino acid and the second amino acid is cyclized with D-proline. The EAs with a C9,10 double bond can readily undergo enolization to form epimers with the opposite chirality at C8 (Komarova & Tolkachev, 2001a; Smith & Shappell, 2002).

The 12 EAs most commonly associated with contaminated cereals and hence with food safety are the lysergic acid derivative, ergometrine, and the ergopeptides, ergocornine, ergocristine, ergotamine, ergocryptine and ergosine, as well as their epimers, ergometrinine, ergocorninine, ergocristinine, ergotaminine, ergocryptinine and ergosinine (EFSA, 2012). Also, ergocryptine and ergocryptinine can occur as both an α- and a β-analogue, although these are seldom distinguished in analysis.

The EAs are generally soluble in organic solvents (methanol, acetonitrile, organic solvent/buffer mixtures) and are charged in acid pH and uncharged in neutral or alkaline pH (Krska & Crews, 2008; Krska et al., 2008; Crews, 2015). Those with a C9,10 double bond have natural fluorescence properties (Flieger, Wurst & Shelby, 1997; Komarova & Tolkachev, 2001b; Scott, 2007). Two factors of importance in their determination are their light sensitivity and the fact that the epimerization reaction at C8 can proceed in both directions and occurs during long storage and readily during chemical analysis, such that both epimers need to be determined (Komarova & Tolkachev, 2001a; Lampen & Klaffke, 2006).

3.2 Description of analytical methods

3.2.1 Introduction

EAs may be extracted with non-polar organic solvents under alkaline conditions or polar solvents under acidic conditions (Komarova & Tolkachev, 2001b; Scott, 2007; Krska & Crews, 2008; EFSA, 2012). Accelerated solvent extraction has also been used (Kokkonen & Jestoi, 2009). Sample purification has been achieved by either liquid/liquid partitioning exploiting the basic tertiary N6 amino group (Scott & Lawrence, 1980), reversed-phase (C18) solid phase extraction (SPE)

(Fajardo et al., 1995), strong cation exchange SPE (Storm et al., 2008; Reinhold & Reinhardt, 2011), mixed cation/reversed-phase SPE (Reinhard, Rupp & Zoller, 2008), Extrelut® columns (Baumann, Hunziker & Zimmerli, 1985), silica gel columns (Rottinghaus et al., 1993) or immunoaffinity columns (IAC) (Kokkonen & Jestoi, 2010). In addition, a molecularly imprinted polymer has been developed for the alkaloids of interest and inserted as polymerized beads in an SPE column (Lenain et al., 2012). An aptamer-functionalized silica gel has been trialled but showed activity for only a limited number of the alkaloids (Rouah-Martin et al., 2014).

The earliest tests for EAs were performed using simple colorimetric reagents, resulting in the determination of total alkaloid content (Robbers et al., 1975; Young, 1981). These tests were performed by adding aqueous succinic acid to the dried extract, followed by modified van Urk's reagent (*p*-dimethylaminobenzaldehyde and aqueous ferric chloride in aqueous sulfuric acid). The resultant blue colour was measured at 580 nm. The method was improved with respect to reaction time and sensitivity by replacing the ferric chloride with sodium nitrite (Michelon & Kelleher, 1963). Subsequent development of chromatographic methods allowed the individual alkaloids to be separated and individually quantified.

Official control of ergot is usually based on the presence by weight of sclerotia in a grain sample as determined by visual inspection. The determination of ricinoleic acid has been used as a surrogate marker for ergot content, as it is a major component of the fatty lipids in *Claviceps* sclerotia (Franzmann et al., 2010). Following lipid extraction and hydrolysis, ricinoleic acid in rye was determined as the silyl derivative by gas chromatography (GC) with flame ionization detection. Although capable of detecting ergot impurities at concentrations as low as 0.01%, there is no correlation with total EAs owing to the variability of the levels of the latter (Franzmann et al., 2010).

Although more a physical technique than analytical chemistry, near-infrared spectroscopy (NIR) has been used to determine EA content in tall fescue (Roberts et al., 2005, 2009). NIR hyperspectral imaging has been used to identify sclerotia in cereal grains (Vermeulen et al., 2009) and has been applied to scanning wheat on a conveyor belt (Vermeulen et al., 2012). The method was further validated for wheat, rye, rapeseed, straw and barley straw (Vermeulen et al., 2013). Extension of the technique to wheat flour showed potential but was variable at low levels with over- and underestimation, possibly as a result of sampling problems (Vermeulen et al., 2017).

All analytical operations need to be performed in subdued light and with quantitation of both epimers. Stock analytical standards should be prepared in aprotic solvents and stored in amber vials below 0 °C. Individual calibrants should be prepared immediately before use or stored at −18 °C as a dried film. Several

reviews of the available analytical methods have been published (Komarova & Tolkachev, 2001b; Scott, 2007; Krska & Crews, 2008; EFSA, 2012; Crews, 2015).

3.2.2 Screening tests

3.2.2.1 Thin layer chromatography (TLC)

Of the chromatographic techniques applied to mycotoxins, TLC was the first, although it has been largely superseded by instrumental HPLC. Separation of the main EAs contaminating cereals was attempted with normal phase TLC using silica- or alumina-coated plates. However, complete separation of all 12 main alkaloids was not achieved, even with two-dimensional TLC (Scott, 2007). Some reports in the older literature describe partial separations of most of the main alkaloids (Reichelt & Kudrnac, 1973; Szepesi, Molnar & Nyiredy, 1979). EAs with a C9,10 double bond exhibit fluorescence and can be determined on a TLC plate by fluorodensitometry. Apart from this, several spray reagents have been developed and were reviewed by Scott (2007).

An alternative TLC method was developed to measure total EAs in rye as a single spot on a high-performance TLC (HPTLC) plate (Oellig & Melde, 2016). The alkaloids were extracted with ammonium acetate buffer and cleaned up by liquid-liquid extraction. The amino HPTLC plates were developed with methanol to separate the alkaloids from matrix components. Quantification was by natural fluorescence.

3.2.2.2 Immunological methods

ELISAs are ideally suited for rapid screening of cereals for total EAs. They are commercially available and can detect all six of the important EAs, as well as their epimers. However, questions have been raised as to whether cross-reactivity is the same for all 12 forms (Schnitzius et al., 2001; Crews, 2015). Besides the commercial ELISAs, some laboratories have developed their own monoclonal antibodies for determination of total EAs (Liesener et al., 2010; Gross, Curtui & Usleber, 2018).

3.2.3 Quantitative methods

3.2.3.1 Gas chromatography (GC)

Although GC has been used to determine many mycotoxins, it is not the method of choice for EAs other than the structurally simpler clavine alkaloids and lysergic acid derivatives (Scott, 1993; Flieger, Wurst & Shelby, 1997). For the ergopeptines with their amide linkage at C8, decomposition tends to occur in hot injector ports and only fragments can be determined, usually by MS (Jegorov et al., 1997).

3.2.3.2 HPLC with fluorescence detection (HPLC-FLD)

Quantitative determination of all 12 of the main EAs associated with contaminated cereals is generally achieved by HPLC with either UV/fluorescence detection or mass spectrometric detection (Scott, 2007; Krska & Crews, 2008; EFSA, 2012; Crews, 2015). The alkaloids are separated on reversed-phase (for example, C18) columns with isocratic or gradient elution programmes. Mobile phases are typically acidified acetonitrile solutions or acetonitrile with basic buffer. A drawback of acetonitrile with basic buffer is that the silica support of the HPLC packing can degrade at an alkaline pH. Some of the earlier HPLC methods only reported a limited number of the alkaloids. UV detection can be employed at wavelengths of 225 to 254 nm (Blaney et al., 2009). However, better sensitivity and selectivity can be achieved by utilizing the natural fluorescence with excitation wavelengths between 235 and 250 nm, or 310 to 330 nm with emission wavelengths 370 or 410 to 425 nm (Muller et al., 2009; Koppen et al., 2013; Schummer et al., 2018). The detection limits reported are typically in the low µg/kg range, although some studies have reported figures as low as 0.01 µg/kg (Ware et al., 2000; Lombaert et al., 2003; Muller et al., 2006). Sensitivity of the instrument clearly plays an important part in achieving low detection limits. More recently, lysergic acid diethylamide (LSD) has been proposed as an internal standard for determination of the 12 major alkaloids in rye flour and rye products (Holderied, Rychlik & Elsinghorst, 2019).

3.2.3.3 HPLC coupled to mass spectrometry (HPLC-MS(/MS))

The coupling of HPLC with MS has resulted in a versatile analytical technique that can incorporate both quantitative results and confirmatory mass spectra and has become the instrumental technique of choice for most mycotoxin analyses. Together with limited sample clean-up designed to remove impurities rather than isolating the analytes (unlike the more traditional SPE or IAC), it provides a platform for the development of multimycotoxin methods incorporating toxins of very different chemistries.

HPLC is performed on reversed-phase columns, frequently with volatile weak acids to provide efficient electrospray ionization in the positive electrospray ionization mode (ESI(+)) at the MS interface. Many of the earliest methods for analysing EAs by LC-MS determined only a limited number of alkaloids, possibly due to the absence of standards, which have only become available relatively recently (Shelby et al., 1997; Lehner et al., 2005; Burk, Hobel & Richt, 2006; Mohamed et al., 2006). Krska et al. (2008) developed an HPLC tandem MS (HPLC-MS/MS) method for the six priority EAs and their epimers. Extracts were purified with dispersive SPE (dSPE) and the 12 compounds were separated within 14 minutes by gradient reversed-phase HPLC. The method was validated for 10

different cereal and food matrices and a similar method was applied to a range of rye products (Crews et al., 2009). A variation of this method was developed that omitted the dSPE extract purification and used dihydroergotamine as an internal standard (Tittlemier et al., 2015). Di Mavungu et al. (2012) optimized and validated an HPLC-MS/MS method for the six major EAs and their epimers using alkaline extraction and simple liquid/liquid purification and applied it successfully to a range of food commodities. Twenty-five EAs, including the main six and their epimers, were included in an ultra-HPLC tandem mass spectrometry (UHPLC-MS/MS) method developed by Guo et al. (2016).

A common feature of many analytes determined by HPLC-MS/MS is the occurrence of matrix effects (signal enhancement or suppression). In the case of the EAs, given the absence of labelled analogues such as those used as internal standards for other mycotoxins, some analysts use matrix-matched standards in which calibrants are prepared in an extract of toxin-free sample (Kokkonen & Jestoi, 2009; Zachariasova et al., 2010). Malysheva et al. (2013) investigated these effects in detail for wheat, barley, rye, triticale and oats. There was a wide variation in the level of signal suppression between the different matrices and the different alkaloids, even within different varieties of the same cereal (rye). Signal suppression was strongly influenced by the extract purification technique and was improved with the use of UHPLC. The use of atmospheric pressure chemical ionization rather than ESI produced strong signal enhancement and an overestimation of alkaloid levels.

The development of multi-mycotoxin analytical methods using HPLC-MS/MS has led to the incorporation of EA into a suite of mycotoxins determined in a single method. One of the earliest was that of Sulyok et al. (2007), which included 12 major alkaloids in a total of 87 analytes in various food samples, including bread. Similarly, five of the priority alkaloids (and their epimers) were determined in a total of 32 toxins in beer using UHPLC coupled to high-resolution MS (Orbitrap®) (Zachariasova et al., 2010). Arroyo-Manzanares et al. (2018) developed a UHPLC-MS/MS method with positive ESI that included the main EAs and their epimers together with 24 other mycotoxins. The aim of their study was to provide a faster and simpler alternative to multi-mycotoxin methods that use both positive and negative polarities and may require two separate chromatographic runs per sample. Sample preparation and analysis time was kept short to minimize epimerization and the method was validated under European Commission guidelines.

The movement towards a more general multi-mycotoxin analytical method using MS detection has resulted in the need to reconsider the traditional extract purification methods. In general, two different approaches have been adopted. The first is the complete removal of the extract purification step and the use of the so-called "dilute-and-shoot" method, in which the extract is merely

diluted with an appropriate solvent (for example, HPLC mobile phase) and directly injected into the HPLC (Sulyok et al., 2010; Zachariasova et al., 2010). The second method is a more general easy-to-use clean-up designed not to remove the mycotoxins from the extract, but to remove interfering substances such as lipids and pigments. This latter method has been termed QuEChERS (quick, easy, cheap, effective, rugged and safe) (Walker et al., 2015; Kowalczyk et al., 2016; Leon et al., 2016; Arroyo-Manzanares et al., 2018). This is a relatively fast method involving salting out with buffer salt such as magnesium sulfate or citrate buffer, which may be followed by dispersive dSPE with magnesium sulfate, primary secondary amine, octadecyl sorbent and neutral alumina.

4. Sampling protocols

The inherent non-homogeneous nature of contamination with EAs (and other mycotoxins) in raw agricultural commodities is a major challenge in obtaining representative samples (Brera, Miraglia & Colatosti, 1998; Kabak, Dobson & Var, 2006). This challenge has been addressed for other mycotoxins (for example, aflatoxins, fumonisins and ochratoxin) by the adoption of suitable sampling protocols. The distribution of EAs is more heterogeneous than that of other mycotoxins (Karlovsky et al., 2016), because contamination does not occur on single kernels but within large sclerotia which are prone to fragmenting. Although this is a known problem, no sampling protocols have been developed specifically for EAs. In some countries, the concentration of ergot sclerotia in unprocessed grains is legally limited (EU 2015b, 0.05 g/kg of ergot sclerotia in grain) and although there are publicly available sampling plans for visual inspection of raw grain for sclerotia occurrence (USDA, 1995; Canadian Grain Commision (CGC), 2015b), the value of these protocols when sampling prior to analytical determination of EAs is very limited.

The problem of sampling for major mycotoxins has been addressed by statistical means and the drawing up of sampling plans. Manuals on sampling for inspection purposes were developed by the United States Department of Agriculture, Agricultural Marketing Service, Federal Grain Inspection Service (USDA, 1995), and the CGC (CGC, 2015a). In Europe the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs are detailed in the Commission Regulation (EC) No 401/2006 (EU, 2006). A manual aimed at addressing sampling procedures and written for both food analysts and regulatory officials, which explains some of the statistical issues, was produced as part of the Joint FAO/IAEA Programme, Nuclear Techniques in Food and Agriculture (Whitaker et al., 2010). Much of the statistical research was

consolidated by FAO in a mycotoxin sampling tool, containing information on 26 mycotoxin–commodity combinations (FAO, 2013).[1]

To further assist in the practical application of sampling for mycotoxin contamination, FAO, in collaboration with the Italian National Institute of Health, produced two training videos (Brera, Miraglia & Pineiro, 2007; Istituto Superiore di Sanità, 2015). Maximum levels for mycotoxins in various foods set by the Codex Alimentarius Commission (CAC) are supported by sampling plans. These plans for cereals and cereal products can be accessed online (FAO/WHO, 2016). At the time of writing, the Codex Committee on Methods of Analysis and Sampling (CCMAS) was reworking the General Guidelines on Sampling (CAC/GL 50-2004).

In the absence of specific sampling plans for EAs, aflatoxin sampling plans are routinely used. Whenever possible, it is recommended that the same sampling method is applied to the same product by buyer and seller. It is further recognized that large-scale sampling plans may not be appropriate or advantageous in smallholder settings, thus presenting an additional challenge.

5. Effects of processing

5.1 Sorting, cleaning and milling

Sorting, sieving, flotation, handpicking, washing and milling, can potentially reduce EA levels. Of these processes, manual and automatic sorting, along with sieving, are the most effective means of reducing fungal contamination (Grenier et al., 2014; CGC, 2015a; Karlovsky et al., 2016). As a major portion of EAs has been associated with small, shrivelled and discoloured material within grain samples (Muthaiyan, 2009; Grenier et al., 2014), a reduction in EA levels of about 34–55% is possible when mould-contaminated grains or seeds are physically removed by sieving, handpicking and separation from intact raw samples (Muthaiyan, 2009). In the rye industry, sclerotia are effectively removed by sorting, thus reducing EA contamination (Young et al., 1983; Miedaner & Geiger, 2015). Therefore, these procedures can be useful for reducing the level of human exposure to EAs (EFSA, 2012).

Conventional grain cleaning equipment (i.e. scalpers, shaker decks, gravitational separators and electronic sensor-based sorters) that removes foreign matter, dust, and broken or shrivelled grains can reduce EA contamination (Belser-Ehrlich et al., 2012; CGC, 2015a). Methods using either high velocity

[1] http://www.fstools.org/mycotoxins/

air cleaning or electronic sensor-based handling have been shown to reduce EA levels by more than 80% (Miedaner & Geiger, 2015).

In common with other mycotoxins, EAs in cereals are not destroyed by milling, which merely distributes them among the milling fractions. In general, fractions intended for human food have reduced levels, whereas those intended for animal feed (for example, bran, shorts and feeds) have elevated levels (Farjado et al., 1995; Franzmann et al., 2011; Tittlemier et al., 2019).

5.2 Thermal and chemical food processing

Heat treatment can reduce EA levels in processed products; the level of reduction is strongly dependent on temperature and treatment duration. Temperatures above 100 °C used for frying, roasting, toasting and extrusion cooking have been reported to reduce EA levels. Studies on the fate of EAs in different flours and during different food processing operations suggested a reduction during baking (Wolff et al., 1988; Fajardo et al., 1995; Scott, 2009).

Merkel et al. reported that EAs are degraded and epimerized during bread baking; thus, the ratio shifts from the -ine form to the -inine form (Merkel et al., 2012). Similar observations on the instability and shift in the ratio of epimeric forms were also reported by others (Baumann et al., 1985; Franzmann et al., 2010). Meleard reported a reduction of up to 60% of EAs during a French baking test at 250 °C of a commercial wheat flour spiked with a sclerotia grind (Meleard, 2016).

Bryla et al. investigated the stability of six *R*-configuration EAs (ergometrine, ergocornine, ergocristine, ergocryptine, ergosine and ergotamine) and their respective *S*-enantiomers (ergometrinine, ergocorninine, ergocristinine, ergocryptinine, ergosinine and ergotaminine) during the process of baking rye bread at 190 °C (Bryla et al., 2019). Although the authors did not observe a reduction of EAs during dough formation, they reported a 22% loss of total EAs during baking; specific degradation products were not identified. Interestingly, the authors also noted that the concentration of *R*-enantiomers decreased from dough formation to baking by 46%, but the concentration of *S*-enantiomers increased by 21%. During the baking process, the ratio of *R*-enantiomers to *S*-enantiomers shifted from 65:35 to 45:55.

Fajardo et al. reported losses of EAs during preparation and cooking of different Asian noodles and spaghetti prepared from highly contaminated wheat flour. However, the analytical method used in this study only reported the *R*-enantiomers (Fajardo et al., 1995). Epimerization to *S*-enantiomers during cooking would not have been detected.

In a well-designed study, Tittlemier et al. prepared spaghetti from durum contaminated with different amounts of ergot sclerotia (0.01–0.1%) monitoring the levels of 10 different EAs during the processing steps (Tittlemier et al., 2019). An analytical method (LC-MS/MS) was used that included four *R/S* enantiomeric pairs (ergosine, ergosinine, ergocristine, ergocristinine, ergocornine, ergocorninine, ergocryptine and ergocryptinine) and two further *R*-enantiomers (ergometrine and ergotamine) as analytes. The authors noted no significant epimerization during spaghetti extrusion at 45 °C, but the cooking process led to a marked shift in enantiomers from *R*- to *S*- forms. Notably, the authors did not detect a reduction in the total EA concentration during the production or cooking processes; EAs were also not detected in the cooking water.

5.3 Fermentation

Reduction of levels of major *R*-enantiomer forms of EAs during malting and brewing has been documented (Schwarz et al., 2007). Steeping appears to solubilize a small amount of EAs, with kilning resulting in a greater decrease (≈30%) and an overall reduction in beer of about 46% of the total EAs, as a result of thermal degradation (Schwarz et al., 2007).

6. Prevention and control

6.1 Preharvest control

Factors influencing the concentration of EAs in plants are poorly documented; however, limited data are available on cereals.

Among the cereals, rye and triticale are the most susceptible to infection by *Claviceps* spp., followed by wheat, barley and oats (Platford & Bernier, 1976; Menzies & Turkington, 2015). Rye and triticale are more susceptible mainly because their flowers are cross-pollinated, whereas wheat, barley and oats are self-pollinated (Gaudet et al., 2000). A French survey of 1919 fields sampled between 2012 and 2014 found that rye was 16 times more contaminated than wheat, and that triticale was twice as contaminated as wheat (Orlando et al., 2017).

Differences between cultivars also play a role in susceptibility, but this has not been thoroughly investigated. A few studies have been conducted on rye (Sosulski & Bernier, 1975; Mainka et al., 2007), wheat (Watkins & Littlefield,

1976; Pageau et al., 1994; Menzies, 2004) and barley (Pageau & Lajeunesse, 2006; Oxley et al., 2009) to identify resistant cultivars.

Wild grasses within or outside fields are the primary source of ergot inoculum due to the wide host range of *C. purpurea*. The above-mentioned French survey showed that fields with grasses, both in the main part of the field and in the field margins, were 50% more contaminated than those without grasses (Orlando et al., 2017).

Because ergot sclerotia usually do not survive longer than a year in the soil (Mitchell & Cooke, 1968; Maunas & Leclère, 2013), crop rotation can be used to control EA contamination. However, this strategy is only effective if grasses are simultaneously controlled. For example, difficulties controlling grasses in oilseed rape fields lead to a 20 to 60% increase in EA contamination in subsequent cereal crops (Orlando et al., 2017).

Similarly, the tillage system can help to control plant contamination, as deep ploughing buries sclerotia in the soil. As a result, ascospores are not formed or they cannot be released into the air in spring. Maumené et al. (2016) described the depth distribution of sclerotia artificially dispersed on the soil surface, under different tillage systems (ploughing, shallow cultivation and a combination of these two techniques) over a 2-year crop sequence. The authors recommended limiting tillage the subsequent year to shallow cultivation, to avoid bringing the buried sclerotia back up to the soil surface. The French field study indicated that fields were slightly less contaminated after deep tillage than after other tillage practices, but there was no statistically significant difference (Orlando et al., 2017).

Lastly, the use of ergot-free or certified seed could significantly improve control, by preventing the introduction of primary inoculum into fields. The treatment of seeds with fungicide has also been identified as a way of decreasing the germination of fungal spores (Shaw, 1988; Puhl et al., 2007; Maunas et al., 2016).

6.2 Postharvest control

There are very few studies on postharvest control of EAs. Cleaning grain during and after harvest by removing sclerotia will reduce EA contamination (Miedaner & Geiger, 2015).

6.3 Decontamination

Effective decontamination measures should be irreversible, products should be non-toxic and grain should keep its nutritional value while maintaining storablity and palatability.

An effective physical decontamination procedure is removal of sclerotia by sieving (Seaman, 1980; Muthaiyan, 2009). Opto-electric sorting is another highly effective way of sorting sclerotia from cereal (Young et al., 1983; Miedaner & Geiger, 2015). Berg et al. (1995) reported winnowing prior to sorting as an effective decontamination procedure. Studies by Franzmann et al. showed that milling, even after carefully applied cleaning practices, only redistributed sclerotia fragments and dust in the grinding stock (Franzmann et al., 2011). Peeling was also reported as a successful EA reducing procedure, but cannot be universally applied (Franzmann et al., 2011).

Young et al. reported that EAs in wheat ergot sclerotia could be reduced by up to 90% by applying chlorine at either 150 °C or 200 °C. Treatment with sulfur dioxide and hydrogen chloride at the same temperatures reduced the amounts of EAs by only 20%. The authors further observed that treatment of wheat sclerotia in an autoclave for 30 minutes at 121 °C prior to feeding to growing chickens reduced toxic effects (Young et al., 1983). Ergot separation from rye grains by flotation in sodium chloride solution also showed promising effects, but the treatment resulted in damp and salty grain (Plante & Sutherland, 1944; Seaman, 1980).

Although EAs are reported to be photosensitive (Stoll & Schlientz, 1955), Young et al. observed that irradiation of ground sclerotia with UV light for 54 hours did not change EA levels (Young et al., 1983). Other techniques including enzymatic or microbial decontamination are reported to have been successfully applied for other mycotoxins (Petruzzi et al., 2014; Karlovsky, 2016), but not for sclerotia or EAs.

A challenge of applying chemicals, irradiation or other procedures is that the processing agents, procedures, or microorganisms used for decontamination must be authorized for use in food (Codex Alimentarius, 2015). Procedures authorized in national food legislation were not found. National context may be nuanced; for example, European Commission Regulation EC 2015/786 defines acceptability criteria for detoxification processes applied to products intended for animal feed (EU, 2015a), but these criteria do not apply to food.

7. Levels and patterns of contamination in food commodities

7.1 Surveillance data

The GEMS/food contaminants database was queried for records relating to the analytical results for ergot alkaloids referring either to the sum of EAs or to the different ergot alkaloids: ergometrine (ergonovine), ergosine, ergocornine, ergotamine, ergocristine, α-ergocryptine, β-ergocryptine and the corresponding -inine (*S*)-epimers (ergometrinine (ergonovinine), ergosinine, ergocorninine, ergotaminine, ergocristinine, α-ergocryptinine and β-ergocryptinine).

Data extracted (n = 203 453 results) were submitted between 2004 and 2019 and originated from four WHO regions: African Region (Benin, Cameroon, Mali and Nigeria), European Region, Region of the Americas (Canada) and the Western Pacific Region (Hong Kong SAR, New Zealand and Singapore). No concentration data for EAs have been submitted to the GEMS/Food contaminants database from countries in the WHO South-East Asia and Eastern Mediterranean regions. Overall, data extracted originated from 30 countries or country groups, representing 13 of the 17 GEMS/Food cluster diets.

The extracted database was cleaned by:

1) removing individual results related to animal feed;
2) removing aggregated data, keeping only results from individual samples;
3) converting all results and the analytical limits: LOD and LOQ to the same units (µg/kg) and ensuring that the naming of foods was consistent;
4) removing results expressed as a summation when analytical results for the individual EAs were available for the same food sample identifier;
5) reclassifying food descriptors for some data to establish consistency within the database (for example, some records of EAs in pasta and cookies were reported in "composite foods" and/or in "snacks and dessert" and these records were reclassified as "bread and other cooked cereal products", and since spelt is a wheat species (*Triticum spelta*) records relating to spelt were reclassified as "wheat";

6) excluding some quantified samples classified in the "herbs and spices" food category, which referred to baking ingredients that are not ready-to-eat;
7) excluding records of grain as crops that referred to samples of unprocessed grains of unknown end-use, as such samples are usually not considered when estimating human dietary exposure;
8) substituting for left-censored data, as recommended in Chapter 6 *Dietary exposure assessment of chemicals in food* (2020) of the EHC 240 "principles and methods for the risk assessment of chemicals in foods" (WHO/IPCS, 2009). At the lower bound (LB), results below the LOQ and LOD were replaced by zero and at the upper bound (UB), results below the LOD were replaced by the LOD and those below the LOQ were replaced by the LOQ.

After cleaning, as indicated in Table 15 the database contained 178 184 data items originating from the WHO African Region (1.4%), Region of the Americas (13.6%), European Region (83.8%) and the Western Pacific Region (1.2%).

For 11 of the EAs considered, 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 (Table 16). Table 17 summarizes data on EA concentrations by region and country from the GEMS/Food contaminants database.

No quantified concentration data were submitted for alcoholic beverages (n = 4198); eggs and egg products (n = 48); fats and oils (n = 1315); fish and others (n = 72); fruits and fruit products (n = 11 471); fruit and vegetable juices (n = 1822); herbs and spices (n = 865); meat and meat products (n = 136); milk and milk products (n = 156); non-alcoholic beverages (n = 81); nuts and oilseeds (n = 21 945); products for special nutritional use (n = 192); snacks and desserts (n = 496); starchy roots and tubers (n = 594); stimulant beverages (n = 72); sugar and confectionery (n = 1572), or vegetables and vegetable products (n = 1368).

Quantified concentrations of EAs were available for some food commodities: cereals and cereal-based products (n = 19 635/124 252); legumes and pulses (n = 33/2513, mainly in dry soya beans), and foods for infants and young children (n = 4/4780, mainly cereal-based infant foods).

As the relative potency of individual EAs is uncertain, the Committee calculated the total EA concentration as the simple sum, for all food samples

Table 15
Summary of EA concentrations from the GEMS/Food contaminants database

EA	No. of records	% positive	Mean positive (µg/kg)	Maximum (µg/kg)
Ergometrine	13 469	14.4	34.7	760
Ergometrinine	11 940	5.0	16.0	234
Ergotamine	13 176	18.9	62.1	3 343
Ergotaminine	11 549	9.6	26.1	339
Ergosine	18 432	9.8	33.2	1 287
Ergosinine	11 485	6.1	22.4	1 606
Ergocristine	20 484	17.3	64.9	9 279
Ergocristinine	13 544	11.2	39.2	3 538
Ergocryptine	13 519	14.6	27.8	661
Ergocryptinine	22 245	5.7	20.0	1 007
Ergocornine	14 516	12.2	34.2	617
Ergocorninine	13 435	6.8	21.8	396
Total EAs (not specified)[a]	390	20.0	46.5	1 200
Overall	**178 184**	**11.1**	**39.9**	**9 279**

[a] Analytical records with no specification of the EA measured were from Hong Kong SAR and Singapore.

Table 16
Summary of maximum EA concentrations in the different WHO regions, from the GEMS/Food contaminants database

	WHO region			
EA	African Region maximum (µg/kg)	European Region maximum (µg/kg)	Western Pacific Region maximum (µg/kg)	Region of the Americas maximum (µg/kg)
Ergometrine	27	760	22	153
Ergometrinine	1	234	2	33
Ergotamine	31	2 131	23	3 343
Ergotaminine	2	339	16	91
Ergosine	29	1 287	10	296
Ergosinine	2	1 066	6	38
Ergocristine	37	9 279	27	951
Ergocristinine	11	3 538	22	414
Ergocryptine	14	661	25	414
Ergocryptinine	6	1 007	5	88
Ergocornine	5	619	8	172
Ergocorninine	3	396	12	58

Table 17
Summary of EA concentrations by region and country from the GEMS/Food contaminants database

Region/country	No. of records	% positive	Mean positive (µg/kg)	Maximum (µg/kg)
African	**2520**	**2.1**	**7.2**	**37**
Americas	**24 236**	**19.7**	**38.7**	**3 343**
Canada	24 236	19.7	38.7	3 343
European	**149 358**	**9.6**	**39.9**	**9 279**
European Union	149 358	9.6	39.9	9 279
Western Pacific	**2 070**	**28.1**	**7.8**	**1 200**
Hong Kong SAR	351	22.2	46.5	1 200
New Zealand	1 680	29.9	1.8	27
Singapore	39	0.0	0.0	0.00
Overall	**178 184**	**11.1**	**39.9**	**9 279**

for which concentration data were available for individual EAs (Tables 18–21). The upper bound (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 for which EAs were reported only as not detected or not quantified, UB estimates were derived by averaging the LODs or LOQs. For example, of the 84 samples of barley reported from the WHO European Region, only one sample was quantified. The mean UB was calculated by adding the value of the quantifed sample to the sum of analytical limits of each individual EA analysed in the 83 other samples, and dividing by the total number of individual EAs analysed in each sample. This approach was used rather than summing the LOD/LOQ of each unquantified individual alkaloid to avoid overly conservative estimates of UB concentrations (Tables 18–21). The Committee did not consider food commodities for which results were reported only as "none detected" or "none quantified".

Owing to the paucity of data for regions other than Europe, a literature search was conducted using Web of Science with the search term "TS=((mycotoxin)+(ergot alkaloids) and (surve* or occurr* or contam*) and (food or feed) NOT(Europe*))". This search recovered only 82 publications (as of January 2021), which were screened for relevance by title, abstract and then the full paper. The results from the GEMS/Food contaminants database and the literature search are summarized below by WHO region.

7.1.1 African Region

A total of 2520 analytical results from 210 food samples from African countries were present in the GEMS/Food contaminants database. The samples were from the African Total Diet Study performed by Ingenbleek et al. across four sub-Saharan countries (Benin, Cameroon, Mali and Nigeria). All samples were analysed for 12 EAs (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and the corresponding -inine epimers); 97.9% of the analytical results were below the LOD (0.03 to 1.1 µg/kg depending on the EA).

Food consumption data were estimated as the daily amount of food consumed (in g per adult male equivalent per day) derived from previously existing household budget surveys generated by national statistics authorities from 72 979 households. In total, 4020 food samples, representing at least 90% of each national total diet by weight, were obtained from a core list of 84 subgroups. A food composite approach was taken, pooling 12 samples of the same core food to form 335 pooled samples. Samples were collected from each of the eight study centres. They were purchased during the rainy season (October 2017) and again during the dry season (February 2018) (Ingenbleek et al., 2020). The study centres included three located in coastal areas (Duala, the Littoral of Benin and Lagos) and five in non-coastal areas (Bamako, the Borgou region of Benin, Kano, North Cameroon and Sikasso) (Ingenbleek et al., 2019). Of the 335 composite samples, 194 composite samples of cereals, tubers, legumes, vegetables, nuts and seeds, dairy products, oils, beverages and miscellaneous foods were considered to be potentially contaminated by mycotoxins and were analysed by LC-MS/MS.

Only bread and other cooked cereal products were found to be contaminated (Table 18). The most contamination was observed in a "bread and other cooked cereal products" sample contaminated with 36.91 µg/kg ergocristine and having a total EA contamination of 165.7 µg/kg.

Except the total diet study by Ingenbleek et al., the results of which are already included in the GEMS/Food contaminants database, the literature search identified no other study that investigated the occurrence of the 12 EAs (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and the corresponding -inine epimers) in African food.

One study investigated the variation of fungal metabolites in sorghum malts used to prepare the traditional Namibian fermented beverages *omalodu* and *otombo*. Six clavine EAs (agroclavine, chanoclavin, elymoclavine, festuclavine, fumigaclavine A and fumigaclavine B) were quantified with a prevalence of 50–100% in malt samples used to prepare both beverages, except for elymoclavine, which was only found in 3% and 38% of *otombo* and *omalodu* malt samples, respectively (Nafuka et al., 2019).

Table 18
Summary of mean and maximal EA concentrations in food from the WHO African Region from the GEMS/Food contaminants database[a]

Food commodities	No. of samples	% <LOD or LOQ	mean LB, µg/kg	mean UB, µg/kg	Max. µg/kg
Cereals and cereal-based products	**59**	**89.8**	**6.2**	**7.0**	**166**
Bread and other cooked cereal products	9	33	40.9	43.0	166
Maize	16	100	0.0	0.5	
Millet	8	100	0.0	0.5	
Rice	16	100	0.0	0.5	
Sorghum	10	100	0.0	0.5	

LB, lower bound; LOD, limit of detection; LOQ, limit of quantitation; UB, upper bound.
[a] 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.

7.1.2 Region of the Americas

A total of 24 236 analytical results from 6240 food samples from the Region of the Americas were present in the GEMS/Food contaminants database. All these data came from Canada and were collected between 2009 and 2018. Most samples (5394) were analysed for three EAs (ergocristine, ergocryptine and ergosine), 286 were analysed for six EAs (ergocornine, ergometrine, ergocristine, ergocryptine, ergosine and ergotamine), 191 for 10 EAs (ergometrine, ergocristine, ergocryptine, ergosine, ergotamine and the corresponding -inine epimers), and 369 for 12 EAs (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and the corresponding -inine epimers). More than 80% of the analytical results were below the LOD (1.7 to 9 µg/kg depending on the EA).

Only cereals and cereal-based products were found to be contaminated (Table 19). The maximum level was observed in a barley sample contaminated with 3343 µg/kg ergotamine and a total EA contamination of 3549 µg/kg.

As for other parts of the world, the literature survey did not identify any additional data.

7.1.3 Eastern Mediterranean and South-East Asia regions

There were no EA analyses submitted to the GEMS/Food contaminants database from the Eastern Mediterranean and South-East Asia regions. The literature survey did not provide any additional data.

7.1.4 European Region

The European Region was the most represented in the GEMS/Food contaminants database, with a total of 149 538 analyses (83.8% of total analyses) from 14 972

Table 19
Summary of mean and maximal EA concentrations in food from Canada, from the GEMS/ Food contaminants database[a]

Food commodities	No. of samples	% <LOD or LOQ	mean LB, µg/kg	mean UB, µg/kg	Max. µg/kg
Cereals and cereal-based products	**5 593**	**72.2**	**33.0**	**40.4**	**3549**
Barley	264	66.3	45.9	54.8	3 549
Bran, unprocessed of cereal grain (except buckwheat, canihua, quinoa)	186	90.3	3.2	11.9	97
Bread and other cooked cereal products	1 425	69.1	16.9	23.9	1 060
Buckwheat	140	95	2.3	7.8	217
Cereals and cereal-based products NES	612	98	0.8	6.0	234
Maize	449	8.4	0.4	5.7	53
Oats	434	90.8	7.2	14.9	481
Rye	132	41.7	195.0	200.5	1 917
Wheat	1 404	38.5	83.9	93.5	1 066

LB, lower bound; LOD, limit of detection; LOQ, limit of quantitation; UB, upper bound.
[a] 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.

samples having reported results for EAs. All samples were from the European Union and had been collected between 2004 and 2019. Most of them were analysed for the 12 EAs (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and the corresponding -inine epimers). Overall, 9.6% of all analytical results from the European Region were positive for EAs.

Only two food commodities were found to be contaminated: cereals and cereal-based products, and legumes and pulses (Table 20). The maximum levels were observed in rye grain (13 783 µg/kg) and undefined cereals and cereal-based products (5649 µg/kg). Within the cereals and cereal-based products, four categories had a relatively high proportion of contaminated test samples, i.e. bread and other cooked cereal products (37.1% of the samples in this category were contaminated; mean LB 29.8 µg/kg and mean UB 61.0 µg/kg); cereal and cereal-based products unidentified (38.6% samples contaminated; mean LB 99.4 µg/kg and mean UB 126.1 µg/kg); rye (45.9% samples contaminated; mean LB 93.4 µg/kg and mean UB 124.6 µg/kg) and wholemeal bread (60.5% samples contaminated; mean LB 68.5 µg/kg and mean UB 114.3 µg/kg). Among the samples of legumes and pulses, 6 of the 67 samples of beans, shelled (immature seeds) were contaminated (mean LB 6.5 µg/kg and mean UB 14.2 µg/kg) as well as 11 of the 109 dried soya bean samples (mean LB 2.3 µg/kg and mean UB 9.1 µg/kg).

Table 20

Summary of mean and maximal EA concentrations in food from the WHO European Region, from the GEMS/Food contaminants database[a]

Food commodities	No. of samples	% <LOD or LOQ	mean LB, µg/kg	mean UB, µg/kg	Max. µg/kg
Cereals and cereal-based products	**9 381**	**66.9**	**63.5**	**89.8**	**13 783**
Barley	84	99	1.6	11.6	133
Bran, unprocessed of cereal grain (except buckwheat, canihua, quinoa)	1	100	0.0	2.0	
Bread and other cooked cereal products	2 334	62.9	29.8	61.0	923
Buckwheat	110	89.1	3.7	11.5	80
Cereal grains NES	519	95	9.5	28.7	1 280
Cereals and cereal-based products NES	4261	61.4	99.4	126.1	5645
Maize	123	100	0.0	16.2	
Millet	41	100	0.0	5.2	
Oats	183	90.7	7.8	20.0	359
Rice	155	100	0.0	15.4	
Rye	910	54.1	93.4	124.6	13 783
Sorghum	1	100	0.0	2.0	
Wheat	549	83.6	15.0	33.7	1101
White bread	72	87.5	4.1	23.5	199
Wholemeal bread	38	39.5	68.5	114.3	552
Legumes and pulses	**197**	**91.4**	**3.5**	**11.7**	**318**
Beans except broad bean and soya bean (green pods and immature seeds)	18	100	0.0	16.7	
Beans, shelled (immature seeds)	67	91	6.5	14.2	318
Lentil (dry)	2	100	0.0	20.0	
Peas (dry)	1	100	0.0	20.0	
Soya bean (dry)	109	89.9	2.3	9.1	36

LB, lower bound; LOD, limit of detection; LOQ, limit of quantitation; UB, upper bound.

[a] 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.

7.1.5 Western Pacific Region

Three countries from the Western Pacific Region supplied data on EAs to the GEMS/Food contaminants database: New Zealand (1650 analytical results from 140 food samples), Hong Kong SAR (351 analytical results from 351 food samples) and Singapore (39 analytical results from 39 food samples). These samples were collected between 2016 and 2019.

The samples from Singapore and Hong Kong SAR were analysed for total EAs with no indication of the specific EAs included, whereas the samples from New Zealand were analysed separately for the 12 EAs (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and the corresponding -inine epimers). All samples from Singapore were below the LOD (10 µg/kg).

Table 21
Summary of mean and maximal EA concentrations in food from the WHO Western Pacific Region, from the GEMS/Food contaminants database[a]

Food commodities	No. of samples	% <LOD or LOQ	mean LB, µg/kg	mean UB, µg/kg	Max. µg/kg
Cereals and cereal-based products	**496**	**67.3**	**9.1**	**9.8**	**1200**
Barley	11	91	49.1	49.4	540
Bread and other cooked cereal products	149	45	7.7	8.2	130
Buckwheat	5	100	0.0	0.5	
Cereals and cereal-based products NES	176	81.3	3.7	4.6	180
Maize	9	100	0.0	0.5	
Millet	3	100	0.0	0.5	
Oats	26	92.3	1.7	3.3	42
Rice	23	95.7	0.4	1.9	10
Rye	29	34.5	15.8	16.1	169
Sorghum	3	100	0.0	0.5	
Spelt	9	66.7	7.0	7.4	31
Wheat	9	100	0.0	0.5	
Wheat flour	17	29.4	4.6	5.2	21
Wheatgerm	3	0	464	464	1200
White bread	6	16.7	15.0	15.1	44
Wholemeal bread	3	66.7	14.0	14.3	42
Legumes and pulses	**10**	**90**	**0.9**	**2.8**	**9**
Lentils (dry)	3	100	0.0	1.7	
Peas (dry)	7	85.7	1.3	3.3	9

LB, lower bound; LOD, limit of detection; LOQ, limit of quantitation; UB, upper bound.
[a] 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.

Only two food commodities were found to be contaminated: cereals and cereal-based products and legumes and pulses (Table 21). The maximum level was observed in a wheatgerm sample from Hong Kong SAR contaminated with 1200 µg/kg total EA. More than half of the bread and other cooked cereal products had a detectable level of EAs (mean LB 7.7 µg/kg and mean UB 8.2 µg/kg). Of the samples of legumes and pulses, one out of the 10 had detectable levels of EAs.

No report on the occurrence of EAs in the Western Pacific Region was identified in the literature search.

7.2 Conclusions

Based on data from the GEMS/Food contaminants database, comparisons of analyses for EA across global regions have indicated that, despite large differences

in the number of analyses reported, the only foodstuffs testing positive for EAs were cereals and cereal-based products and, to a lesser extent, legumes and pulses. Most of the analytical records were supplied from the European Region. Results were also obtained from the African Region (Benin, Cameroon, Mali and Nigeria), the Region of the Americas (Canada) and the Western Pacific Region (Hong Kong SAR, New Zealand and Singapore). 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 Europe (mean UB level 89.8 µg/kg) and in the Region of 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). 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.

8. Food consumption and dietary exposure estimates

8.1 Concentrations in food used in the dietary exposure estimates

The GEMS/food contaminants database was used as the source of information. The query as well as the cleaning steps are described in section 7.1. Estimates of dietary exposure derived by the Committee only considered food commodities for which quantified levels of any individual EAs were reported.

8.2 Food consumption data used in the dietary exposure estimates

In addition to national and regional estimates of dietary exposure published in the literature, the Committee derived international or regional estimates of dietary exposure to EAs using the GEMS/Food cluster diets. Considering that cereals and cereal-based products are foods consumed on a regular basis and considering that wheat and wheat-based products represent the main foods contributing to chronic dietary exposure to EAs across national estimates, the Committee considered that the use of GEMS/Food cluster diets rather than the use of summarized individual food consumption data (Chronic individual food consumption database – Summary statistics (CIFOCOss)) data was sufficient to capture international estimates of dietary exposure to EAs.

The GEMS/Food cluster diets provide mean per capita food consumption values based on FAO food balance sheet data for raw commodities and some semi-processed commodities for 17 clusters of countries (Sy et al., 2013). Relevant food consumption data for each of the 17 GEMS/Food cluster diets categorized by WHO regions are presented in Table 22. Although some clusters defined in 2013 do not map exactly to a single WHO region, the Committee considered that the differences noted in clustering were limited to a small number of countries and were not likely to affect the chronic dietary estimates.

8.3 Assessments of dietary exposure

Estimates of national and international chronic and acute dietary exposure to EAs reported from the scientific or grey literature, or derived by the Committee were all based on total EA concentration calculated as the simple sum of individual EAs. Table 23 summarizes national and international estimates of chronic dietary exposure to EAs from the literature or derived by the Committee.

8.3.1 National or regional estimates of chronic dietary exposure from the published literature

Few publications or reports on dietary exposure to EAs have appeared in the scientific or grey literature. The Committee evaluated studies on dietary exposure to EAs in sub-Saharan African countries, European countries and New Zealand.

(a) WHO African Region

Twelve EAs were detected in foods processed from wheat (five of six bread samples), with a mean concentration of 62.4 µg/kg, ranging from not detected to 165.7 µg/kg, for the sum of 12 EAs. The treatment of left-censored data for estimation of dietary exposure (LB–UB) was only applied for products in which alkaloids were detected. Assessment of dietary exposure to the sum of EAs was not carried out in the original study (only assessment of dietary exposure to each individual EA was performed for all food commodities including those where EAs were not quantified) and was subsequently estimated by the Committee, based on information available from FAO. Dietary exposure estimates for the adult population are summarized for mean and high percentile (P95) for the eight study centres (Table 23).

(b) WHO European Region

Both chronic and acute human dietary exposure to EAs were assessed for European countries (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2012). Dietary exposure to EAs was estimated using occurrence data submitted by

Table 22

Summary of consumption data for 17 GEMS/Food cluster diets grouped by WHO region

	Food consumption for WHO GEMS/Food cluster diet in g/person per day, grouped by WHO region																
	Eastern Mediterranean			European						African			Americas		Western Pacific		South-East Asia
Food commodities	G01	G04	G06	G02	G07	G08	G10	G11	G15	G03	G13	G16	G05	G12	G14	G17	G09
Grains and grain-based products																	
Barley, including flour and grits	12.5	0.1	0.6	7.6	0.6	2.6	2.0	1.0	1.9	0.1	5.5	0.0	1.3	0.1	0.0	0.0	0.4
Buckwheat, including flour	0.0	0.0	0.1	0.3	0.0	0.6	0.3	0.0	0.0	0.0	0.0	0.0	0.1	0.0	2.8	0.0	0.1
Cereals preparations NES (and flour)	0.9	9.1	0.7	0.9	3.3	1.6	8.2	1.5	4.2	0.7	0.7	0.1	2.0	5.0	0.6	1.9	0.7
Maize, including maize flour and sweet corn	23.4	36.3	61.6	36.1	17.8	14.7	17.1	5.8	29.6	87.8	94.4	56.0	48.0	53.1	8.1	28.1	21.1
Oats, including rolled oats	0.0	1.1	0.0	3.9	4.1	3.4	2.7	1.7	1.5	0.1	0.2	0.1	0.5	1.7	0.0	0.0	0.1
Rice, including milled, husked, broken and flour	35.4	87.7	72.6	11.9	16.8	12.8	58.3	13.1	14.9	72.8	43.8	15.2	150.7	67.9	222.0	60.2	263.2
Rye, including rye flour	0.1	0.1	1.7	15.5	2.6	28.3	5.2	1.2	11.2	0.1	0.0	0.0	0.0	0.0	0.0	0.7	0.2
Wheat, including flour, macaroni and bread	301.8	226.1	343.5	271.3	199.1	193.7	185.8	171.7	216.3	30.5	57.8	20.5	137.0	134.4	87.8	105.6	106.4
Wheatgerm	0.0	0.0	0.0	0.0	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
Pulses, nuts and oilseeds																	
Beans (green pods and immature seeds)	1.6	1.2	7.8	0.6	2.2	5.2	1.6	16.9	4.1	0.5	0.3	0.0	0.9	0.2	3.1	0.0	4.2
Peas (dry)	1.6	1.5	0.2	3.2	3.8	1.3	2.3	2.7	3.5	0.9	1.5	3.6	2.9	3.8	2.5	0.7	0.9
Soya beans (dry)	0.6	0.0	0.3	0.0	0.0	0.3	3.9	0.0	0.3	0.4	2.8	3.2	1.6	5.8	0.1	0.0	6.6

NES, not otherwise specified.

Table 23
Summary statistics of chronic dietary exposure to the sum of EAs (ng/kg bw per day) across eight studies in sub-Saharan African countries

Dietary exposure (ng/kg bw per day)	Cameroon		Benin		Mali		Nigeria	
	Duala	Garoua	Cotonou	Borgou	Bamako	Sikasso	Lagos	Kano
Mean LB sum 12 EAs	37	10	0.3	0.1	28	5	92	34
Mean UB sum 12 EAs	42	11	0.3	0.1	31	6	94	35
P95 LB sum 12 EAs[a]	52	24	1	1	54	12	177	91
P95 UB sum 12 EAs[a]	73	25	1	1	57	13	179	94

LB, lower bound; P95, 95th percentile; UB, upper bound.
[a] Highest P95 individual EAs + sum of the mean of the 11 other EAs.

Member States, combined with food consumption information from the EFSA Comprehensive European Food Consumption Database (EFSA, 2017). The main results reported by EFSA are summarized below.

The initial data set with 12 675 samples representing 123 367 analytical results on EAs was collected between 2004 and 2016 in 22 European countries: 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. The analytical results for EAs referred to the 12 main *Claviceps purpurea* EAs: ergometrine, ergosine, ergocornine, ergotamine, ergocristine, ergocryptine, and the corresponding -inine (*S*)-epimers (ergometrinine, ergosinine, ergocorninine, ergotaminine, ergocristinine and ergocryptinine). Additional analytical data on the two isomers (α- and β-) of ergocryptine/ergocryptinine were reported either as individual results or as the sum of both isomers.

The left-censored data were treated by the substitution method as recommended in the *Principles and methods for the risk assessment of chemicals in food* (FAO/WHO, 2009). At the LB, results below the LOQ and LOD were replaced by zero; at the UB, the results below the LOD were replaced by the LOD and those below the LOQ were replaced by the value reported as the LOQ. Additionally, as a point estimate between the two extremes, a middle bound (MB) scenario was calculated by assigning a value of LOD/2 or LOQ/2 to the left-censored data.

The highest levels of EAs were reported in rye and rye-containing commodities, particularly in raw agricultural or minimally processed commodities (for example, "rye milling products", 198–239 µg/kg, LB–UB). Among processed foods, the highest levels of EAs were found in "mixed wheat and rye bread and rolls" (33–82 µg/kg), "rye bread and rolls" (29–67 µg/kg) and

"rye flakes" (35–83 µg/kg), with the range representing the LB–UB scenarios in all cases.

For chronic dietary exposure assessment, the Committee considered food consumption data and body weight information from 35 recent dietary surveys carried out in 19 European countries present in the latest version of the comprehensive database. Occurrence and consumption data were linked at the lowest food level possible. The mean and the high (P95) chronic dietary exposures were calculated by combining mean occurrence values for EAs with the average daily consumption for each food at individual level in each dietary survey. Dietary exposure estimates were calculated for each dietary survey and age class. In addition, the different food commodities were grouped within each food category to better assess their contribution to the total dietary exposure to EAs.

Considering both the LB and the UB scenarios, the estimates for mean dietary exposure ranged from 10 ng/kg bw per day (minimum LB) in diverse age classes to 470 ng/kg bw per day (maximum UB) for "toddlers". Estimates for P95 dietary exposure ranged from 20 ng/kg bw per day (minimum LB) in diverse age classes to 860 ng/kg bw per day (maximum UB) for "toddlers". Estimates of chronic dietary exposure are summarized in Table 24.

The main contributors to dietary exposure to EAs were different types of bread and rolls, particularly those containing or made exclusively of rye. In some countries such as Austria, the contribution of "mixed wheat and rye bread and rolls" to the exposure to EAs represented up to 84% (MB) of the total dietary exposure. The consumption of rye-derived processed foods was also important in Nordic countries, where the consumption of "rye bread and rolls" contributed 61% of the total dietary exposure and up to 65% for "mixed wheat and rye bread and rolls" in some dietary surveys. "Wheat bread and rolls" were also a major contributor to the dietary exposure to EAs (up to 80% of the total, MB) in some countries. However, in general, these countries were not among those with the highest dietary exposure estimates for EAs. Other food commodities that contributed to the exposure to EAs were "breakfast cereals" in general, and "cereal flakes" in particular, with contributions up to 43% at the MB.

(c) WHO Pacific Region

New Zealand Food Safety runs an ongoing mycotoxin surveillance programme, which in 2017–2018 included survey and assessment of dietary exposure to EAs in the New Zealand food supply (NZFS, 2020).

The analytical results for the dietary exposure assessment were taken from a survey of cereal-based foods, with a particular focus on rye-based foods. The study analysed each sample for the 12 EAs: ergometrine, ergometrinine,

Table 24
Summary statistics of chronic dietary exposure to ergot alkaloids (ng/kg bw per day) across European dietary surveys and different age classes

		Mean dietary exposure (ng/kg bw per day)					
		Lower bound			Upper bound		
Age class	*N*	Min.	Median	Max.	Min.	Median	Max.
Infants (<12 months)	6	10	20	80	30	150	340
Toddlers (12 to <36 months)	10	30	60	120	180	250	470
Other children (36 months to <10 years)	18	20	50	170	140	200	460
Adolescents (10 to <18 years)	17	10	30	150	70	120	290
Adults (18 to <65 years)	17	10	20	50	60	90	180
Elderly (65 to <75 years)	14	10	20	50	50	90	140
Very elderly (over 75 years)	12	10	20	60	50	90	160
		95th percentile dietary exposure[a] (ng/kg bw per day)					
Infants (<12 months)	5	50	–[b]	190	290	–[b]	760
Toddlers (12 to <36 months)	7	70	180	300	380	590	860
Other children (36 months to <10 years)	18	50	120	390	290	420	790
Adolescents (10 to <18 years)	17	30	70	330	140	270	560
Adults (18 to <65 years)	17	20	40	120	120	180	360
Elderly (65 to <75 years)	14	20	40	100	110	170	280
Very elderly (over 75 years)	9	20	30	90	120	150	260

BW, body weight; Max., maximum; Min., minimum; N, number of surveys.
[a] The 95th percentile estimates obtained on dietary surveys/age classes with fewer than 60 observations may not be statistically robust. Those estimates were not included in this table.
[b] A minimum number of six dietary surveys are required to estimate a statistically robust median.

ergotamine, ergotaminine, ergosine, ergosinine, ergocornine, ergocorninine, ergocryptine, ergocryptinine, ergocristine and ergocristinine. Total EA concentrations were calculated as the sum of the 12 individual EA results for each food sample. For left-censored data (<LOQ), the UB was assigned a value of the LOQ to any EA detected at >LOD and <LOQ and a value of the LOD to any EA detected at <LOD, whereas the LB was assigned a concentration value of zero to any EAs below the LOQ. The LB–UB concentration estimates for each sample were used to derive the arithmetic means of LB–UB for each food type. For LB, mean total EA concentrations of all flour samples followed the order rye > spelt > oats > wheat. The order of this data set was comparable to the results reported by EFSA (2017).

Over half of the samples (60%) contained quantifiable levels of EAs (LOQs were in the range 0.5–1.25 µg/kg for the individual EAs). Just over one-third of samples (35%) contained quantifiable concentrations of total EAs ≤10 µg/kg. Concentrations of total EAs in the range 11–50 µg/kg were found in 20% of samples and the remaining 5% of samples had concentrations of total EAs

>50 µg/kg. The highest concentration was observed in a sample of rye flour. In the foods studied, ergotamine and ergotaminine were by far the most prevalent EAs.

Individual food consumption data were derived from the 2009 Adult Nutrition Survey (4721 respondents aged 15 years and over) and the 2002 National Children's Nutrition Survey (3098 respondents aged 5–15 years). Both surveys used a single-pass 24-hour dietary recall. Use of a single day of dietary recall tends to inflate the upper percentiles of the food consumption distribution, compared to habitual long-term consumption levels. Mean UB estimates of dietary EA exposure were 9.4 ng/kg bw per day for adults and 25 ng/kg bw per day for children. The main contributors to dietary EA exposure were wheat bread, wheat flour and multigrain bread.

NZFS concluded that the dietary exposure assessment for EAs in New Zealand is approximately 10-fold lower than in similar European studies. This is primarily because of the lower EA concentrations measured in cereal and rye-based foods.

8.3.2 National or regional estimates of chronic dietary exposure derived by the Committee

Additional national estimates of dietary exposure may be derived by the Committee. Considering that estimates of chronic dietary exposure for EAs are available for European countries, sub-Saharan African countries and New Zealand, and considering that those national estimates were of the same order of magnitude as those performed by the Committee with corresponding GEMS/Food cluster diets, the Committee decided not to derive additional national estimates of dietary exposure to EAs (for example, for countries in the WHO Region of the Americas).

8.3.3 International estimates of chronic dietary exposure derived by the Committee

International estimates of dietary exposure to EAs were derived by the Committee using food consumption information from the WHO GEMS/Food cluster diets. The concentration data for EAs in foods used for these international estimates of dietary exposure are shown in Tables 17–20. Concentration data on EAs in foods from the GEMS/Food contaminants database were pooled for countries within the same WHO region. Mean (LB and UB) concentrations for food commodities were then combined with food consumption information for the clusters associated with that WHO region (see Table 22) to derive international estimates of dietary exposure. Based on the assumption of equipotency for each individual EA, the Committee derived estimates of dietary exposure for the sum of EAs. The Committee decided not to derive estimates of dietary exposure for

clusters within WHO regions where 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 WHO Eastern Mediterranean Region and cluster G09 associated with the WHO South-East Asia Region.

The Committee derived international estimates of chronic dietary exposure using the 13 GEMS/Food cluster diets for the WHO African, Americas, European and Western Pacific regions (Table 22) with the concentration data for EAs in foods from the same WHO regions and food commodities as displayed in Tables 17–20.

Table 25 provides the estimated total and per commodity LB–UB dietary exposure to EAs for the GEMS/Food cluster diets in ng/kg bw per day. The GEMS/Food cluster diets are mean per capita estimates of food consumption and do not allow estimation of high percentiles of dietary exposure. However, the Committee used a previously established approximation in which the 90th percentile is approximately twice the mean dietary exposure (FAO/WHO, 2009). A standard body weight of 60 kg was used for all data reported in all GEMS/Food clusters to assess exposure per kg of body weight.

LB–UB mean estimates of dietary exposure to EAs ranged from 12.2 ng/kg bw per day (G14, Western Pacific) to 184.2 ng/kg bw per day (G02, Europe). LB–UB mean estimates of dietary exposure to EAs ranged from 24.4 ng/kg bw per day (G14, Western Pacific) to 368.5 ng/kg bw per day (G02, Europe). Wheat and wheat products are the main foods contributing to dietary exposure to EAs in all clusters (ranging from 67 to 100%). Rye and rye products also contributed to dietary exposure in all clusters, but to a lesser extent (ranging from 2 to 33%).

8.3.4 Dietary chronic exposures summary

National and international estimates of dietary exposure to EAs described in the previous sections are summarized in Table 26.

Across national and international estimates, LB–UB estimates of mean dietary exposure were in the range 10–470 ng/kg bw per day for children and 0.1–180 ng/kg bw per day for adults. High percentile LB–UB estimates of dietary exposure (P90 or P95) were in the range 28–860 ng/kg bw per day for children and 1–369 ng/kg bw per day for adults. For all national and international estimates the main foods contributing to dietary exposure were wheat and wheat-based-products.

The Committee noted that the dietary exposure estimates for GEMS/Food clusters within WHO regions for which national estimates were available (African, European and Western Pacific regions, Table 25) were of approximately the same order of magnitude as the corresponding national estimates. The

Table 25

Estimated LB–UB dietary exposure to the sum of EAs in the GEMS/Food cluster diets

	Estimated LB–UB dietary exposure to EAs in the GEMS/Food cluster diets (grouped by WHO region) in ng/kg bw per day																							
	European												African						Americas				Western Pacific	
Food commodities	G02		G07		G08		G10		G11		G15		G03		G13		G16		G05		G12		G14	
All foods	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Mean[a]	89.3	184.2	60.8	127.9	81.4	153.2	65.9	138.0	50.6	106.4	74.5	151.6	20.8	23.2	39.4	42.5	14.0	15.3	116.0	152.3	113.0	142.7	12.2	18.6
High (90th percentile)[b]	178.6	368.5	121.6	255.9	162.8	306.4	131.8	276.1	101.3	212.9	149.0	303.2	41.6	46.4	78.7	85.0	28.0	30.6	232.0	304.6	226.0	285.5	24.4	37.3
Grains and grain-based products																								
Barley, including flour and grits	0.2	1.5	0.0	0.1	0.1	0.5	0.1	0.4	0.0	0.2	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.2	0.0	0.0	0.0	0.0
%	0.2	0.8	0.0	0.1	0.1	0.3	0.1	0.3	0.1	0.2	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.8	0.0	0.0	0.1	0.1
Buckwheat, including flour	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
%	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Cereals preparations, NES (and flour)	1.1	1.4	4.2	5.4	2.0	2.6	10.5	13.5	2.0	2.5	5.3	6.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.6	0.0	0.0
%	1.2	0.8	6.9	4.2	2.5	1.7	15.9	9.8	3.9	2.4	7.1	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.4	0.3	0.3
Maize, including maize flour and sweetcorn	0.0	7.3	0.0	3.6	0.0	3.0	0.0	3.5	0.0	1.2	0.0	6.0	0.0	0.7	0.0	0.8	0.0	0.5	0.3	4.6	0.4	5.1	0.0	0.1
%	0.0	4.0	0.0	2.8	0.0	2.0	0.0	2.5	0.0	1.1	0.0	4.0	0.0	3.1	0.0	1.8	0.0	3.0	0.3	3.0	0.3	3.6	0.0	0.4
Oats, including rolled oats	0.3	0.9	0.4	0.9	0.3	0.8	0.2	0.6	0.2	0.4	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.4	0.0	0.0
%	0.4	0.5	0.6	0.7	0.4	0.5	0.4	0.4	0.3	0.4	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.0	0.0
Rice, including milled, husked, broken and flour	0.0	1.8	0.0	2.6	0.0	2.0	0.0	9.0	0.0	2.0	0.0	2.3	0.0	0.6	0.0	0.4	0.0	0.1	0.0	12.6	0.0	5.7	1.5	7.1
%	0.0	1.0	0.0	2.0	0.0	1.3	0.0	6.5	0.0	1.9	0.0	1.5	0.0	2.5	0.0	0.8	0.0	0.8	0.0	8.2	0.0	4.0	12.6	38.1

Table 25 (continued)

	Estimated LB–UB dietary exposure to EAs in the GEMS/Food cluster diets (grouped by WHO region) in ng/kg bw per day																							
	European												African						Americas				Western Pacific	
Food commodities	G02		G07		G08		G10		G11		G15		G03		G13		G16		G05		G12		G14	
Grains and grain-based products (continued)	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Rye, including flour	14.5	19.3	2.4	3.2	26.5	35.3	4.9	6.5	1.1	1.5	10.4	13.9	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
%	16.2	10.5	4.0	2.5	32.5	23.0	7.4	4.7	2.2	1.4	14.0	9.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
Wheat, including flour, macaroni and bread	73.1	151.2	53.7	111.0	52.2	107.9	50.1	103.5	46.3	95.7	58.3	120.5	20.8	21.9	39.4	41.4	14.0	14.7	114.5	133.4	112.3	130.9	10.6	11.2
%	81.9	82.1	88.3	86.8	64.1	70.5	76.0	75.0	91.3	89.9	78.2	79.5	100.0	94.4	100.0	97.3	100.0	96.1	98.7	87.6	99.4	91.7	86.4	60.3
Wheatgerm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
Pulses, nuts and oilseeds																								
Beans (green pods and immature seeds)	0.0	0.1	0.1	0.3	0.3	0.7	0.1	0.2	1.1	2.4	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
%	0.0	0.0	0.2	0.2	0.4	0.5	0.2	0.2	2.2	2.3	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Peas (dry)	0.0	0.6	0.0	0.8	0.0	0.3	0.0	0.5	0.0	0.5	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
%	0.0	0.3	0.0	0.6	0.0	0.2	0.0	0.3	0.0	0.5	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Soya beans (dry)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
%	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.4	0.7

%: percentage of dietary exposure to EAs that is due to consumption of the associated food commodity to the total dietary exposure from all foods, based on LB estimates of dietary exposure to EAs; bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; UB: upper bound.

[a] The range of dietary exposure estimates refers to LB and UB estimates of mean dietary exposure. 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 exposure estimates are based on a 60-kg body weight.

[b] High percentiles are an approximation of the 90th percentile dietary exposure, calculated as twice the mean dietary exposure (FAO/WHO, 2009).

Table 26

Summary of national and international LB–UB estimates of dietary exposure to EAs from the literature and derived by the Committee

Country/ WHO region	Food concentration data used	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (P90 or P95) in ng/kg bw per day [b]	Major contributors	Reference
WHO Americas[c]	GEMS/Food contaminants	Per capita consumption	Adult	113–152 (226–305)	Wheat and wheat-based products	Derived by this Committee
Benin	Survey mean (Ingenbleek et al., 2019)	Household budget survey	Adult (18 to <65 years)	0.1–0.3 (1)	Wheat and wheat-based products	Derived by this Committee
Cameroon	Survey mean (Ingenbleek et al., 2019)	Household budget survey	Adult (18 to <65 years)	10–42 (24–73)	Wheat and wheat-based products	Derived by this Committee
Mali	Survey mean (Ingenbleek et al., 2019)	Household budget survey	Adult (18 to <65 years)	5–31 (12–57)	Wheat and wheat-based products	Derived by this Committee
Nigeria	Survey mean (Ingenbleek et al., 2019)	Household budget survey	Adult (18 to <65 years)	34–94 (91–179)	Wheat and wheat-based products	Derived by this Committee
WHO African[c]	GEMS/Food contaminants	Per capita consumption	Adult	14–42 (28–85)	Wheat and wheat-based products	Derived by this Committee
New Zealand	Survey mean	National food consumption surveys (24-hour dietary recall)	Children (5–15 years) Adults (>15 years)	12–25 (28–59) 4.6–9.4 (12–22)	Wheat breads Wheat flour-containing foods Multigrain breads	(NZFS, 2020)
WHO Western Pacific[c]	GEMS/Food contaminants	Per capita consumption	Adult	12–19 (24–37)	Wheat and wheat-based products Rice and flour	Derived by this Committee
Europe (22 countries)[a]	EFSA database	National food consumption surveys (at least two survey days)	Infants (<12 months) Toddlers (12 to <36 months) Other children (36 months to <10 years) Adolescents (10 to <18 years) Adults (18 to <65 years) Elderly (65 to <75 years) Very elderly (over 75 years)	10–34 (50–760) 30–470 (70–860) 20–460 (50–790) 10–290 (30–560) 10–180 (20–360) 10–140 (20–280) 10–160 (20–280)	Wheat breads and rolls Breads and rolls containing or made exclusively of rye Breakfast cereals and cereal flakes in particular	(EFSA, 2017)

Table 26 (continued)

Country/ WHO region	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (P90 or P95) in ng/kg bw per day [b]	Major contributors	Reference
WHO European[c]	GEMS/Food contaminants	Per capita consumption	Adult	51–184 (101–369)	Wheat and wheat-based products Rye and flour	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.

[b] The range of dietary exposure estimates refers to LB and UB estimates of mean dietary exposure. National and international dietary exposure to EAs were reported in the 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 (FAO/WHO, 2009).

concentration data used to derive international estimates are exactly the same as those used to derive national estimates for the African and European regions.

8.4 Assessments of acute dietary exposure

8.4.1 National estimates of acute dietary exposure from the published literature

Except for European countries, no acute dietary exposure estimates for EAs have been reported in the scientific literature.

Acute dietary exposure to EAs was estimated by EFSA using a probabilistic approach. Single-day food consumption information from 41 recent dietary surveys carried out in 23 European countries was used. Acute exposure was assessed for each reporting day by multiplying the total daily consumption for each food by one occurrence level randomly drawn among the individual results available for that type of food. EFSA considered EA content in the food samples as the sum of up to 12 individual EAs. Most of the available data were left-censored (only 11% of the analytical results were quantified). Acute dietary exposure was estimated using the MB scenario for sample EA concentrations, as the UB scenario would represent an overly conservative exposure. The MB scenario was calculated by assigning a value of LOD/2 or LOQ/2 to the left-censored data. Respective exposures from the different foods consumed that day were then summed and finally divided by the individual's body weight. This process was iterated 1000 times for each reporting day. For each of these end-points, the 95% confidence interval was defined as the 2.5th and 97.5th percentiles obtained from the 1000 iterations.

The range of acute exposure estimates (average and P95, MB scenario) to EAs across European dietary surveys with their corresponding confidence intervals (2.5th and 97.5th percentiles) are presented in Table 27.

Mean acute exposure ranged from 20 ng/kg bw per day in "infants" up to 320 ng/kg bw per day in "other children". The highest estimate of MB P95 acute dietary exposure was for a dietary survey within the age class "other children" (980 ng/kg bw per day).

The food types making the greatest contribution to acute dietary exposure to EAs were the same as those identified for chronic dietary exposure: "mixed wheat and rye bread and rolls" and "rye bread and rolls". For high consumers, a single consumption of "mixed wheat and rye bread and rolls" can lead to acute exposure estimates up to 740 (95% CI = 590–930) ng/kg bw per day while for "rye bread and rolls", the exposure can reach values up to 640 (95% CI = 600–690) ng/kg bw per day.

Table 27
Summary statistics of acute dietary exposure to EAs (ng/kg bw per day) from European dietary surveys and different age classes

	Mean acute dietary exposure (ng/kg bw per day)			
Age class	**Number of dietary surveys**	**Minimum (95% CI)**	**Median**	**Maximum (95% CI)**
Infants (<12 months)	6	20 (10–40)	80	210 (200–210)
Toddlers (12 to <36 months)	11	110 (110–110)	150	300 (290–300)
Other children (36 months to <10 years)	20	80 (80–80)	130	320 (300–330)
Adolescents (10 to <18 years)	20	40 (40–50)	70	230 (210–240)
Adults (18 to <65 years)	22	30 (30–30)	60	170 (160–170)
Elderly (65 to <75 years)	16	30 (30–30)	50	130 (110–140)
Very elderly (over 75 years)	14	30 (30–40)	50	120 (100–150)
	95th percentile acute dietary exposure (ng/kg bw per day)			
Infants (<12 months)	5[a]	260 (250–270)	–	650 (620–680)
Toddlers (12 to <36 months)	10	350 (310–380)	500	790 (760–830)
Other children (36 months to <10 years)	20	270 (250–300)	400	980 (900–1060)
Adolescents (10 to <18 years)	20	130 (110–160)	260	770 (670–890)
Adults (18 to <65 years)	22	100 (100–110)	210	490 (460–530)
Elderly (65 to <75 years)	16	100 (90–110)	160	370 (290–480)
Very elderly (over 75 years)	14	100 (90–110)	160	390 (270–510)

95% CI: 95th percentile confidence interval.
[a] A minimum number of six dietary surveys are required to estimate a statistically robust median.

8.4.2 National estimates of acute dietary exposure derived by the Committee

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

8.5 Summary of global dietary exposure estimates

Across national studies and international estimates, LB–UB estimates of mean dietary exposure were in the range 0.01–0.47 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 (P90 or P95) were in the range 0.03–0.86 µg/kg bw per day for children and <0.01–0.37 µg/kg bw per day for adults. The main foods contributing to dietary exposure for all national and international estimates were wheat and wheat-based products.

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, together with a relatively symmetrical distribution of their occurrence values. 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.

9. Dose–response analysis and estimation of toxicity/ carcinogenic risk

9.1 Identification of key data for risk assessment

Data from studies in experimental animals described in section 2.2.2 and from clinical and epidemiological studies in humans described in section 2.4 were identified and evaluated for their relevance in assessing the human health risk and suitability for dose–response modelling and in the derivation of the 95% lower confidence limits of the benchmark dose (BMDL) as point of departure.

9.1.1 Pivotal data from biochemical and toxicological studies

Limited toxicological data were available on naturally occurring EAs. The longest exposure period was in a 13-week toxicity study conducted in rats treated with ergotamine tartrate (Speijers et al., 1993). No long-term toxicity study of specific naturally occurring EAs was available.

Seven key studies in experimental animals were identified by the Committee as having data that might be applicable for dose–response modelling. In addition to the 13-week toxicity study conducted in rats with ergotamine tartrate (Speijers et al., 1993), three short-term toxicity (4 weeks) studies in rats treated with ergotamine tartrate (Speijers et al., 1992), ergometrine maleate (Peters-Volleberg, Beems & Speijers, 1996) and α-ergocryptine (Janssen et al., 1998, 2000a,b) and two short-term toxicity studies (4 weeks) in pigs given feed contaminated with ergot sclerotia (Oresanya et al., 2003, Maruo et al., 2018) were also considered. A further study conducted in pigs exposed for 50 days to feed contaminated with ergot sclerotia was also considered (Dignean, Schiefer & Blair, 1986).

(a) Rats

Ergotamine tartrate administered at concentrations of 0, 4, 20, 100 or 500 mg/kg diet showed concentration-dependent effects on body and relative organ weight,

feed intake, and histopathological and biochemical parameters in female and male rats (Speijers et al., 1992). The concentration-dependent incidence of tail abnormality (redness of the tail tip, which in some cases progresses to necrosis and degenerative changes in the longitudinal skeletal muscle) assessed in this study at the two highest dietary concentrations was identified as the most relevant adverse health effect from this study for dose–response modelling.

Ergotamine maleate administered at concentrations of 0, 2, 10, 50 or 250 mg/kg diet caused significant effects, mainly at the highest concentration (relative organ weights, histopathological changes and effects on T4 levels (Peters-Volleberg, Beems & Speijers, 1996). Prolactin levels were decreased in animals given the two highest concentrations. Tail abnormalities were not observed and this study was not considered further for dose–response modelling.

The study by Janssen et al. (1998, 2000a,b) using concentrations of 0, 4, 20, 100 or 500 mg α-ergocryptine/kg diet showed concentration-dependent effects on body weight, body weight gain, feed intake and feed efficiency as well as on relative organ weights, which exhibited some non-monotonicity. Concentration-dependent effects were also observed in several parameters of blood and urine chemistry and after microscopical examination of tissues at autopsy, including a detailed examination of the tail. Focal muscle degeneration in the tail was considered relevant for dose–response modelling. Concentration-dependent changes also occurred in carbohydrate metabolism and thyroid and pituitary functions. Significant decreases in serum prolactin were observed, in particular, in females where the levels were statistically significantly decreased in animals that received concentrations of 20 and 500 mg/kg feed. The concentration-response of prolactin was, however, non-monotonic in animals that received 100 mg/kg feed. In these animals, the mean was about twice that of the next lower concentration of 20 mg/kg feed. Only a few of the suite of dose–response models could be fitted satisfactorily in a tentative benchmark dose (BMD) analysis of the data on serum prolactin in females. The highly variable prolactin levels seen in this study were anticipated in females, probably because of their unknown estrus cycle status at the time of blood collection. Serum prolactin levels in female rats typically increase during the early stages of estrus. It was also noted that the prolactin levels in this study were mainly within the normal range. The prolactin levels in males were much lower and the fitted curves were flatter than those of the females. The Committee concluded that the data available on the suppression of prolactin were not suitable for determining a BMDL using the BMD approach and considered the data on tail muscle abnormalities observed in this study for dose–response modelling.

(b) **Pigs**

The Committee noted that a dose–response evaluation of data on pigs exposed to ergot sclerotia at different concentrations is restricted to a concentration–response analysis. This is because the calculations of doses of specific EAs would not be possible from the limited information available in the published reports. Results of concentration–response modelling in pigs might, however, be informative when these results are compared with concentration–response modelling from other animals.

In the 50-day study by Dignean, Schiefer & Blair (1986), pigs were fed an experimental diet that included ergot sclerotia at concentrations of 0, 0.225, 0.45, 0.9 or 1.8 g sclerotia/kg diet. Although the extent and severity of haematological, hepatic, urinary and histological alterations were associated with increasing concentrations of sclerotia in the diet, the Committee could not identify relevant end-points suitable for concentration–response modelling, particularly because only two animals (one female and one male) were assigned to each concentration.

Ergot sclerotia added to the basal diet of groups of pigs in small but increasing amounts (0, 0.05, 0.10, 0.25, 0.50 and 1.00%) (n = 32 per group) was associated with a concentration-dependent decrease of body weight, feed intake and feed efficiency as well as with the suppression of prolactin and urea nitrogen (Oresanya et al., 2003). The Committee considered prolactin reduction as relevant but also noted the absence of a clear concentration–response relationship.

A recently published study of ergot sclerotia in pigs (n = 24 per group) compared a control group with two groups receiving concentrations of 1.2 or 2.5 g sclerotia/kg diet with mean EA concentrations of 2.36 and 5.05 mg/kg diet, respectively (Maruo et al., 2018). The Committee identified a clear concentration–response relationship between the occurrence and severity of histopathological lesions in the intestine and liver. Lesion scores for animals given concentrations of 2.36 and 5.05 mg/kg diet were statistically significantly different from those of the control animals in the three subsamples of n = 6 pigs per group. The Committee obtained from the authors individual data for the pigs (body weights, feed intake and the frequency and severity of jejunal lesions). A dimensionless individual jejunal lesion score ((1, 1, 1, 2, 4, 5) for controls and (3, 4, 5, 7, 7, 9) and (4, 4, 9, 9, 10, 12) for 1.2 or 2.5 g sclerotia/kg diet, respectively) based on different histopathological observations and representing frequency and severity of lesions showed a clear concentration–response relationship. The mean scores (SD) were 2.33 (1.75), 5.83 (2.23) and 8 (3.29) for the control group and the two concentration groups, respectively. The Committee considered using a BMD approach to calculate a reference concentration from these data. Application of Benchmark Dose Software version 3.2 of the United States Environmental Protection Agency (BMDS 3.2) to both the individual and the summary data

resulted in wide benchmark concentration (BMC) intervals for benchmark response (BMR) values of 10% or larger. Most of the BMCL values (the LB of the BMC interval calculated as a BMDL) were orders of magnitude lower than the low sclerotia concentration of 1.2 mg/kg feed. Model averaging was not possible given the sparsity of the data (n = 6 per group and only two concentrations). The Committee noted that the test material in this study was feed contaminated with ergot sclerotia in which mixtures of EAs were present and the proportions differed depending on whether the feed was for the low- or high-concentration group. The relevance of this mixture with regard to human dietary exposure was difficult to assess, taking into account the variability in food contamination data according to the region of the world.

Considering the difficulties, the Committee refrained from dose–response modelling of this dataset using a BMD approach and decided that the dose–response assessment should revert to the LOAEL of 100 µg EAq/kg bw calculated by the authors (see section 2.2.2).

In the 13-week study by Speijers et al. (1993), female and male rats were exposed to ergotamine tartrate administered at concentrations of 0, 5, 20 or 80 mg/kg diet. Concentration-dependent effects were observed for body weight gain and feed intake, relative organ weight, haematological, biochemical and histopathological effects. The Committee identified the concentration-dependent incidence of muscular atrophy in the caudal longitudinal muscles of the tail of animals as the most relevant adverse health effect for dose–response modelling.

9.1.2 Pivotal data from human clinical/epidemiological studies

The Committee considered the human data on the clinical use of EAs to be relevant for establishing a health-based guidance value (HBGV). As certain effects induced by EAs in humans can be due to acute or short-term exposures, the Committee decided it would be appropriate to establish an acute oral reference dose from the available data. Ergotamine and ergometrine have been used in human medicine for the treatment of migraine headache and for management of the third stage of labour and postpartum blood loss. Ergometrine is the most toxic of the naturally occurring EAs based on LD_{50} values. Humans appear to be more sensitive to some effects of ergometrine, including on uterine smooth muscle contraction, than experimental animals, and ergometrine maleate is more active than ergotamine as a uterine-stimulating agent.

The initial oral dosage of ergometrine maleate to induce smooth muscle contractions of the uterus is typically 0.2 mg and can be increased to 0.4 mg, 2–4 times daily for up to 7 days. Side-effects, including vomiting and nausea, are well-characterized in the normal therapeutic dose range. Overdosages may cause

seizures and gangrene as well as more severe gastrointestinal symptoms, vascular effects, dizziness or loss of consciousness, and cardiovascular effects.

Relatively higher doses of ergotamine tartrate are used to treat migraine (vasoconstriction) compared with the therapeutic doses of ergometrine maleate. The usual oral dose is 2 mg, which can be repeated until maximums of 6 mg per day, 8 mg per attack, 12 mg per week and two courses per month are reached. Common side-effects, which typically occur as a result of prolonged use, include nausea and vomiting, and effects consistent with ergotism (i.e. cardiovascular effects, gangrene, confusion and convulsions). In rare cases, pleural and peritoneal fibrosis and fibrosis of the cardiac valves have been reported.

9.2 General modelling considerations

The Committee identified the tail muscular atrophy effect in experimental animals as the critical effect suitable for dose–reponse modelling. This tail muscular atrophy can be explained by vasoconstriction. The most consistent toxicity findings for ergotamine tartrate and α-ergocryptine were tail muscular atrophy in female rats in 4-week and 13-week studies. Data from clinical uses of ergotamine and ergometrine in humans were not considered suitable for quantitative dose–reponse modelling.

9.2.1 Selection of data

For acute toxicity, data from short-term studies were considered by the Committee for dose–response modelling, based on tail muscular atrophy or degeneration in rats (Speijers et al., 1992; Janssen et al., 2000a,b).

For long-term toxicity, data from the subchronic study in rats were considered by the Committee for dose–response modelling, based on tail muscular atrophy (Speijers et al., 1993).

9.2.2 Measure of exposure

EA doses were derived from EA concentrations using available information on initial and final body weights and food intake data separately for each sex and each dose group and the control group. In some cases, initial body weights were calculated by subtracting from the reported final body weight an estimated body weight gain calculated by the authors (for example, when based on modelling of all measured weights over the duration of the experiment). An average weekly food intake was calculated when mean weekly food intake was available. The Committee calculated doses using the respective molecular weights when exposure was reported in terms of the concentrations of EA salt (for ergotamine tartrate, see Table 28).

Table 28
Dose–response data for the two 4-week studies and the 13-week study identified for dose–response modelling using the benchmark dose approach for EAs[a]

Speijers et al., 1992 ergotamine tartrate in diet for 4 weeks 0, 100, 500 mg/kg diet Approximately six males and six females per group		
	Ergotamine (mg/kg bw per day)	**Incidence of tail abnormality**
females	0, 7.3, 41	0/4, 0/6, 3/6
males	0, 7.5, 37	0/3, 2/6, 4/5

Janssen et al., 1992 α-ergocryptine in diet for 4 weeks 0, 4, 20, 100, 500 mg/kg diet six males and six females per group		
	α-Ergocryptine (mg/kg bw per day)	**Incidence of muscular degeneration**
females	0, 0.37, 1.7, 8.9, 60	0/6, 0/6, 0/6, 0/5, 4/6
males	0, 0.34, 1.4, 6.6, 44	0/6, 0/6, 0/6, 2/6, 6/6

Speijers et al., 1993 Ergotamine tartrate in diet for 13 weeks 0, 5, 20, 80 mg/kg diet 10 males and 10 females per group		
	Ergotamine (mg/kg bw per day)	**Incidence of tail abnormality**
females	0, 0.48,1.99, 7.63	0/10, 0/10, 2/10, 7/10
males	0, 0.42, 1.65, 6.18	1/10,1/10,1/10,7/10

[a] The Committee calculated the doses of ergotamine from the concentrations of ergotamine tartrate in the diet using the reports of the authors. Doses of α-ergocryptine were calculated by the authors.

9.2.3 Measure of response

In all three studies, statistically significant dose-dependent effects were noted. The Committee concluded that the data on these three studies were relevant for BMD modelling and a BMR of 10% extra risk was used for these quantal dose–response data.

9.2.4 Selection of mathematical model

The BMD approach was used for dose–response modelling (DRM) on the data from the experimental animals selected for the risk assessment of EAs using the guidance of FAO/WHO (2020), Chapter 5.

BMDS 3.2 was applied for DRM (https://www.epa.gov/bmds) on the data considered suitable for this purpose. The models available in BMDS 3.2 for dichotomous (quantal) response were used as the default set of models and Bayesian model averaging (Bayesian MA) was used for the calculation of

BMD confidence intervals (BMD-CI). The BMR default value of 10% extra risk was selected for the dichotomous critical end-point of incidence of tail muscle degeneration or muscular atrophy in Sprague-Dawley rats investigated over 4 and 13 weeks. For the quantitative end-points of prolactin levels in Sprague-Dawley rats and the jejunal lesion score in pigs, the relative deviation from the level at dose zero of 10% or higher was used as the BMR.

The benchmark dose at 10% extra risk (BMD_{10}) estimates and the BMD_{10}-CI of Bayes MA are reported below in detail for the two 4-week studies (Speijers et al., 1992; Janssen et al., 2000a,b) and the 13-week study by Speijers et al. (1993), separately for females and males. The results of Bayesian MA are summarized in Table 29 and details are given in Tables 31 and 32 when using the BMDS 3.2 default of non-informative priors (11% prior probability assigned to each of the nine models used to calculate the BMD and the BMD-CI. Figure 3 shows the overall curve fitting of the suite of models for Bayesian MA by BMDS 3.2 for the 13-week study by Speijers et al. (1993) for females and males. Figure 4 shows the fitted curve of the model with the largest posterior probability, i.e. the highest weight, among the nine single Bayesian models used for Bayesain MA with its individual BMD_{10} and BMD_{10}-CI since no comprehensive graphics on the overall outcome results of BMD model averaging BMD-CI were available.

From the result of Bayesian MA, the lower confidence bound ($BMDL_{10}$) corresponding to the BMR of 10% extra risk was selected as the reference point for hazard characterization.

9.3 BMD analysis

Table 29 shows the BMD_{10} and the BMD_{10}-CI with upper and lower 95% confidence limits ($BMDL_{10}$ and $BMDU_{10}$) using Bayesian MA. The data from the two 4-week studies on ergotamine tartrate and α-ergocryptine provided the lowest reference point ($BMDL_{10}$) of 1.3 mg/kg bw from males in the Janssen et al. (2000a) study, based on muscular degeneration in the tail, secondary to vasoconstriction.

The Committee considered the dose–response data from the 13-week study by Speijers et al. (1993) on muscular atrophy in the tail as suitable for calculating a reference point for chronic exposure in humans. Female and male Sprague-Dawley rats were exposed to ergotamine tartrate at three doses equivalent to a range from 0.4 to 7.6 mg ergotamine/kg bw per day (Table 28). Bayesian MA performed with BMDS 3.2 provided a BMD_{10}-CI of 0.61–3.20 mg ergotamine/kg bw per day for females and 0.63–3.53 mg ergotamine/kg bw per day for males. The Committee established an overall $BMDL_{10}$ of 0.6 mg/kg bw per day for ergotamine for the subchronic exposure of rats.

Table 29

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

Ergot alkaloid/ concentration in the diet	Sex	Dose (mg/kg bw per day)	BMD (mg/kg bw per day)	BMD-confidence interval (mg/kg bw per day)	Reference
4-week studies					
Ergotamine tartrate	Females	0, 7.3, 41	16.6	5.25–64.7	Speijers et al. (1992)
0, 100, 500 mg/kg diet	Males	0, 7.5, 37	6.37	1.68–28.4	
α-Ergocryptine 0, 4, 20,	Females	0, 0.37, 1.7, 8.9, 60	18.1	5.36–37	Janssen et al. (2000a)
100, 500 mg/kg diet	Males	0, 0.34, 1.4, 6.6, 44	3.88	1.29–9.59	
13-week study					
Ergotamine tartrate	Females	0, 0.48,1.99, 7.63	1.54	0.61–3.20	Speijers et al. (1993)
0, 5, 20, 80 g/kg diet	Males	0, 0.42, 1.65, 6.18	1.68	0.63–3.53	

BMDS 3.2, Benchmark Dose Software from the United States Environmental Protection Agency, version 3.2.

Table 30

Benchmark dose (BMD) analysis of the tail abnormality observed in the 4-week study in female and male rats (Speijers et al., 1992) using BMDS 3.2[a]

	Females			Males		
	BMDS 3.2 Bayesian MA		MA weights probability (%) rounded	BMDS 3.2 Bayesian MA		MA weights probability (%) rounded
Model	BMD	BMD-CI posterior		BMD	BMD-CI posterior	
Probit	17.5	9.08–61.0	17	6.93	3.57–30.2	12
Logit	22.8	9.43–inf	17	9.03	3.74–66.5	15
LogProbit	19.4	7.67–33.9	10	11.3	2.01–27.1	7
LogLogistic	13.6	3.81–26.9	12	6.01	0.713–19.8	9
Weibull	17.3	6.41–33.9	17	10.3	1.99–28.4	8
Gamma	15.6	5.12–76.3	6	6.78	1.34–87.7	5
Multistage 2	6.92	3.91–11.9	2	4.41	2.27–8.33	11
Multistage 1	6.71	3.72–12.9	not used	4.08	2.11–8.81	not used
Quantal linear	8.36	3.77–24.8	12	3.54	1.78–13.1	28
Hill dichotomous	15.0	3.68–68.4	10	4.07	0.435–42.3	4
Model average	**16.6**	**5.25–64.7**		**6.37**	**1.68–28.4**	

BMDS 3.2, Benchmark Dose Software from the United States Environmental Protection Agency, version 3.2; MA, model average.
[a] BMR=10% for extra risk, all results rounded to two significant digits after decimal for doses in mg/kg bw per day.

Detailed results are shown in Tables 30–32 indicating acceptable fits for all nine models incorporated in the Bayesian model averaging. The posterior probabilities used for model averaging by the BMDS 3.2 software ranged between 6 and 15%. A cross-check with results obtained by independent software available

Table 31

Benchmark dose (BMD) analysis of the tail muscular degeneration in the 4-week study in female and male rats (Janssen et al., 2000a) using BMDS 3.2[a]

	Females			Males		
	BMDS 3.2 Bayesian MA		MA weights probability (%) rounded	BMDS 3.2 Bayesian MA		MA weights probability (%) rounded
Model	BMD	BMD-CI posterior		BMD	BMD-CI posterior	
Probit	23.4	13.0–39.1	26	6.54	3.01–12.0	14
Logit	21.9	12.6–44.4	8	6.89	3.29–12.7	5
LogProbit	22.5	7.96–41.5	12	4.26	1.58–13.0	3
LogLogistic	15.9	4.57–39.6	12	3.71	1.01–11.6	2
Weibull	19.9	7.06–37.2	11	4.60	1.37–14.5	7
Gamma	14.6	5.11–35.6	5	3.40	1.14–8.58	5
Multistage 4	9.71	5.32.16.5	not used	4.46	2.20–8.63	not used
Multistage 3	9.39	5.11–15–9	not used	4.25	2.10–8.18	not used
Multistage 2	8.71	4.83–15.1	5	4.00	1.98–7.35	28
Multistage 1	7.93	4.44–15.0	not used	3.12	1.72–5.98	not used
Quantal linear	8.27	4.00–20.4	12	2.19	1.07–4.74	33
Hill dichotomous	15.0	3.68–68.4	8	3.23	1.03–8.33	3
Model average	**18.1**	**5.36–37.4**		**3.88**	**1.29–9.59**	

BMDS 3.2, Benchmark Dose Software from the United States Environmental Protection Agency, version 3.2; MA, model average.
[a] BMR=10% for extra risk, all results rounded to two significant digits after decimal for doses in mg/kg bw per day.

Table 32

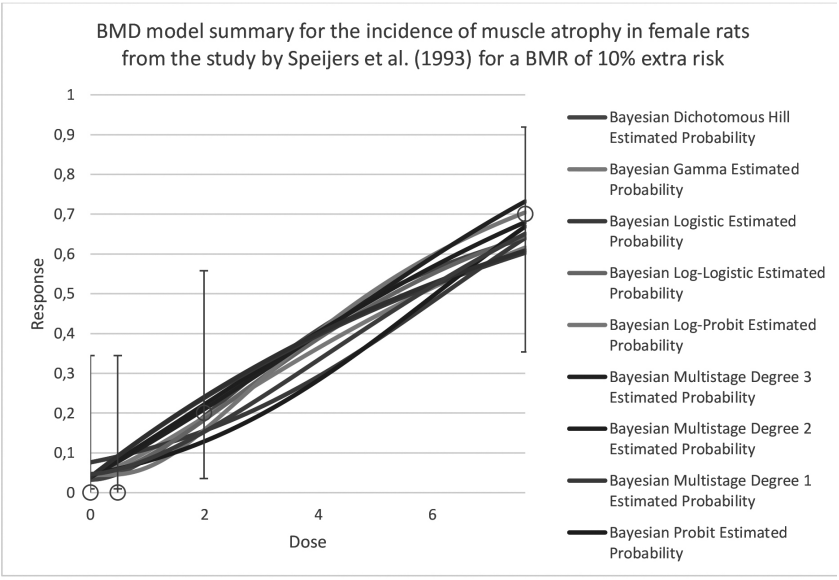
Benchmark dose (BMD) analysis of the tail muscular atrophy observed in the 13-week study in female and male rats (Speijers et al., 1993) using BMDS 3.2[a]

	Females			Males		
	BMDS 3.2 Bayesian MA		MA weights probability (%) rounded	BMDS 3.2 Bayesian MA		MA weights probability (%) rounded
Model	BMD	BMD-CI posterior		BMD	BMD-CI posterior	
Probit	2.20	1.43–3.35	15	1.60	1.05–2.67	15
Logit	2.23	1.44–3.43	8	1.71	1.09–3.26	13
LogProbit	1.85	0.85–4.24	7	2.42	1.08–4.18	14
LogLogistic	1.55	0.58–3.27	13	1.80	0.62–3.45	14
Weibull	1.86	0.75–3.75	11	2.07	0.82–3.67	14
Gamma	1.42	0.59–2.86	7	1.47	0.58–3.25	6
Multistage 3	1.15	0.68–2.00	not used	1.02	0.60–1.67	not used
Multistage 2	1.06	0.63–1.74	12	0.94	0.55–1.55	6
Multistage 1	0.91	0.57–1.56	not used	0.83	0.50–1.48	not used
Quantal linear	0.90	0.52–1.74	21	0.85	0.47–1.90	12
Hill dichotomous	1.44	0.54–3.05	7	1.73	0.57–3.49	6
Model average	**1.55**	**0.61–3.20**		**1.70**	**0.63–3.53**	

BMDS 3.2, Benchmark Dose Software from the United States Environmental Protection Agency, version 3.2; MA, model average.
[a] BMR=10% for extra risk, all results rounded to two significant digits after decimal for doses in mg/kg bw per day.

Fig. 3

Fit of the suite of models available in BMDS 3.2 for Bayesian modelling used for model averaging of the dose–response assessment of ergotamine in the 13-week study by Speijers et al. (1993)[a]



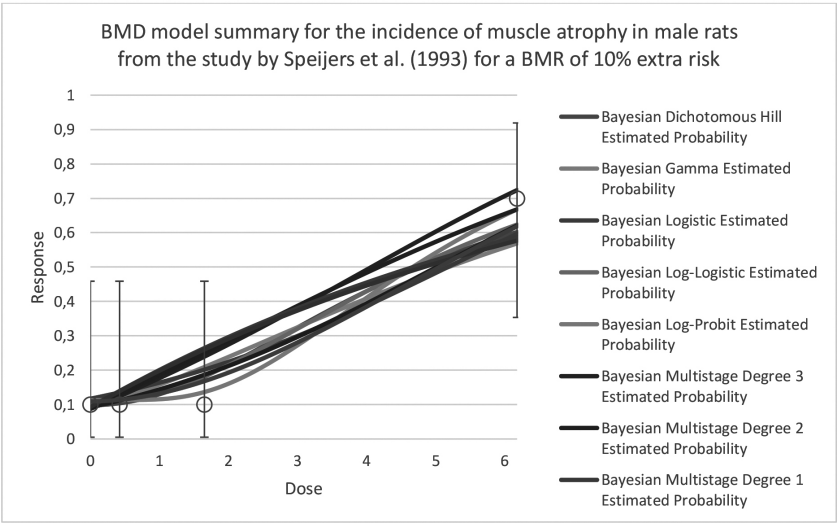
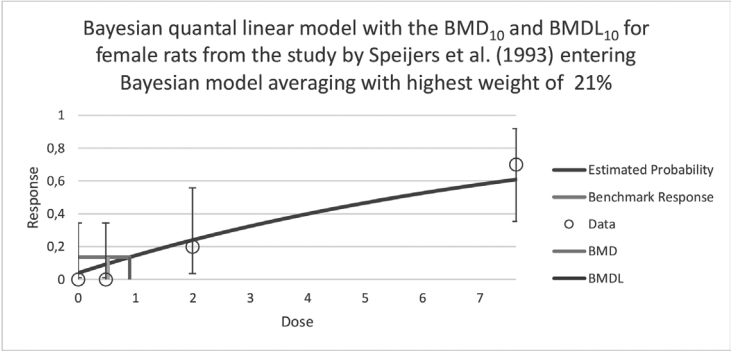
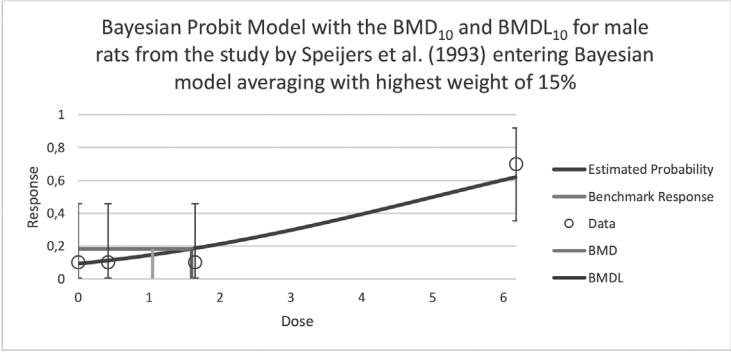


Fig. 4

Fit of the model with highest weight (largest posterior probability) included in the Bayesian model averaging of BMDS 3.2 of US EPA of the dose–response assessment of ergotamine in the 13-week study by Speijers et al. (1993) shown in Figure 3 and Table 32





from EFSA (https://shiny-efsa.openanalytics,eu/bmd) based on a slightly different set of models was also applied to the male and female data, both separately and combined. This resulted in similar reference points for the response of muscular atrophy in rats as an end-point for vasoconstriction in tails.

The Committee noted that applying a default uncertainty factor (UF) of 100 for intra- and interspecies differences together, a UF of 2 for the use of the 13-week study for chronic toxicity in the absence of long-term data on chronic toxicity, and an additional UF of 3 (to take into account the limitations of the available toxicity data) converts the overall $BMDL_{10}$ of 0.6 mg/kg bw per day to a tolerable daily intake (TDI) of 1 µg/kg bw per day.

10. Comments

10.1 Biochemical aspects

No reports of kinetic studies are available for most of the naturally occurring EAs (for example, 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 (Nimmerfall & Rosenthaler, 1976; Eckert et al., 1978). 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 (Nimerfall & Rosenthaler, 1976; Eckert et al., 1978). In humans, excretion of various radiolabelled EAs including some dihydro-derivatives in urine, was low (range 1 to 5%) *(*Schmidt & Fanchamps, 1974; Meier & Schreier, 1976; Little et al., 1982; Maurer & Frick, 1984; Ronca et al., 1996*)*. 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) (Schumann et al., 2009).

Apart from their 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% (Leist & Grauwiler, 1973; Reddy et al., 2020*)*.

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 (de Groot et al., 1994). 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 (Meier & Schreier, 1976; Aellig & Nüesch, 1977; Ala-Hurula et al., 1979a; Little et al., 1982).

There is no information on the likely presence of ergometrine in the milk of lactating women (EMEA, 1999). 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 (Erkkola et al., 1978; Vogel et al., 2004).

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 (Horowitz, Dart & Gomez, 1996) and ritonavir (Liaudet et al., 1999). 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 (Eckert et al., 1978). Maurer & Frick (1984) 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 (Chen et al., 2002; Bicalho et al., 2005). This metabolite, 8'-OH-dihydroergotamine, has been shown to have approximately the same potency as dihydroergotamine for vasoconstrictive activity in human volunteers (Aellig, 1984).

10.2 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 (Griffith et al., 1978). 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 (Thorat et al., 2019; Reddy et al., 2020) 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 (4-week) toxicity studies have been conducted in rats treated with ergotamine tartrate (Speijers et al., 1992), ergometrine maleate (Peters-Volleberg, Beems & Speijers, 1996) and α-ergocryptine (Janssen et al., 1998, 2000a, b) and in pigs given feed contaminated with ergot sclerotia (Oresanya et al., 2003; Maruo et al., 2018). A further study was conducted in pigs exposed for 50 days to feed contaminated with ergot sclerotia (Dignean, Schiefer & Blair, 1986).

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 (Speijers et al., 1992). 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 (Peters-Volleberg, Beems & Speijers, 1996). 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 (Peters-Volleberg, Beems & Speijers, 1996).

α-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 (Janssen et al., 1998, 2000a, b). 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 (Janssen et al., 1998). 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 (Janssen et al., 2000a).

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 (Speijers et al., 1992; Peters-Volleberg, Beems & Speijers, 1996; Janssen et al., 2000a, b). 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 (Maruo et al., 2018). 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 (Oresanya et al., 2003). The Committee noted that there was no dose–response relationship in the prolactin reduction (Oresanya et al., 2003).

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 (Dignean, Schiefer & Blair, 1986). 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 (Speijers et al., 1993). 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 37). 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 (Fitzhugh, Nelson & Calvery, 1944), 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 (Zeiger et al., 1987). α-Ergocryptine did not induce sister chromatid exchange in Chinese hamster ovary cells (Dighe & Vaidya, 1988). Agroclavine (a precursor compound) showed a weak mutagenic response when activated with rat liver S9 in *S.* Typhimurium strains (Glatt et al., 1987). However, ergometrine maleate and ergotamine tartrate induced chromosomal damage in human leukocytes in vitro (Jarvik & Kato, 1968; Dighe & Vaidya, 1988; Roberts & Rand, 1977a). Ergotamine tartrate and ergometrine maleate induced sister chromatid exchange in Chinese hamster ovary cells (Dighe & Vaidya, 1988). Semi-synthetic dihydrogenated derivatives (dihydroergocristine and α-dihydroergocryptine) also gave negative results in tests with *S.* Typhimurium strains (Dubini et al., 1990; Adams et al., 1993).

In vivo, ergotamine tartrate showed no genotoxic potential in the micronucleus test after intraperitoneal injection to mice and Chinese hamster (Matter, 1976) and gave negative results in the dominant lethal test after intraperitoneal injection in mice (Roberts & Rand, 1978; Matter, 1982). Semi-synthetic dihydrogenated derivatives (dihydroergocristine and α-dihydroergocryptine) also gave negative results in the in vivo mouse micronucleus assay after oral administration (Dubini et al., 1990; Adams et al., 1993). However, ergotamine tartrate was reported to induce a significant number

of chromosomal aberrations in bone marrow cells after intraperitoneal injection in mice (Roberts & Rand, 1977b).

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 (Carlsen Zeilmaker & Shelesnyak, 1961; Deanesly, 1968; Carpent & Desclin, 1969; Mantle, 1969; Grauwiler & Schön, 1973; Floss, Cassady & Robbers, 1973; Schön, Leist & Grauwiler, 1975; Griffith et al., 1978; Holstege & Traven 2014). The effects vary according to the EA (Griffith et al., 1978). 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 (Ben-Jonathan, LaPensee & LaPensee, 2008). EAs inhibit prolactin secretion and impair lactation in rodents and humans (Mantle, 1968; Shaar & Clemens, 1972; Griffith et al., 1978; Flint & Ensor, 1979; Kopinski et al., 2007, 2008).

The pharmacological mechanisms associated with ergot toxicity are complex and have not been fully delineated (Holstege & Traven 2014). They include peripheral vasoconstriction, peripheral adrenergic blockade, reduced secretion of prolactin and stimulation of uterine smooth muscle (Peters-Volleberg, Beems & Speijers 1996).

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) (for example, 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 (Zajdel et al., 2015). Ergotamine displayed high affinity for adrenergic (α1, α2), 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 (Mantegani, Brambilla & Varasi, 1999).

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 (Griffith et al., 1978). 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 (Speijers et al., 1992, 1993).

Cherewyk et al. (2020) 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 (Müller-Schweinitzer, 1984).

EAs have an agonist effect on the α-adrenergic receptors. Activation of α1-adrenergic receptors produces anorexia. Reduced appetite was observed in rat studies (Speijers et al., 1992, 1993; Janssen et al., 2000a, b) and in rabbit studies (Canty et al., 2014; Solano-Baez et al., 2018) 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.

10.3 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 (2015)). 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 (Poole et al., 2018; Britt et al., 2019; Cowan et al., 2018, 2019; Klotz et al., 2019). The effects of EAs on lactation vary with the livestock species. Consumption of EAs reduces milk yield in cattle, horses and sheep (Poole & Poole, 2019). Poole & Poole (2019) 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.

10.4 Observations in humans

10.4.1 Biomarkers

A small number of studies of EA exposure biomarkers were identified. One study measured EAs in serum and urine and collected 24-hour dietary recall data that were used to estimate dietary exposure for 600 men and women participating in the European Food Consumption Validation Project (DeRuyck et al., 2020). 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.

10.4.2 Clinical observations

Ergotamine tartrate is used to treat migraine and cluster headaches (Silberstein & McCrory, 2003; Tfelt-Hansen & Diener, 2014). 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) (Martindale, 2010).

The adverse effects of ergotamine are related to its vasoconstrictive properties and its effects on the CNS. 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) (Martindale, 2010). In rare cases, pleural and peritoneal fibrosis and fibrosis of the cardiac valves have been reported (Martindale, 2010). 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 (for example, 1 mg ergotamine plus 100 mg caffeine daily for 5 days) (Navarro et al., 2017), and up to several decades in

patients treated for migraine (for example, 1–3 mg ergotamine plus caffeine for 30 years (Maréchaux et al., 2015).

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 (Sanders-Bush & Mayer, 2006). 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 (Sanders-Bush & Mayer, 2006). 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 (Martindale, 2010). Overdosages may cause seizures and gangrene as well as more severe gastrointestinal symptoms, vascular effects, dizziness or loss of consciousness and cardiovascular effects (Martindale, 2010).

10.4.3 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) (Velentgas et al., 2004; Wammes-van der Heijden et al., 2006). 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 (Robblee et al., 2020), and that most of the symptoms reported after overdose in another study were due to interactions with CYP3A4 inhibitors (Srisuma, Lavonas & Wananukul, 2014). 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)) (Armenian & Kearney, 2014).

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 (Bánhidy et al., 2007). 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) (Wainscott, Volans & Wilkinson, 1974). 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 (Medveczky, Puhó & Czeizel, 2004; Ács et al., 2006).

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 (De Groot et al., 1996). 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 (AHFS, 1995; EFSA, 2012). 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) (Armenian & Kearney, 2014).

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) (EFSA, 2012). 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 (King, 1979; EFSA, 2012) 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 (Urga et al., 2002; EFSA, 2012; Belser-Ehrlich et al., 2013). 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) (Krishnamachari & Bhat, 1976; WHO-ICPS, 1990; EFSA, 2012).

10.5 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 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 available 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 multimycotoxin HPLC-MS/MS methods. The development of multimycotoxin 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.

10.6 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.

10.7 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 (for example, 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 (Tittlemier et al., 2019).

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

10.8 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.

10.9 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 (3343 µg/kg), the highest level 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 33 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.

Table 33

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			

Table 33 (continued)

	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)**
Wholemeal bread	NA				38	39.5	68.5	114.3	3	66.7	14.0	14.3	NA			
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			
Soya bean (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.

10.10 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 were all based on total EA concentration calculated as the simple sum of individual EAs. Table 34 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 33. 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 (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.

10.10.1 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.

10.11 Dose–response analysis

10.11.1 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 35). 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).

10.11.2 Dose–response data in animals

The key studies in experimental animals identified by the Committee are summarized in Table 36.

Table 34

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	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) Toddlers (12 to <36 months) Other children (36 months to <10 years) Adolescents (10 to <18 years) Adults (18 to <65 years) Elderly (65 to <75 years) Very elderly (over 75 years)	0.01–0.03 (0.05–0.76) 0.03–0.47 (0.07–0.86) 0.02–0.46 (0.05–0.79) 0.01–0.29 (0.03–0.56) 0.01–0.18 (0.02–0.37) 0.01–0.14 (0.02–0.28) 0.01–0.16 (0.02–0.28)	EFSA, 2017
Western Pacific Region (New Zealand)	Children (5–15 years) Adults (>15 years)	0.01–0.03 (0.03–0.06) <0.01–0.01 (0.01–0.02)	NZFS, 2020
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 exposure 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 (FAO/WHO, 2009).

Table 35

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
Adverse effects, neonate *(Vasoconstriction, CNS, respiratory, renal)*[c]	0.2	0.15	0.04–0.05[b]	EFSA, 2012
Adverse effects, children <7 years old *(Gastrointestinal symptoms)*[e]	0.4 (median dose) 0.2–2 (range	0.3	0.015–0.02[d]	Armenian & Kearney, 2014

Table 35 (continued)

Ergot alkaloid (EA) *(Effect)*	Dose (as mg EA salt/person)	Dose (as mg EA/person)	Dose (as mg EA/kg bw)	Reference
Ergotamine tartrate				
Therapeutic dose, adult[f] *(Vasoconstriction cerebral arteries)*	1–2	0.9–1.8	0.015–0.03[a]	Martindale, 2010
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

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 36
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
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, Beems & Speijers, 1996
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, 2000a,b
Ergot sclerotia	Pig	Not determined	0.1 mg EAs/kg bw per day[c]	Jejunum lesions	4 weeks	Maruo et al., 2018
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

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.
[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.

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

Table 37
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 0, 100, 500 mg/kg diet	 Females Males	Ergotamine 0, 7.3, 41 0, 7.5, 37	 16.6 6.37	 5.25–64.7 1.68–28.4	Abnormality 0/4, 0/6, 3/6 0/3, 2/6, 4/5	Speijers et al., 1992
α-Ergocryptine concentration 0, 4, 20, 100, 500 mg/kg diet	 Females Males	α-Ergocryptine 0, 0.37, 1.7, 8.9, 60 0, 0.34, 1.4, 6.6, 44	 18.1 3.88	 5.36–37.4 1.29–9.59	Muscle degeneration 0/6, 0/6, 0/6, 0/5, 4/6 0/6, 0/6, 0/6, 2/6, 6/6	Janssen et al., 2000a
13-week study						
Ergotamine tartrate concentration 0, 5, 20, 80 mg/kg diet	 Females Males	Ergotamine 0, 0.48,1.99, 7.63 0, 0.42, 1.65, 6.18	 1.55 1.69	 0.61–3.20 0.63–3.53	Muscular atrophy 0/10, 0/10, 2/10, 7/10 1/10, 1/10, 1/10, 7/10	Speijers et al., 1993

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

available information on experimental animals from the two 4-week studies on ergotamine tartrate (Speijers et al., 1992) and on α-ergocryptine (Janssen et al., 2000a), and from the 13-week study on ergotamine tartrate (Speijers et al., 1993) (Table 37).

Dose–response modelling on data from experimental animals was performed by means of the BMD analysis using the US EPA software BMDS 3.2[3] (Table 37). Models available in BMDS 3.2 for dichotomous (quantal) response were used as the default set of models and Bayesian MA was used for the calculation of BMD confidence intervals (BMD-CI) following recent JECFA guidance (EHC 240 Chapter 5 in FAO/WHO 2020). 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.

11. 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.

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

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 (Speijers et al., 1993) 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 HBGV, and that this may indicate a human health concern.

11.1 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.

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Previous cargoes – solvents and reactants

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A. ASSESSMENT OF SUBSTANCES PROPOSED AS PREVIOUS CARGOES

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 the Codex Committee on Fats and Oils (CCFO) in 1987 (CAC, 1987). 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 (CAC, 2001); 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 (SCF) 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. The present evaluation by JECFA addresses the following solvents and reactants: acetic anhydride, *sec*-butyl acetate, tertbutyl acetate, *n*-pentane and cyclohexane, which are substances on the current list of chemicals acceptable as previous cargoes by CCFO.

2. Background

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 (USDA, 2019). 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.

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.

2.3 Global transport of fats and oils

Transportation by sea is regulated by the International Maritime Organization (IMO). 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 1) 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.

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 the European Food Safety Authority (EFSA).

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 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 SCF. Having

Table 1
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] Group 1 was considered at this meeting. For the evaluations of the other substances see the report of the ninetieth meeting (Annex 1, reference *246*).
[b] Discussed with Group 2 – alcohols.

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 the ninetieth and ninety-first meetings, a list of 23 substances was proposed to FAO/WHO (Table 1) 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. 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 (FAO/WHO, 2006, Appendix II) and drafted the meeting report with FAO/WHO (2007).

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 acceptable daily intake (ADI) or tolerable daily intake (TDI) should be greater than or equal to 0.1 mg/kg bw in order to provide sufficient protection for children and high-intake 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 FAO/WHO, 2006).

The criteria as adopted by CAC 34 (2011) are listed in Table 2.

Table 2
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.

4. Basis of evaluation

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.

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.

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) (FAO/WHO 2006).

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.

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 ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to **0.1 mg/kg bw per day**.

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 (WHO, 2017). 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 (WHO, 2012). 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 (Boobis et al., 2017; Arcella et al., 2019).

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

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 during 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) (US EPA, 2020), 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

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 3). Dietary exposures from these different sources should be considered in exposure assessment.

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

Table 3
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
1,4-Butanediol (1,4-butylene glycol)	Used in food contact material
Calcium ammonium nitrate solution	Calcium, nitrate and ammonium are ubiquitous in the human diet
Calcium lignosulfonate liquid (lignin liquor; sulfite lye), molecular weight not specified	Calcium lignosulfonate (40-65) is used as a food additive, an additive in animal feed and as an ingredient in pesticides
Calcium nitrate (CN-9) solution	Calcium and nitrate are ubiquitous in the human diet
iso Decyl alcohol (isodecanol)	None
Myristyl alcohol (1-tetradecanol; tetradecanol)	Flavouring agent, formulation agent, lubricant, release agent
iso Nonyl alcohol (isononanol)	None
iso Octyl alcohol (isooctanol)	Used in food contact material
Tridecyl alcohol (1-tridecanol)	Used in food contact material
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[a]	Occurs naturally in foods
Methyl tertiary butyl ether (MTBE)	Drinking-water
Mineral oil, medium and low viscosity, class II and III	Used in food contact material, direct food additive
Montan wax	Food additive
1,3-Propylene glycol	Used in place of 1,2-propanediol as a food additive
Propylene tetramer (tetrapropylene, dodecene)	None
Soybean oil epoxidized	Used in food contact material
Ethyl tertiary butyl ether (ETBE)	Drinking-water

[a] Discussed with Group 2 – Alcohols.

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.

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 (IARC), 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 ninetieth 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 margin of exposure (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.

5. Recommendations

The Committee recommended that the 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 (for example, MOE approach).
- Where there are additional sources of dietary exposure to the previous cargo chemical substances, they should be considered in the exposure assessment.

B. EVALUATION OF SOLVENTS AND REACTANTS

This section considers the suitability of certain solvents and reactants as an immediate previous cargo for a subsequent cargo of an edible fat or oil. These substances are included on the FOSFA and NIOP acceptable previous (prior) cargo lists. Separate subsections consider acetic anhydride, *sec*-butyl acetate, *tert*-butyl acetate, *n*-pentane and cyclohexane.

I. Acetic anhydride

1. Explanation

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

Acetic anhydride has not been previously considered by JECFA. In 1997 the Scientific Committee on Food (SCF) and EFSA (2012) 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.

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 noted established metabolic pathways for acetic

[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 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).

acid 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.

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 (for example, 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.

2. Chemical and technical considerations

The physicochemical characteristics of acetic anhydride are listed in Table 4.

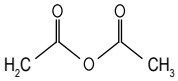
2.1 Manufacture and uses of acetic anhydride

The following manufacturing processes for industrial synthesis of acetic anhydride are described in the literature.

2.1.1 Acetic acid process (ketene process)

The ketene process for the production of acetic anhydride was the main manufacturing process up to the 1970s. It proceeds in two steps: the thermal cleavage of acetic acid to form ketene (at 730–750 °C, 15–20 kPa) and the reaction of ketene with acetic acid.

Table 4
Physical and chemical characteristics of acetic anhydride

Chemical name	Acetic anhydride
Synonyms	Ethanoic anhydride; acetyl acetate; acetic acid, 1,1′-anhydride; ethanoyl ethanoate; acetic acid anhydride; acetyl acetate; acetyl oxide; acetic oxide
CAS number	108-24-7
Chemical structure	
Molecular formula; molar mass	$C_4H_6O_3$; 102.09 g/mol
Description	Colourless liquid with a strong, pungent, vinegar-like odour
Melting point	−73.1 °C
Boiling point	139.5 °C
Solubility	Soluble in water. In aqueous solution, acetic anhydride hydrolyses and acetic acid is formed

$$CH_3COOH \rightarrow CH_2{=}C{=}O + H_2O$$
$$CH_2{=}C{=}O + CH_3COOH \rightarrow (CH_3CO)_2O$$

Triethyl phosphate is commonly used as a dehydration catalyst for the water formed in the first step. Aqueous 30% ammonia is employed as solvent in the second step (Held et al., 2000; Wagner, 2002).

2.1.2 Acetaldehyde oxidation process

Liquid-phase catalytic oxidation of acetaldehyde can be directed by appropriate catalysts, such as transition metal salts of cobalt or manganese, to produce anhydride. Either ethyl acetate or acetic acid may be used as reaction solvent. Acetaldehyde oxidation generates peroxyacetic acid, which then reacts with more acetaldehyde to yield acetaldehyde monoperoxyacetate (CAS No. 7416-48-0). Under sufficient pressure to permit a liquid phase at 55–56 °C, the acetaldehyde monoperoxyacetate decomposes nearly quantitatively into anhydride and water in the presence of copper.

$$CH_3CHO + O_2 \rightarrow CH_3COOOH$$
$$CH_3COOOH + CH_3CHO \rightarrow CH_3COOOCH(OH)CH_3$$
$$CH_3COOOCH(OH)CH_3 \rightarrow (CH_3CO)_2O + H_2O$$

The catalyst is a mixture of three parts copper acetate to one part cobalt acetate by weight (Wagner, 2002).

2.1.3 Carbonylation of methyl acetate process

Acetic anhydride is produced by carbonylation of methyl acetate:

$$CH_3CO_2CH_3 + CO \rightarrow (CH_3CO)_2O$$

This process involves the conversion of methyl acetate to methyl iodide and an acetate salt. Carbonylation of the methyl iodide in turn yields acetyl iodide, which reacts with acetate salts or acetic acid to give the product. Rhodium and lithium iodides are employed as catalysts. Because acetic anhydride is not stable in water, the conversion is conducted under anhydrous conditions (Zoeller et al., 1992). The catalyst system for the methyl acetate carbonylation process involves rhodium chloride trihydrate (CAS No. 13569-65-8), methyl iodide (CAS No. 74-88-4), chromium metal powder and an aluminium oxide support or a nickel carbonyl complex with triphenylphosphine, methyl iodide and chromium hexacarbonyl (Wagner, 2002).

The chief industrial application of acetic anhydride is for acetylation reactions in the production of cellulose acetates (The Merck Index, 2000; Wagner, 2002). Acetic anhydride is also used in the manufacture of fibres, plastics, pharmaceuticals, dyes and explosives (PubChem online database). In food applications it is used in the starch industry as an acetylation compound in production of modified starches: INS No. 1414, INS No. 1420 and INS No. 1422 (Annex 1, references *230* and *231*).

2.2 Impurities and secondary contaminants

Technical-grade acetic anhydride (maximum 97%), often contains colour bodies, heavy metals, phosphorus and sulfur compounds. Anhydride manufactured by acetic acid pyrolysis sometimes contains ketene polymers, for example, acetylacetone, diketene and dehydroacetic acid, and particulate carbon or soot is occasionally encountered. Polymers of allene, or its equilibrium mixture, methylacetylene-allene, are reactive impurities which, if exposed to air, slowly autoxidize to dangerous peroxidic compounds (Wagner, 2002).

The Committee noted that fats and oils for human consumption have to conform to the maximum levels for heavy metals in the Codex *General Standard for Contaminants and Toxins in Food and Feed*, CXS-193-1995, and it is therefore not necessary to estimate exposures to heavy metals in food oils due to carryover from previous cargoes of acetic anhydride. Additional impurities in acetic anhydride, depending on the method of manufacture, include ketene polymers (for example, acetylacetone, diketene, 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; therefore, exposure to ketene polymers, polymers of allene (or its equilibrium mixture), and particulate carbon or soot cannot be estimated.

2.3 Reactivity and reactions with fats and oils

On contact with water (for example, during tank washing) acetic anhydride hydrolyses to acetic acid (SCF, 1997). 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 (Wagner, 2002).

2.4 Methods of analysis

No test methods for analysing acetic anhydride in fats and oils were found in the literature. Residues of acetic acid can be detected in cleaning water and wipe samples via liquid chromatography with tandem mass spectrometry (LC-MS/MS) or ion chromatography (IC). The claimed best detection limit of the IC method with conductivity detection for acetic acid was reported to be around 0.012–0.6 ppm (Krata et al., 2009; Hodgkins et al., 2011). The method for analysis of trace levels of acetic acid in wastewater samples by ultra-high-performance liquid chromatography (UHPLC) with a high sensitivity photodiode array detector (PDA) (Nexera X2 system, SPD-M30A, HS capillary flow cell) with a limit of detection (LOD) of 0.3 ppm and limit of quantification (LOQ) of 1.0 ppm is described by Shimadzu (2014).

3. Biological data

3.1 Biochemical aspects

Acetic anhydride readily hydrolyses to acetic acid in the presence of water. The washing of cargo containers between transport of different cargoes, and moisture within the edible oil are 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. Consequently, for the purposes of this assessment, systemic exposure is expected to be predominantly to acetic acid. Acetic acid is a product of normal metabolism in humans, and it is expected to be readily absorbed, metabolized and rapidly excreted. In a study on human

volunteers, Smith, Jeukendrup & Ball (2007) reported that ingestion of a solution containing carbon-13-labelled sodium acetate (120 mg/kg bw acetate) resulted in a transient increase in plasma and urinary acetate concentrations. Plasma acetate concentrations peaked at 60 minutes and returned to baseline levels between 90 and 120 minutes following ingestion. Based on analysis of the breath and urine following ingestion, approximately 80% of the administered acetic acid is expected to be exhaled and less than 1% is expected to be excreted in the urine. Similarly, rats exposed to radiolabelled acetic acid in the diet were reported to excrete 50% as CO_2 (EC, 2012). In dogs exposed to sodium acetate via intravenous injection, acetic acid was rapidly eliminated with elimination half-lives of 3.0 ± 0.5, 4.0 ± 0.3, 4.1 ± 0.4 and 5.0 ± 0.5 minutes following doses of 3, 4, 5 and 6 mmol/kg (equivalent to approximately 183, 240, 300 and 360 mg/kg), respectively (Freundt, 1973). Elimination from the cerebrospinal fluid (CSF) is slightly slower (after administration of 6 mmol/kg an elimination rate of 14.6 ± 5.9 minutes was recorded in dogs; Freundt, 1973).

3.2 Toxicity in experimental animals

3.2.1 Acute toxicity

Owing to its chemical reactivity, acetic anhydride is highly corrosive (PubChem[1]) and the reported median lethal dose (LD_{50}) values in rats range from 630 mg/kg bw (ECHA, 2020) up to 1800 mg/kg bw (Smyth, Carpenter & Weil, 1951; OECD, 1997). According to the rat study summarized in ECHA (2020), symptoms reported following acute oral exposure are dyspnoea, apathy, lying down, staggering, shaking, trembling, diarrhoea, hypertension, arched back and general poor condition. Postmortem examinations of animals that died during the study revealed acute dilation in the heart, hardening in the stomach lining and bloody ulceration. Although exposure to acetic acid at high concentrations is also irritating and corrosive, acute oral toxicity of acetic acid in rats and mice is low with estimated LD_{50} values >3000 mg/kg bw (Annex 1, reference *132*).

3.2.2 Short-term and long-term studies of toxicity and carcinogenicity

No short-term or long-term studies investigating the oral toxicity of acetic anhydride were identified.

Acetic acid

JECFA (Annex 1, references *33* and *132*), EFSA (2013), EC (2012) and Environment and Climate Change Canada and Health Canada (2019) briefly summarized the

[1] https://pubchem.ncbi.nlm.nih.gov/compound/Acetic-anhydride

results of repeated-dose oral toxicity studies in various experimental animals. However, none of the raw data for the studies summarized by these agencies are readily accessible and the studies were performed before Good Laboratory Practice (GLP) and other guidelines were established. Brief summaries of the available information are provided below.

Hemmingway & Sparrow (1942) reported on rats exposed to doses of approximately 4200 to 4800 mg/kg bw per day sodium acetate (approximately 3074 to 3514 mg/kg bw acetate) via gavage for 14 days. The effects observed were decreased body weight, blistering paws, reddened noses and death. Leung & Paustenbach (1990) and the EC (2012) mentioned the results of a study by Mori (1952), indicating that rats exposed to 4500 mg/kg bw per day acetic acid in the diet for 30 days experience gastric lesions. However, Mori (1952) noted that, owing to its volatility, quantifying the acetic acid ingested by the rats was impossible. Mori (1952) reported that rats were exposed to glacial acetic acid at a concentration of 50 cm^3/kg of rice. In addition to the 30-day experiment, Mori (1952) investigated the lesions in the stomach of albino rats maintained on diets of rice containing glacial acetic acid. For the chronic portion of the study, five male rats were exposed to acetic acid at a concentration of 10 cm^3/kg of rice for 18 days followed by 20 cm^3/kg for 157 days and finally 50 cm^3/kg for up to an additional 177 days (between 200 and 352 days in total). Four out of five of the chronically exposed male rats showed gastric lesions. In a separate study, three out of three female and one out of two male rats exposed to 50 cm^3/kg for 166 days showed gastric lesions and five out of five female rats exposed to 50 cm^3/kg for 200 days showed gastric lesions.

Kondo et al. (2001) administered 6% acetic acid (~290 mg/kg bw per day) to groups of six male "spontaneously hypertensive" rats via the diet using a prepared acetic acid solution, a commercial rice vinegar product or water (control) for 8 weeks. Changes in blood pressure, heart rate, body weight, food and water consumption, renin, angiotensin II, aldosterone and prostaglandin E2 levels were monitored. According to the authors, acetic acid significantly decreased blood pressure and renin activity and no adverse effects were reported. Environment and Climate Change Canada and Health Canada (2019) used these results to establish a no-observed-adverse-effect level (NOAEL) of 290 mg/kg bw per day.

The summary of the study by Wysokinska (1952; Polish language article), provided by JECFA (Annex 1, reference *33*), indicates that daily exposure of rats by gavage to 3 mL of a 10% solution of acetic acid (equivalent to approximately 1000 mg/kg bw per day) for 90 days results in decreased haemoglobin and erythrocyte counts in rats. Based on the dose and concentration of acetic acid used in this gavage study, it is likely that these effects are a result of haemorrhage from severe irritation at the site of first contact.

The United States Environmental Protection Agency (US EPA, 2010) summarized the results of an unpublished 90-day repeated-dose oral toxicity test (experimental animal species not reported). No toxicological effects regarding mortality, clinical observations, neurotoxicity assessment, haematology, clinical chemistry, organ weights or macroscopic or microscopic observations were noted. Based on a decrease in body weight following exposure to 390 mg/kg bw per day, a NOAEL of 195 mg/kg bw per day was identified. The US EPA (2010) suggested that the reduction in weight gain was probably due to reduced appetite and a corresponding reduction in feed consumption.

Similarly, Sollamann (1921)[1] reported that rats exposed to acetic acid in drinking water at concentrations of 0.5% (equivalent to approximately 410 mg/kg bw per day) for 9 to 15 weeks showed reduced body weight gain, loss of appetite and decreased food consumption (previously reviewed by the Committee in 1966/1967 (Annex 1, reference *12*). Administration of acetic acid in the drinking water at doses of between 8 and 210 mg/kg bw per day did not induce changes in food and water consumption.

Pardoe (1952) used sodium acetate as a vehicle control for evaluating the effects of lead acetate: male rats were exposed to 350 mg/kg bw per day sodium acetate (approximately 256 mg/kg bw per day acetate) via gavage for 135 days. Since the purpose of Pardoe's (1952) study was to investigate the toxicity of lead acetate, no control group was included as a basis for evaluating the effects of acetic acid specifically. Nevertheless, according to the available information, no significant effects were reported. JECFA (Annex 1, reference *132*) used the results from this study to identify a NOAEL[2] of 350 mg/kg bw per day for acetic acid.

EFSA's (2013) assessment of acetic acid briefly mentions the results of an 8-month gavage study in rats (no original citations provided) in which rats exposed to 150 mg/kg bw (three doses per week) demonstrated hyperplasia of the forestomach and oesophagus without evidence of tumour formation.

Lamb & Evvard (1919) reported that dietary exposure of pigs to acetic acid at successive doses of 0, 240, 720, 960 and 1200 mg/kg bw per day for 30-day periods each (total of 150 days exposure) resulted in no significant differences in growth rate, weight gain, early morning urinary ammonia and terminal blood pH when compared to controls (previously reviewed by the Committee in 1966/1967 (Annex 1, reference *12*). Based on a 6-month dietary study in pigs EFSA (2013) identified a NOAEL of 450 mg/kg bw per day (no additional details given).

According to EFSA (2013) and Environment and Climate Change Canada and Health Canada (2019), acetic acid shows no carcinogenic potential.

[1] Note: Similar results were reported by EC (2012) and cited as: Henschler D (1973). Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten (Maximale Arbeitsplatz-Konzentrationen), 20. Lieferung, Essigsäure. Weinheim: VCH Verlagsgesellschaft.

[2] At the time called NOEL.

However, Alexandrov et al. (1989) reported hyperplasia in the forestomach and oesophagus in outbred white male rats exposed to 0.5 mL of 3% acetic acid solution (~ 38 mg/kg bw per day assuming a body weight of 0.4 kg and 3% = 30 mg/mL), via gavage, three times per week, for 8 months.

Following co-administration with *n*-nitrososarcosin ethyl ester, a known oesophageal carcinogen in rats, acetic acid promoted tumour formation in the oesophagus (Alexandrov et al., 1989). Rotstein & Slaga (1988) reported that although acetic acid is a very weak tumour promoter in the multistage mouse skin model, it was very effective at enhancing tumour progression following initiation with dimethylbenz[a]anthracene (Rotstein & Slaga, 1988).

3.2.3 Reproductive and developmental toxicity

There are no data on the reproductive or developmental toxicity of acetic anhydride following oral exposure but there are data on the effects of inhalation exposure. The OECD (1997) summarized the results of a developmental toxicity study in pregnant rats exposed to vapour concentrations of 0, 25, 100 or 400 ppm acetic anhydride for 6 hours/day during gestation days (GD) 6 to 15. Fetotoxicity (details not specified) associated with maternal effects (severe respiratory tract irritation and body weight reductions) were observed at 100 ppm. Based on "substantial irritation of the respiratory tract", a lowest-observed-adverse-effect-level (LOAEL) for maternal effects of 25 ppm was identified. Since no fetal effects were noted at the LOAEL, a NOAEL for developmental effects of 25 ppm was identified. The OECD (1997) summary also mentions that following 13 weeks of exposure to 0, 1, 5 or 20 ppm acetic anhydride vapour for 6 hours/day and 5 days/week, no adverse effects on the reproductive organs of male or female rats were observed.

Reproductive and developmental toxicity of acetic acid

Developmental toxicity following oral exposure to acetic acid has been investigated in mice, rats and rabbits (Food and Drug Research Labs, 1974). Female CD-1 mice, Wistar rats and Dutch-belted rabbits were administered doses of 0, 16, 74.3, 345 and 1600 mg/kg bw per day apple cider vinegar (table strength 5%) by oral gavage from GD 6 to 15 (mice and rats) or days 6 to 18 (rabbits) and euthanized on GD 17 (mice), 20 (rats) or 29 (rabbits). In pregnant mice, the body weights of the dams dosed with 345 and 1600 mg/kg bw per day were decreased compared to the negative control group. Although there were no effects on fetal weights, an increase in the number of litters containing dead fetuses and fetuses with incomplete ossification of the sternebrae were observed following exposure of dams to 1600 mg/kg bw per day. Consequently, a maternal NOAEL of 74.3 mg/kg bw per day and a developmental toxicity NOAEL of 345 mg/kg bw per day was identified. No toxicologically relevant effects or abnormalities were observed

in rats at any dose and a NOAEL of 1600 mg/kg bw per day for reproductive and developmental toxicity was identified. Although no evidence of developmental toxicity was observed in rabbits up to doses of 1600 mg/kg bw per day, maternal toxicity (reduced body weight in animals given 1600 mg/kg bw per day) and evidence of reproductive toxicity (decreased pregnancy rate, smaller numbers of live fetuses, corpora lutea and implant sites, and increased resorptions) was observed at doses as low as 74.3 mg/kg bw per day. However, given the sensitivity of rabbits to the bactericidal effects of acetic acid on the gastrointestinal flora (EFSA, 2013; Environment and Climate Change Canada and Health Canada, 2019), the Committee considered the data on rabbits as not relevant to human health risk assessment.

3.2.4 Genotoxicity

Acetic anhydride has produced negative results in in vitro and in vivo genotoxicity testing. The equivocal results reported by Seifried et al. (2006), were probably due to the acidic nature of acetic anhydride, as it has been demonstrated that acetic acid reduces the pH of the culture medium and produces false-positive results in vitro (Morita et al., 1990). Table 5 summarizes the available information on the genotoxicity of acetic anhydride.

Acetic acid genotoxicity

Acetic acid is considered non-genotoxic (Annex 1, reference *132*); however, due to its acidic nature, chromosomal aberrations have been observed in vitro when the pH/osmolality of the culture medium is not controlled. Acidic substances that decrease the pH of the culture medium have been shown to induce false-positive results in in vitro systems (Morita, Takeda & Okumura, 1990). Table 6 summarizes the available information on the genotoxicity of acetic acid.

3.2.5 Allergenicity

The Committee did not identify any studies in animals that indicated that acetic anhydride elicits an allergic response upon oral exposure. There are also no data available that indicate that it contains a known food allergen. However, there is information on humans that indicates that acetic anhydride and acetic acid are capable of inducing allergic reactions (see section 3.3).

3.2.6 Impurities

Some of the impurities identified in section 2.2 are reactive and potentially genotoxic (for example, diketene).[1]

[1] As determined using the online Toxtree software: https://apps.ideaconsult.net/data/ui/toxtree

Table 5
Summary of some in vitro and in vivo studies on the genotoxicity of acetic anhydride

Test system and testing conditions	Treatment	Result	Reference
Gene mutation in *Salmonella* Typhimurium (TA98, TA1537, TA1538, TA100, TA1535)	Not reported Cytotoxic: 0.1 µL/plate (S9+); 1.0 µL/plate (S9–)	Negative (S9– and S9+)	OECD (1997)
Gene mutation in *Salmonella* Typhimurium (TA98, TA1537, TA1538, TA100, TA1535)	8 to 5000 µg/plate Cytotoxic: 3333 µg/plate (S9+); 667 µg/plate (S9–)	Negative (S9– and S9+)	OECD (1997) Seifried et al. (2006)
Gene mutation in *Salmonella* Typhimurium (TA98, TA1537, TA100, TA1535)	3.3 to 1000 µg/plate Cytotoxic: 900 µg/plate (S9+); 190 µg/plate (S9–)	Negative (S9– and S9+)	Mortelmans et al. (1986) as cited in OECD (1997)
Gene mutation in *Salmonella* Typhimurium (G46, C3076, D3052, TA98, TA1537, TA1538, TA100) and *Escherischia coli* (WP2 and WP2 uvrA)	Not reported	Negative (S9– and S9+)	McMahon, Cline & Thompson (1979)
Gene mutation in mouse lymphoma cells (*L5178Y TK*$^{+/-}$)	0.04 to 0.3 µL/mL[a] Cytotoxic: 0.05 µL/mL (S9+); 0.1 µL/mL (S9–)	Negative (S9+) Equivocal (S9–)	OECD (1997) Seifried et al. (2006)
In vivo rat micronucleus test following 13 weeks of inhalation exposure	0, 1, 5 or 20 ppm for 6 hrs/day, 5 days/week for 13 weeks	Negative	OECD (1997)

[a] Seifried et al. (2008) corrected the units reported in Seifried et al. (2006) for the results of the mouse lymphoma assay.

Table 6
Summary of some in vitro genetic toxicity studies on acetic acid

Test system and testing conditions	Treatment	Result	Reference
Gene mutation in *Salmonella* Typhimurium (TA98, TA97, TA100, TA1535)	100–6666 µg/plate	Negative (S9– and S9+)	Zeiger (1992)
Sister chromatid exchange in human lymphocytes	2.5–10 mM (~150–601 mg/L)	Positive (S9–)[a]	Morita, Takeda & Okumura (1990)
Chromosome aberration in Chinese hamster ovary K1 cells	4–14 mM (~240–841 mg/L) 10–30 mM (~601–1802 mg/L)	Positive (S9– and S9+) Positive (S9– and S9+)[b]	Mohammadzadeh-Aghdash et al. (2018)
DNA fragmentation in human umbilical vein endothelial cells	487.71 µM sodium acetate (IC_{50})	No fragmentation	Abd-Elhakim et al. (2018)
Comet assay with male rat peripheral lymphocytes	50, 100 and 200 mmol/L	Positive	OECD (1997) Seifried et al. (2006)

[a] Only a slight increase was observed above 5 mM, which was correlated with a decrease in pH of the culture medium.
[b] Positive results were only observed at the highest concentrations evaluated in the absence and presence of S9 after buffering the culture medium and the authors concluded that the clastogenic effect of acetic acid in cultured cells is attributable to acidification of the culture medium.

3.3 Observations in humans

Acute exposure of humans to acetic anhydride causes severe eye, skin and respiratory tract irritation, and death at high concentrations (Sinclair, 1994; OECD, 1997). Additionally, Yokota, Takeshita & Morimoto (1999) reported that

amines and acetic anhydrides are capable of inducing both IgE-mediated asthma and IgG-mediated late respiratory responses in sensitized chemical workers. EFSA (2012) suggested that the hypersensitivity reactions reported in sensitized workers may be due to acetic anhydride's ability to react with amino acids and potentially form haptens. However, EFSA (2012) concluded that there is currently no indication that acetic anhydride is an allergen or an adjuvant when present in liquid, such as when used as a previous cargo.

Acetic acid

Acetic acid is highly irritating to the skin and mucous membranes. Leung & Paustenbach (1990) reported blackening and hyperkeratosis of the skin of the hands, conjunctivitis, pharyngitis and erosion of the exposed teeth in workers exposed to acetic acid vapour at concentrations of 80 to 200 ppm over 7 to 12 years. Furthermore, the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (German MAK Commission, 2011) and EC (2012) reported that accidental ingestion of 20 and 50 g (equivalent to approximately 286 to 714 mg/kg bw) of concentrated acetic acid is lethal in humans, with survivors showing signs of oesophageal constriction. EFSA (2013) suggested that adverse effects in humans following exposure to acetic acid are related to the corrosive action of acetic acid at high concentrations (for example, ulceration of skin and mucous membranes, gastrointestinal and/or respiratory tract damage) and that the sensitization potential appears to be low based on human experience. The EC (2012) suggest that, based on occupational exposures, individuals may become accustomed to the irritating effects of acetic acid over time.

Boehncke & Gall (1996) reported a case of a type I hypersensitivity-like reaction in a 68-year-old individual who, shortly following consumption of ethanol (for example, from medication, beer, wine or other alcoholic beverages) or salad dressings containing acetic acid, consumed on separate occasions, experienced conjunctivitis, angioedema, dyspnoea and urticarial rashes. The authors observed that the patient's serum IgE was significantly elevated (327 U/mL versus <150 U/mL) and that following a standardized skin prick test, positive reactions were noted for acetic acid concentrations ranging from 9.6 to 1.2% but not for ethanol. An extensive list of other possible allergens all produced negative results in skin prick testing.[1] To rule out the possibility of irritation, skin prick testing with acetic acid at the same concentrations was conducted in five healthy volunteers. None of the five volunteers showed positive reactions to acetic acid.

[1] i.e. Acetaldehyde, several seasonal and perennial allergens, meat, fish, crustaceans, fruits, vegetables, nuts, cereals (malt, barley, rice, rye, wheat, as well as hop and brewer's yeast), food preservatives (sodium salicylate 5%, hydroxybenzoic acid 1%; both diluted in distilled water and in pharmaceutical quality), tartrazine, and several brands of beer and red and white wine.

Przybilla & Ring (1983) reported the case of a healthy 22-year-old woman who experienced generalized itching, facial flushing, dizziness, abdominal pain, numbness in the mouth, and dyspnoea and collapse on several occasions after drinking wine, beer, rum or vinegar. Skin prick tests were negative for ethanol, wine and beer; however, positive results were obtained for vinegar and acetic acid (9.6 and 0.96%). In contrast, skin prick tests for 10 healthy volunteers were negative for acetic acid. Following oral challenge with 1 mL of ethanol or 50 mL of beer the patient experienced urticaria, facial flushing, itching of the mucous membranes, hoarseness, dyspnoea, tachycardia and painful uterine cramps. Serum IgE levels in this patient were significantly elevated (i.e. 690 kU/L). Boehncke & Gall (1996) proposed that, owing to its size, acetic acid itself is unlikely to function directly as an antigen, but more likely as a hapten.

Kondo et al. (2009) conducted a parallel-group, randomized, double-blind, placebo-controlled trial in a cohort of 175 obese Japanese people aged 25 to 60 years to investigate the effects of acetic acid on body fat mass. Following exposure to 0, 15 or 30 mL of apple vinegar (equivalent to 0, 750 or 1500 mg acetic acid) for 12 weeks, Kondo et al. (2009) reported decreased body weight, body mass index, visceral fat area, waist circumference and serum triglyceride levels in both treatment groups compared to controls. Additionally, the high-dose group showed significantly lower systolic blood pressure at the 12-week measurement. EFSA (2011) reported decreased systolic blood pressure in another Japanese study with 750 mg/kg bw per day acetic acid following 2 weeks of consumption (original article in Japanese). According to the US National Library of Medicine's clinical trials database, a 12-week trial[1] in pre-hypertensive Americans aged 30–65 years has been planned to investigate the blood pressure lowering effects of a fruit-flavoured beverage containing diluted Mizkan rice vinegar with and without 750 mg acetic acid. Although there appears to be some evidence for the blood pressure lowering effects of acetic acid in animals (Kondo et al., 2001) and humans, EFSA (2011) concluded that a sustained effect on blood pressure is unlikely since acetic acid is rapidly absorbed and excreted following consumption.

4. Occurrence and exposure

Acetic anhydride is approved in some countries as an acetylation agent for use in the preparation of modified food starches and acetylated mono-glycerides. Acetic anhydride is rapidly hydrolysed to acetic acid, a constituent

[1] https://clinicaltrials.gov/ct2/show/study/NCT03596099

present naturally in vinegar (at up to 15%) and other foods (EFSA, 2018). Environment and Climate Change Canada and Health Canada (2017) 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 (2017) determined that exposure to acetic anhydride from packaging uses is not expected since there are 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 A4.3). 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 (LSRO) data (LSRO, 1977).

5. Comments

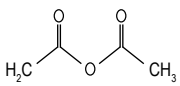
5.1 Chemical and technical considerations

The chemical and technical considerations for acetic anhydride are summarized in Table 7.

5.2 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 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 (Freundt, 1973; Smith, Jeukendrup & Ball, 2007).

Table 5
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.

5.3 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}) in rats ranges from 630 mg/kg bw (ECHA, 2020) up to 1800 mg/kg bw (OECD, 1997). 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. The Committee had 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*; Pardoe, 1952). 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 (Food and Drug Research Labs, 1974).

The Committee concluded that the available in vitro and in vivo information on acetic anhydride and acetic acid does not raise concerns for genotoxicity. Although acetic acid has been associated with tumour promotion (Rotstein & Slaga, 1988; Alexandrov et al., 1989), 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.

5.4 Allergenicity

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.

5.5 Impurities

The Committee noted that fats and oils for human consumption have to conform to the maximum levels for heavy metals set out in the Codex *General Standard for Contaminants and Toxins in Food and Feed*, CXS-193-1995, 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 (for example, 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.

5.6 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 (EFSA, 2018). Environment and Climate Change Canada and Health Canada (2017) 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 (2017) 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 Occurrence and exposure). 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 (LSRO) (1977) data.

6. 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 (LSRO, 1977), 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 (Wagner, 2002). 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 (for example, diketene and dehydroacetic acid), 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.

II. *sec*-Butyl acetate

1. Explanation

The Committee had 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) 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 (2012a) 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. 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) completed 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 (for example, 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 I.

2. Chemical and technical considerations

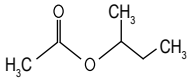
The physicochemical characteristics of *sec*-butyl acetate are listed in Table 8.

2.1 Manufacture and uses of *sec*-butyl acetate

The most common process for manufacturing *sec*-butyl acetate is esterification of acetic acid with *sec*-butanol using sulfuric acid as a catalyst. The removal of water by azeotropic separation forces the reaction to completion. Common azeotropic agents are cyclohexane, benzene and toluene. The acid catalyst is neutralized and the ester purified by distillation (Lewis, 2007; Cheung at al., 2012). Esterification of *sec*-butanol with acetic anhydride offers an alternative route for producing *sec*-butyl acetate. Owing to its high reactivity, acetic anhydride readily forms esters with alcohols. The reaction is irreversible and the esterification goes to completion without eliminating products or water (O'Neil, 2001; Cheung at al., 2012). *sec*-Butyl acetate can also be prepared from 2-butene under pressure and heated with an excess of glacial acetic acid containing 10% sulfuric acid. Instead of sulfuric acid, boron trifluoride-diethyl ether may be used as a catalyst (Cheung at al., 2012).

sec-Butyl acetate is used as a solvent in nitrocellulose lacquers, thinners, enamels, leather finishes, acyclic polymers and vinyl resins (Cheung at al., 2012; PubChem online). In the EU, *sec*-butyl acetate is approved for use

Table 8
Physical and chemical characteristics of *sec*-butyl acetate

Chemical name	Butyl acetate, *sec-*; *sec*-butyl acetate
Synonyms	Butan-2-yl acetate; 2-butyl acetate; *sec*-butyl ethanoate
CAS number	105-46-4
Chemical structure	
Molecular formula; molar mass	$C_6H_{12}O_2$; 116.16 g/mol
Description	Colourless liquid with a fruity scent. It produces irritating vapour. Liquid and vapour are highly flammable
Melting point	–99 °C
Boiling point	112 °C
Flash point	17 °C (closed cup)
Solubility	Slightly soluble in water, soluble in ethanol and diethyl ether

as a flavouring substance for food (FL No. 09.323) (Regulation (EC) 1334/2008; Commission Implementing Regulation (EU) No 872/2012).

2.2 Impurities and secondary contaminants

sec-Butyl acetate may contain acetic acid up to 0.2% as an impurity (Kodak, 1985).

2.3 Reactivity and reactions with fats and oils

Hydrolysis of *sec*-butyl acetate results in formation of acetic acid and *sec*-butanol, which in the presence of acid may undergo transesterification with lipids producing a mixture of glycerol and fatty acid *sec*-butyl esters. However, these reactions are slow and require excess alcohol and temperatures above 100 °C (Schuchardt et al., 1998).

2.4 Methods of analysis

No test methods for analysing *sec*-butyl acetate in fats and oils were found in the literature. *sec*-Butyl acetate can be analysed by gas chromatography with flame ionization detection (GC-FID) or GC-MS (PubChem, SciFinder – CAS online databases).

3. Biological data

3.1 Biochemical aspects

There are no studies on the biochemical aspects of *sec*-butyl acetate following oral exposure and no physiologically-based pharmacokinetic models were identified. However, based on its physicochemical properties (molecular weight = 116.16 g/mol; log n-octanol/water partition coefficient (K_{ow}) = 1.72; PubChem), *sec*-butyl acetate is expected to be readily absorbed into the systemic circulation following oral exposure. Once absorbed, *sec*-butyl acetate is expected to be rapidly hydrolysed (within minutes) to acetic acid and *sec*-butanol in the blood, liver, small intestine and respiratory tract (WHO/IPCS, 2005). *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 (Traiger & Bruckner, 1976; Dietz et al., 1981; WHO/IPCS, 1987). Peng et al. (1995) also demonstrated that in vitro, *sec*-butyl acetate undergoes hydroxylation to an unstable hemiketal (2-hydroxy-2-acetoxybutane) followed by a non-hydrolytic cleavage to 2-butanone by cytochrome P450 2B4.

Dietz et al. (1981) demonstrated that the concentrations of 2-butanone and its metabolites in rats orally dosed with *sec*-butanol are comparable to concentrations obtained after oral dosing with 2-butanone. Therefore, it is expected that *sec*-butanol and 2-butanone will exhibit similar toxicological profiles.

3.2 Toxicological studies

3.2.1 Acute toxicity

Sec-Butyl acetate exhibits low acute oral toxicity with a reported oral LD_{50} of 3200 mg/kg bw in rats (WHO/IPCS, 2005). According to a US National Institute for Occupational Safety and Health *Pocket guide to chemical hazards*,[1] symptoms possibly induced by *sec*-butyl acetate include eye irritation, headache, drowsiness, upper airway dryness, dry skin and narcosis.

Sec-butanol/2-butanone

Similarly, *sec*-butanol exhibits low acute oral toxicity, with reported oral LD_{50} values of 2193 mg/kg bw in rats and 6480 mg/kg bw in rabbits (ECETOC, 2003). According to the German MAK Commission,[2] acute exposure to *sec*-butanol

[1] https://www.cdc.gov/niosh/npg/npgd0073.html

[2] https://onlinelibrary.wiley.com/doi/full/10.1002/3527600418.mb7892e0019

(all routes) leads to central nervous system depression and typical symptoms of acute alcohol intoxication/narcosis. The severity of intoxication is greater for *sec*-butanol than for ethanol. 2-Butanone also exhibits low acute oral toxicity in mice and rats, with reported LD_{50} values of > 2000 mg/kg bw (US EPA, 2003).

3.2.2 Short-term and long-term studies of toxicity and carcinogenicity

No relevant oral toxicity studies were identified for *sec*-butyl acetate, *sec*-butanol or 2-butanone.

3.2.3 Reproductive and developmental toxicity

No reproductive or developmental toxicity data were identified for *sec*-butyl acetate. However, some toxicological information is available on the reproductive and developmental toxicity of *sec*-butanol and 2-butanone.

Sec-butanol

A two-generation reproductive and developmental oral toxicity study with the primary metabolite *sec*-butanol was conducted in rats (Cox et al., 1975, as cited in US EPA, 2003a; unpublished study). In the US EPA (2003a) evaluation of the original study, various deficiencies were highlighted (for example, lack of measurements of estrous cyclicity, sperm parameters, weights of uterus, epididymides, seminal vesicles and brain; and incomplete clinical chemistry, haematology and histopathology). Nevertheless, this study was chosen as the critical study in the derivation of the US EPA (2003a) reference dose (RfD) for 2-butanone. Since a copy of the original study was not available for the present assessment, details of the US EPA (2003a) evaluation are provided below.

Weanling FDRL-Wistar stock male and female rats (F_0; 30 per sex and group) were exposed to *sec*-butanol at concentrations of 0, 0.3, 1.0 or 3.0% for 8 weeks in drinking water prior to mating, and during gestation of two separate F1 generations. The doses received by the F_0 generation up to day 10 after birth (postnatal day (PND) 10) of the F_{1A} pups were reported as 538, 1644 or 5089 mg/kg bw per day in males and 594, 1771 and 4571 mg/kg bw per day in females. Maternal toxicity (reduced body weight gain) accompanied by developmental effects in the F_{1A} pups (increased fetal mortality and reduced fetal and pup body weights) were reported following exposure to the highest concentration in drinking water (3% solution; doses of 5089 mg/kg bw per day in males and 4571 mg/kg bw per day in females). Therefore, F_0 generation parents in the high-dose group and F_{1A} offspring were given drinking water instead of *sec*-butanol between PND 10 and PND 21 of lactation. Dosing was resumed after PND 21 at a lower concentration (2.0% instead of 3.0%) for 2 weeks post-lactation. The estimated average daily intake was 3384 mg/kg bw per day for

males and 3122 mg/kg bw per day for females. Dosing at the other drinking water concentrations (0.3 and 1.0%) continued as per the experimental protocol. Two weeks post-lactation of F_{1A}, the F_0 generation was re-mated to produce a second set of first-generation offspring (F_{1B}). The pregnancies of 20 dams were terminated on gestational day 20 and the F_{1B} fetuses were evaluated for reproductive and developmental toxicity. Dosing continued for selected F_{1A} pups (8 pups per litter and dose) from all dose groups postweaning, at drinking water concentrations of 0, 0.3, 1.0 or 2.0% *sec*-butanol. At 12 weeks of age F_{1A} animals were mated to produce the second generation (F_2) litters. On PND 21, the F_{1A} parents and F_2 offspring were sacrificed.

A reduction in body weight gain (~15%) was reported in the F_0 generation at the highest dose (3.0% concentration) compared to controls. There was also an increased incidence of the number of F_0 male rats that failed to copulate with F_0 females (0% (1/30), 0.3% (2/30), 1% (0/30), and 3% (6/30) in the highest dose group (3.0%) compared to controls (20% versus 3%). The US EPA analysed the data for F_0 male copulatory success rate, which suggested the highest dose (3%) of *sec*-butanol had a possible impact on male performance. Data were not available to determine the copulatory failure rate for the other generations in this study. No other toxicologically relevant adverse effects on reproductive parameters were noted in the F_0 generation.

Adverse developmental effects observed in the F_{1A} and F_{1B} offspring from the F_0 generation consisted of:

- increased early and late fetal death at the highest dose (3.0%) for the F_{1A} generation when compared to controls;
- a significant reduction in F_{1A} pup body weights at the highest dose (3.0%) on PND 4 (~22%) and PND 21 (~39%) and at the 1.0% dose on PND 21 compared to controls; and
- decreased F_{1B} fetal weight (10%; not statistically significant) at the 2.0% dose with a dose-related trend.

No toxicologically significant increases in the incidence of skeletal malformations were noted in the F_{1B} offspring. Toxicologically relevant histopathological findings in the adult F_{1A} generation after approximately 23 weeks (for example, 3 weeks in utero, 3 weeks indirectly via lactation, 9 weeks maturation, 2 weeks mating, 3 weeks gestation, 3 weeks weaning) of exposure were limited to abnormalities in the kidneys (i.e. tubular cast formation and foci of tubular degeneration and regeneration) of males from all dose groups. Similar tubular effects were not observed in females. Similar to the F_{1A} pups, reduced body weights were reported in the F_2 offspring from the highest dose group (2.0%) on PND 4 (5%) and 21 (13%) compared to controls. No body weight

changes for F_2 pups in the other dose groups (0.3% and 1.0%) were observed. A NOAEL of 594 mg/kg bw per day (0.3% *sec*-butanol) was identified based on decreased pup body weight in the F_{1A} generation at a dose of 1771 mg/kg bw per day (1% *sec*-butanol). Based on the decreased body weights measured in F_{1A} pups on PND 21, the US EPA (2003a) calculated a lower 95% confidence limit on the benchmark dose for a 5% response ($BMDL_{05}$) of 657 mg/kg bw per day using US EPA's Benchmark Dose Software (BMDS, version 1.3.1).

In another developmental toxicity study, reduced fetal weights were also observed in the offspring of rats following inhalation exposure of the dams to maternally toxic doses of *sec*-butanol (Nelson et al., 1989). In this study, pregnant Sprague-Dawley rats (15 per group) were exposed (whole body) to concentrations of 0, 3500, 5000 or 7000 ppm *sec*-butanol via the inhalation route for 7 hours per day from gestational days 1 to 19. Maternal toxicity was observed at all doses based on reduced food intake and decreased body weight gain. Exposure of dams to concentrations of 5000 ppm and 7000 ppm led to significantly reduced fetal body weights. A significant increase in resorptions per litter, decreased number of live fetuses per litter, and an increased number of fetuses with skeletal variations were also observed in the offspring of dams exposed to 7000 ppm. The study authors described the skeletal variations as typical of fetotoxicity, in particular reduced ossification. A no-observed-adverse-effect-concentration (NOAEC) for developmental toxicity of 3500 ppm was identified.

2-Butanone

No relevant reproductive or developmental oral toxicity studies on 2-butanone were identified. However, developmental toxicity in rats and mice has been observed following inhalation exposure to 2-butanone. Inhalation exposure (whole body) of pregnant Sprague-Dawley rats to approximately 3000 ppm 2-butanone, for 7 hours per day during gestational days 6 to 15 resulted in decreased weight gain in dams and increased incidence of skeletal variations in offspring (Deacon et al., 1981). In Swiss CD-1 mice, inhalation exposure (whole body) to approximately 3000 ppm 2-butanone for 7 hours per day during gestational days 6 to 15 led to increased relative liver weights in the dams, a small but statistically significant decrease in male fetal body weights, and an increased incidence of misaligned sternebrae (Schwetz et al., 1991). In neither study were adverse effects on dams and development observed at concentrations of 400 and 1000 ppm 2-butanone. Based on the results of these studies, a NOAEC of 1000 ppm for maternal and developmental toxicity in rats and mice was identified.

Table 9
Summary of selected in vitro genotoxicity studies on *sec*-butanol

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Up to 10 000 µg/plate Plate incorporation	Negative (S9– and S9+[a])	ECETOC (2003)
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537, TA1538) and *Escherichia coli* (WP2uvrA/pKM 101)	Up to 4000 µg/plate Plate incorporation	Negative (S9– and S9+[a])	Brooks, Meyer & Hutson (1988)
Gene mutation in *Saccharomyces cerevisiae* JD1	10–5000 µg/mL	Negative (S9– and S9+[a])	Brooks, Meyer & Hutson (1988)
Chromosome aberration in Chinese hamster ovary cell line	Up to 5000 µg/mL	Negative (S9– and S9+)	Brooks, Meyer & Hutson (1988)

[a] Aroclor 1254-induced rat liver S9 mix.

3.2.4 Genotoxicity

No studies on the genotoxicity of *sec*-butyl acetate were identified. *Sec*-butanol did not raise concerns for genotoxicity based on the available information from in vitro studies (Table 9). There is some evidence that 2-butanone (Table 10) elicits aneuploidy in yeast assays (not part of the standard battery of genotoxicity tests listed in WHO/IPCS, 2020) without metabolic activation. However, the findings were inconsistent with other studies evaluating similar end-points using the standard battery of in vitro and in vivo genotoxicity tests. The Committee concluded that based on the available in vitro and in vivo information, *sec*-butyl acetate does not raise concerns for genotoxicity.

3.2.5 Allergenicity

There were no studies identified on the allergenic potential of *sec*-butyl acetate. *Sec*-butanol and 2-butanone did not induce skin sensitization reactions in the guinea-pig maximization tests (ECETOC 2003; ECHA, 2020a,b).

3.2.6 Impurities

No impurities of concern were identified.

3.3 Observations in humans

No data on the effects of *sec*-butyl acetate on humans were identified.

Table 10
Summary of selected in vitro and in vivo genotoxicity studies on 2-butanone

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA102)	Up to 5000 µg/plate	Negative (S9– and S9+)	Jung et al. (1992)
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	0.05–32 µL/plate	Negative (S9– and S9+)	O'Donoghue et al. (1988)
Gene mutation in *Salmonella* Typhimurium (TA97, TA98, TA100, TA1535, TA1537)	100–10 000 µg/plate	Negative (S9– and S9+)	Zeiger et al. (1992)
Gene mutation in *Escherichia coli* (WP2 and WP2uvrA)	31.25–4000 µg/plate	Negative (S9– and S9+)	ATSDR (2020)
Gene mutation in *Saccharomyces cerevisiae*	10–5000 µg/plate	Negative (S9– and S9+)	ATSDR (2020)
Induction of mitotic aneuploidy in *Saccharomyces cerevisiae*	3.54%	Positive (S9–)	WHO/IPCS (1993) Zimmermann et al. (1985)
Chromosomal aberrations in rat liver cells (RL4)	250–1000 µg/mL	Negative (S9–) No data (S9+)	ATSDR (2020)
Unscheduled DNA synthesis in rat hepatocytes	0.1–5.0 µL/mL	Negative (S9–) No data (S9+)	O'Donoghue et al. (1988)
Morphological transformation in BALB/3T3	9–18 µL/mL	Negative (S9–) No data (S9+)	O'Donoghue et al. (1988)
Gene mutation in mouse lymphoma cells (*L5178Y TK*$^{+/-}$)	0.67–12 µL/mL	Negative (S9– and S9+)	O'Donoghue et al. (1988)
Micronucleus frequency in V79 Chinese hamster fibroblasts	280 mM (32 525 µg/mL)	Negative (S9–) No data (S9+)	Kreja & Seidel (2002)
DNA damage (Comet assay) in V79 Chinese hamster fibroblasts	112 and 560 mM (13 009 and 65 050 µg/mL)	Negative (S9–) No data (S9+)	Kreja & Seidel (2002)
DNA damage (Comet assay) in A549 cells	112 and 560 mM	Negative (S9–) No data (S9+)	Kreja & Seidel (2002)
Studies based on in vivo systems			
Bone marrow micronucleus test in CD-1 mouse	1.96 mL/kg via intraperitoneal injection	Negative	O'Donoghue et al. (1988)
Bone marrow micronucleus test in Chinese hamsters	411 mg/kg via intraperitoneal injection	Negative	Basler (1986)

4. Occurrence and exposure

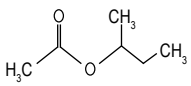
sec-Butyl acetate is naturally present in vinegar at concentrations up to 67 mg/kg (UNEP/ILO/WHO, 2005). No data were identified on exposure to *sec*-butyl acetate from vinegar consumption. However, it may be estimated using EFSA (2018) data on acetic acid exposure from vinegar consumption, which were based on an assumed concentration of 6% acetic acid in the vinegar. The EFSA (2018) estimates of mean acetic acid exposure from vinegar consumption range

across surveys from 0–2.8 mg/person per day for infants to 2.3–123.3 mg/person per day for the elderly. Therefore, the maximum mean intake of vinegar is 0.05 g/day for infants (2.8 mg acetic acid/day × 1 g/1000 mg × 100 g vinegar/6 g acetic acid) and 2 g/day for the elderly population. Assuming a vinegar intake of 0.05 g/day by infants, a *sec*-butyl acetate concentration of 67 mg/kg in vinegar, and a body weight of 5 kg for infants (the body weight indicated for infants aged 0–12 months by EFSA, 2012c), maximum mean exposure to *sec*-butyl acetate from vinegar consumption is 0.01 mg/kg body weight per day. Assuming a vinegar intake of 2 g/day for the elderly, *sec*-butyl acetate concentrations of 67 mg/kg in vinegar, and a body weight of 60 kg, maximum mean exposure to *sec*-butyl acetate from vinegar consumption by the elderly is 0.03 mg/kg body weight per day.

sec-Butyl acetate has a fruity odour (UNEP/ILO/WHO, 2005), and is approved for use as a flavouring agent (09.323) in Europe (EFSA, 2008, 2017). The maximized survey-derived daily intake (MSDI) of *sec*-butyl acetate, calculated based on production volumes (corrected by a factor of 0.6 to account for incomplete survey data), was estimated at 0.0012 µg/capita per day (EFSA, 2017). Based on the MSDI of 0.0012 µg/day and an adult body weight of 60 kg, the exposure to *sec*-butyl acetate from use as a flavouring agent in Europe is equivalent to 2 E-08 mg/kg bw per day. A modified theoretical added maximum daily intake (mTAMDI) for *sec*-butyl acetate present in foods as a flavouring agent, calculated on the basis of standard portion sizes and normal use levels in beverages and foods, was estimated at 3900 µg/person per day (EFSA, 2017). Based on the mTAMDI of 3900 µg/day and on an adult body weight of 60 kg, the exposure to *sec*-butyl acetate from flavouring agent uses in Europe is equivalent to 0.07 mg/kg bw per day. No data are available on exposure of infants and young children to *sec*-butyl acetate from its 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 A4.3). Exposures to *sec*-butyl acetate from vinegar and from its use as a flavouring agent 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.

Table 11
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.

5. Comments

5.1 Chemical and technical considerations

The chemical and technical considerations for *sec*-butyl acetate are summarized in Table 11.

5.2 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 (WHO/IPCS, 2005). *sec*-Butanol is then expected to undergo rapid metabolism by alcohol dehydrogenase, primarily to 2-butanone, and be excreted by either exhalation

or in the urine, or to undergo further metabolism to 3-hydroxy-2-butanone and 2,3-butanediol (Traiger & Bruckner, 1976; Dietz et al., 1981; WHO/IPCS, 1987).

5.3 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 (WHO/IPCS, 2005). For *sec*-butanol, the oral LD_{50} was greater than 2000 mg/kg bw in rats and rabbits (ECETOC, 2003). No short-term or long-term oral toxicity data are available for *sec*-butyl acetate.

Due 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 (Cox et al., 1975, as cited by the US EPA, 2003a; 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 F_1 generations. The doses of the F_0 generation up to day 10 after the birth of the F_{1A} pups were reported based on average daily intakes as 538, 1644 or 5089 mg/kg bw per day in males and 594, 1771 and 4571 mg/kg 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 per day in males and 4571 mg/kg per day in females). Although reduced pup body weights were observed in F_{1A} pups at the 1% dose on PND 4 and 21, reduction in pup body weights at the same dose in the F_2 generation was not observed. Based on these results, the Committee identified a NOAEL of 594 mg/kg per day (0.3% *sec*-butanol) based on decreased pup body weight in the F_{1A} 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 $BMDL_{05}$ of 657 mg/kg bw per day based on decreased pup weights in the F_{1A} pups on PND 21 (Cox et al., 1975, as cited in US EPA, 2003; unpublished study).

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

5.4 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 (ECETOC, 2003; ECHA, 2020a,b).

5.5 Impurities

No impurities of concern were identified.

5.6 Assessment of dietary exposure

sec-Butyl acetate is naturally present in vinegar at concentrations up to 67 mg/kg (WHO/IPCS, 2005). Maximum mean exposure to *sec*-butyl acetate from vinegar consumption, calculated based on data provided by EFSA (2018), 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 (WHO/IPCS, 2005) and is approved for use as a flavouring agent (09.323) in Europe (EFSA, 2008, 2017). 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 A4.3). 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.

6. 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, 2003). *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.

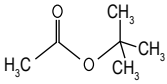
Therefore, *sec*-butyl acetate meets the criteria for acceptability as a previous cargo for edible fats and oils.

III. *Tert*-butyl acetate

1. 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) 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, EFSA (2012a) 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.

Table 12
Physical and chemical characteristics of *tert*-butyl acetate

Chemical name	Butyl acetate, tert-; tert-butyl acetate
Synonyms	Acetic acid tert-butyl ester; t-butyl acetate; tert-butyl ethanoate
CAS number	540-88-5
Chemical structure	
Molecular formula; molar mass	$C_6H_{12}O_2$; 116.16 g/mol
Description	Colourless liquid with a fruity odour. It produces highly flammable irritating vapour.
Melting point	97.8 °C
Boiling point	16.6–22.2 °C (closed cup)
Boiling point	Practically insoluble in water, soluble in ethanol, ethyl ether, chloroform and acetic acid.
Solubility	Soluble in water. In aqueous solution, acetic anhydride hydrolyses and acetic acid is formed

For the present assessment, previous assessments (monographs) completed 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 (for example, *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 I.

2. Chemical and technical considerations

The physicochemical characteristics of *tert*-butyl acetate are listed in Table 12.

2.1 Manufacture and uses of *tert*-butyl acetate

tert-Butyl acetate is produced from isobutylene reacting with acetic acid in the liquid phase with vanadium pentoxide-impregnated silica as catalyst and with heat to increase the yield (SciFinder – CAS, PubChem online databases). Esterification of *tert*-butanol and acetic acid produces *tert*-butyl acetate. The catalyst is sulfuric

acid. The removal of water by azeotropic separation forces the reaction to completion. Common azeotropic agents are cyclohexane, benzene and toluene. The acid catalyst is neutralized and the ester purified by distillation (Lewis, 2007; Cheung at al., 2012).

tert-Butyl acetate is used as a solvent in adhesives, sealants and paints, and as a gasoline additive. There is no information on its use in the food industry.

2.2 Impurities and secondary contaminants

No impurities of concern have been identified in *tert*-butyl acetate.

2.3 Reactivity and reactions with fats and oils

Hydrolysis of *tert*-butyl acetate results in formation of acetic acid and *tert*-butanol, which in the presence of acid may undergo transesterification with lipids producing a mixture of glycerol and fatty acid *tert*-butyl esters. The reactions, however, are slow and require excess alcohol and temperatures above 100 °C (Schuchardt et al., 1998).

2.4 Methods of analysis

No test methods for analysing *tert*-butyl acetate in fats and oils have been found in the literature. *tert*-Butyl acetate can be analysed by GC-FID or GC-MS (PubChem, SciFinder – CAS online databases).

3. Biological data

3.1 Biochemical aspects

No 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 (molecular weight = 116.16 g/mol; log K_{ow} = 1.76; PubChem[1]), *tert*-butyl acetate is expected to be readily absorbed systemically following oral exposure. In contrast to other butyl acetate isomers, *tert*-butyl acetate is not expected to be readily hydrolysed in the blood, liver, small intestines and respiratory tract, and is a poor substrate for alcohol

[1] https://pubchem.ncbi.nlm.nih.gov/compound/*Tert*-butyl-acetate

dehydrogenase (WHO/IPCS, 2005; EFSA, 2012b). For example, the hydrolysis half-life of *tert*-butyl acetate is 270 minutes when exposed to human blood and 300 minutes when exposed to the blood of Sprague-Dawley rats; compared with 12 and 4 minutes respectively for *n*-butyl acetate (Essig, Groth & Freundt, 1989). Essig, Groth & Freundt (1989) suggest that this differential rate of hydrolysis is due to the steric hindrance of the *tert*-butyl moiety. Since limited kinetic data are available for *tert*-butyl acetate following oral exposure, the data reported following inhalation exposure are briefly summarized to provide a qualitative description of *tert*-butyl acetate's likely metabolic and elimination pathways.

In an inhalation study in Sprague-Dawley rats, Cruzan & Kirkpatrick (2006) demonstrated that the predominant route of excretion is via the urine (i.e. 89.2% of the radioactivity was recovered in the urine versus 2.7 and 4.8% in the faeces and air, respectively). Cruzan & Kirkpatrick (2006) also suggested that following exposure to low concentrations of *tert*-butyl acetate (for example, 100 ppm), conversion to 2-hydroxymethylisopropyl acetate is higher than conversion to *tert*-butanol, whereas at high concentrations of *tert*-butyl acetate (for example, 1000 ppm), conversion to *tert*-butanol prevails over conversion to 2-hydroxymethylisopropyl acetate. Additionally, Groth & Freundt (1994), in a study on Sprague-Dawley rats, showed elevated blood concentrations of both *tert*-butyl acetate and *tert*-butanol following inhalation exposure (via tracheal cannula) to 900 ppm *tert*-butyl acetate. *tert*-Butanol concentrations in the blood exceeded concentrations of *tert*-butyl acetate near the end of the 5-hour exposure period. Similar results (i.e. decreasing *tert*-butyl acetate and increasing *tert*-butanol concentrations in blood over time) were reported by Essig, Groth & Freundt (1989).

According to Cruzan & Kirkpatrick (2006), following inhalation exposure of rats to 100 ppm *tert*-butyl acetate, 24.2% of 2-hydroxymethylisopropyl acetate and 13.8% of *tert*-butanol is excreted in the urine after 6 hours, whereas 57.1% is excreted as 2-hydroxyisobutyric acid. Since both 2-hydroxymethylisopropyl acetate and *tert*-butanol are capable of being converted to 2-hydroxyisobutyric acid (the metabolite with the highest percentage of excretion in the urine) the exact relative contribution of hydroxylation of the *tert*-butyl moiety versus hydrolysis cannot be determined. Hydroxylation and glucuronidation of the acetate moiety is considered a minor metabolic pathway because only 1.3 and 0.6% of *tert*-butyl-2-hydroxyacetate glucuronide is measured in the urine 6 hours after inhalation exposure to 100 and 1000 ppm *tert*-butyl acetate, respectively. Bernauer et al. (1998) suggest that metabolism of *tert*-butanol in rats is different to that in humans. For example, following oral administration of 5 mg/kg bw carbon-13-labelled *tert*-butanol, a human volunteer excreted primarily 2-methyl-1,2-propanediol and 2-hydroxyisobutyric acid in the urine. Based on parallel data in rats, Bernauer et al. (1998) suggested that *tert*-butyl alcohol sulfate is a

major urinary metabolite in rats following exposure to *tert*-butanol, whereas 2-hydroxyisobutyric acid was the major metabolite excreted in human urine.

Nevertheless, based on the kinetic data in rats exposed by inhalation to *tert*-butyl acetate, the metabolism of *tert*-butyl acetate probably occurs by both the carboxylesterase and cytochrome P450 pathways resulting primarily in the production of *tert*-butanol. Based on the data summarized by Cruzan & Kirkpatrick (2006), the Office of Environmental Health Hazard Assessment (OEHHA) (2018) suggested that 71% of *tert*-butyl acetate is metabolized to *tert*-butanol (i.e. 13.8% urinary *tert*-butanol + 57.1% urinary 2-hydroxyisobutyric acid). The OEHHA (2018) and EFSA (2012a) also suggest that *tert*-butyl acetate is widely distributed following absorption and is eliminated primarily via the urine as metabolites.

3.2 Toxicological studies

3.2.1 Acute toxicity

Tert-butyl acetate exhibits low acute oral toxicity in rats, with estimated LD_{50} values ≥3420 mg/kg bw (WHO/IPCS, 2005; ECHA, 2020c). Clinical signs of toxicity (for example, lethargy, ataxia, flaccid muscle tone, dyspnoea, loss of righting reflex, prostration, and piloerection, tremors and coma) associated with acute oral exposure to a high dose (for example >2000 mg/kg bw) are probably related to irritation and central nervous system depression.

Tert-butanol exhibits low acute oral toxicity with estimated LD_{50} values ≥3500 mg/kg bw in rats and rabbits (National Toxicology Program; NTP, 1997). The critical acute effects observed following oral exposure were signs of "alcoholic intoxication" and hepatotoxicity with the sedative and intoxicating effects of *tert*-butanol being reported to be similar but more potent than those of ethanol (NTP, 1997).

3.2.2 Short-term and long-term studies of toxicity and carcinogenicity

No information on short-term or long-term oral toxicity of *tert*-butyl acetate is available. The inhalation toxicity studies with *tert*-butyl acetate are considered to be of limited use quantitatively. However, in the absence of more robust information on the short-term and long-term effects following oral exposure to *tert*-butyl acetate, effects observed following repeated-dose inhalation exposure are considered further below.

Faber et al. (2014) describe the results of two 13-week inhalation toxicity studies in male and female rats (Crl:CD(SD)) and male and female mice (Crl:CD1(IGR)) exposed to 0, 100, 400 or 1600 ppm *tert*-butyl acetate via whole-body exposure for 6 hours per day and 7 days per week. In rats, exposure to

1600 ppm *tert*-butyl acetate for 13 weeks induced increased locomotor activity (but no change in functional observation battery in week 3), decreased body weight, decreased food consumption, increased liver, adrenal gland and kidney weights, and histopathological lesions in the kidney. Except for the kidney effects observed in all treated male rats, no other toxicologically relevant effects were noted in rats exposed to doses of 100 and 400 ppm. Hyaline droplet accumulation and basophilic tubules appeared to be related to accumulation of α-2u-globulin (as measured using immunohistopathology and enzyme-linked immunosorbent assay (ELISA) methods) and are considered by the Committee as a male rat specific effect that is not relevant to human health risk assessment. In the 13-week inhalation toxicity study in mice, no effects on the kidney were noted. Treatment-related effects were limited to hyperactivity (but no change in functional observation battery on days 62 and 63) in the groups that received the 400 and 1600 ppm doses. Furthermore, liver effects (increased absolute and relative liver weights in males and females; centrilobular hepatocellular hypertrophy in 1/10 females) were noted in the group exposed to 1600 ppm. Male mice also showed decreased T4, without any change in TSH or T3 or histopathological lesions in the thyroid, an effect that may have been a result of increased liver enzyme activity.

Tert-butanol (metabolite)

A 13-week oral (drinking water) toxicity study of *tert*-butanol was conducted with male and female F344 rats (10 per sex and dose) at doses of 0, 2.5, 5, 10, 20 or 40 mg/mL administered *ad libitum* in deionized water for 94 to 95 days. Doses equal to 0, 235, 496, 804, 1599 or 3589 mg/kg bw per day in males and 261, 503, 7658, 1452 or 3500 mg/kg bw per day in females were achieved. Deaths were recorded of all males dosed with 3589 mg/kg bw per day and 6/10 females dosed with 3500 mg/kg bw per day. Statistically significant decreases in body weight were observed at all doses except in males in the lowest dose group and in females in the highest dose group. The dose-related clinical signs of toxicity observed in males and females were ataxia, hypoactivity and blood in the urine, with ataxia and hypoactivity being the earliest signs of toxicity. Increases in absolute and relative kidney and liver weights were noted in both males and females in the highest dose group and in males in the second highest dose group. Similarly, gross necropsy revealed treatment-related renal lesions (urinary tract calculi, dilation of the ureter and renal pelvis, or thickening of the urinary bladder mucosa) in both males and females from the high-dose group and males from the second highest dose group. Microscopically, the gross lesions were related to increased severity of nephropathy in males and increased mineralization of the kidney in females at all doses. Additionally, inflammation and hyperplasia of the transitional epithelium of the urinary bladder were observed in both males

and females in the highest dose groups and in males in the second highest dose group. Based on increased mineralization in the kidneys of females at all doses, a LOAEL of 261 mg/kg bw per day was identified (NTP, 1995).

In a parallel 13-week study in male and female B6C3F1 mice (10 per sex and dose), *tert*-butanol was administered *ad libitum* via the drinking water at concentrations of 0, 2.5, 5, 10, 20 or 40 mg/mL. In male mice this resulted in doses of approximately 0, 350, 640, 1590, 3940 or 8210 mg/kg bw per day. In female mice, doses of approximately 0, 500, 820, 1660, 6430 or 11 620 mg/kg bw per day were achieved. Over the course of the 13-week study, two males and one female from the highest dose group died as a result of *tert*-butanol exposure and animals in the high-dose group showed emaciation. Males given the high dose also exhibited ataxia and hypoactivity. Significantly reduced body weights (up to 25% lower) were noted in males in the two highest dose groups and females in the highest dose group. Apart from some evidence of slight dehydration (slightly increased haemoglobin and haematocrit) no other toxicologically relevant changes were noted from clinical chemistry or haematological observations. At terminal necropsy, the absolute and relative kidney weights of females from the high-dose group were significantly increased. Hyperplasia and chronic inflammation were also observed in the urinary bladder of males from the two highest dose groups and females from the highest dose group. No other toxicologically significant effects were noted. A NOAEL of 1590 mg/kg bw per day was identified based on significantly decreased body weights and hyperplasia and chronic inflammation of the urinary bladder in males that received the higher doses (NTP, 1995).

In the long-term rat study, male F344 rats (50 per dose) were exposed to 0, 1.25, 2.5 or 5 mg/mL in drinking water (approximately equal to 90, 200 or 420 mg/kg bw per day) for 2 years. Female F344 rats (50 per dose) were exposed to slightly higher concentrations of 0, 2.5, 5 or 10 mg/mL (equal to approximately 180, 330 or 650 mg/kg bw per day). An additional subgroup of 10 animals per dose and sex was exposed for 15 months and sacrificed for interim evaluation. Survival of male and female rats from the highest dose groups was significantly lower than that of control animals. Also, decreased mean terminal body weights were observed in the males from all treatment groups and in females from the highest dose group. Percentage probability of survival at the end of the study was 20, 12, 8 or 2% in males given doses of 0, 90, 200 or 420 mg/kg bw per day, respectively. In females, the percentage survival at the end of the study was 56, 48, 44 or 24% at doses of 0, 180, 330 and 650 mg/kg bw per day, respectively. The terminal mean body weights of treated males were 85, 83 and 76% in the 90, 200 and 420 mg/kg bw per day dose groups, respectively. In females, the figures were 98, 96 and 79% for the 180, 330 and 650 mg/kg bw per day dose groups, respectively. At the 15-month interim evaluation, a dose-responsive increase in absolute and relative kidney weights was noted in treated males and females accompanied by increased

severity of nephropathy (NTP, 1995; Hard et al., 2019). Statistically significant increases in relative kidney weights following 15 months of exposure were noted in males dosed with 200 and 420 mg/kg bw per day and statistically significant increases in absolute and relative kidney weights were observed in females from all of the treatment groups. In addition, a renal tubule adenoma was observed in one male from the high-dose group at the interim sacrifice. Following terminal necropsy at 2 years, the incidence of renal tubule adenoma or carcinoma in all treated males had increased above concurrent control and historical control values (8% versus 0–2%) and analysis of additional step sections from the males revealed a dose-responsive increase in proliferative lesions (hyperplasia and neoplasia). Proliferative effects in the renal tubules of females were limited to hyperplasia in females from the highest dose group; however, the severity of nephropathy was significantly increased in females from all dose groups and in males from the highest dose group. Severity grades of 1.6, 1.9, 2.3 and 2.9 for chronic progressive nephropathy were assigned for females dosed with 0, 180, 330 and 650 mg/kg bw per day, respectively (statistical significance was noted for all treatment groups). The incidence rates for hyperplasia were 0/50, 0/50, 3/50 and 17/50 for females dosed with 0, 180, 330 and 650 mg/kg bw per day, respectively (statistical significance was only reached at the highest dose). The proliferative changes were also accompanied by inflammation (suppurative) of the kidneys in females from the two highest dose groups. The incidence rates were 2/50, 3/50, 13/50 and 17/50 for females dosed with 0, 180, 330 and 650 mg/kg bw per day, respectively. Although *tert*-butanol did not induce carcinogenicity in females, it did induce renal tubule adenomas or carcinomas (combined) in males exposed to doses ≥90 mg/kg bw per day (NTP, 1995). Based on the renal effects observed in both male and female animals, LOAELs of 90 mg/kg bw per day for males and 180 mg/kg bw per day for females were identified (NTP, 1995).

In the chronic mouse study, male and female B6C3F1 mice (60 per sex and dose) were exposed to much higher concentrations in drinking water (0, 5, 10 or 20 mg/L) than in the rat study. The resulting doses were 540, 1040 or 2070 mg/kg bw per day in male mice and 510, 1020 or 2110 mg/kg bw per day in female mice. Survival of the males in the high-dose group and terminal body weights in females given the high dose were significantly decreased. The percentage probability of survival was 45, 62, 58 and 28% in males dosed with 0, 540, 1040 or 2070 mg/kg bw per day, respectively. Terminal body weights of the treated females were 98, 97 and 88% of the control body weights for animals in the 510, 1020 and 2110 mg/kg bw per day dose groups, respectively. Male and female mice from the highest dose groups also showed increased incidence of chronic inflammation of the urinary bladder. An associated statistically significant increase in the incidence of transitional epithelial hyperplasia of the urinary bladder of male mice from the high-dose group was also observed.

Additionally, male mice in the highest dose group showed an increased incidence of fatty changes in hepatocytes and follicular cell hyperplasia was significantly increased in all treated male mice. Female mice also showed a statistically significantly increased incidence of follicular cell hyperplasia at doses of 1020 and 2110 mg/kg bw per day. The incidence rates for follicular cell hyperplasia in males were: 5/60, 18/59, 15/59 and 18/57 at doses of 0, 540, 1040 or 2070 mg/kg bw per day, respectively. In females, the incidence rates were: 19/58, 28/60, 33/59 and 47/57 at doses of 0, 510, 1020 or 2110 mg/kg bw per day, respectively. Follicular cell hyperplasia was accompanied by an increased incidence of thyroid follicular cell adenoma in females exposed to 2110 mg/kg bw per day, whereas male mice showed only a marginally increased incidence of thyroid follicular cell adenoma or carcinoma at doses ≥1040 mg/kg bw per day. Based on the increased incidence of follicular cell hyperplasia, a LOAEL of 540 mg/kg bw per day was identified for males and a NOAEL of 510 mg/kg bw per day was identified for females (NTP, 1995).

Based on the results of the NTP (1995) drinking water studies summarized above, the critical non-neoplastic effects associated with repeated oral exposure to *tert*-butanol are lesions in the urinary tract, with rats appearing more sensitive than mice and males appearing more sensitive than females. Similar to the conclusions of Faber et al. (2014) regarding the inhalation toxicity of *tert*-butyl acetate, the observed non-neoplastic histopathological changes in the kidney of male rats exposed to *tert*-butanol are considered a male rat specific effect related to α-2u-globulin, with limited relevance to human health risk assessment (US EPA, 2017; Hard et al., 2019). The Committee noted that the proliferative effects in the thyroid glands of mice were not observed following 13 weeks of exposure to a high dose of *tert*-butanol (NTP, 1995; 1997) or *tert*-butyl acetate (Faber et al., 2014). Furthermore, Blanck et al. (2010) suggested that the proliferation of thyroid follicular cells in mice is due to a non-genotoxic mode of action associated with increased liver enzyme activity at high doses.

In addition to the subchronic (13-week) and chronic (2-year) drinking water studies, the NTP (1997) conducted 18-day and 13-week inhalation toxicity studies in mice and rats with target *tert*-butanol concentrations ranging from 113 to 7000 ppm. Target concentrations were 113, 225, 450, 900 or 1750 ppm in rats and mice exposed for 13 weeks; and 450, 900, 1750, 3500 or 7000 ppm in rats and mice exposed for 18 days. The critical effects noted by the NTP (1997) following 13 weeks of inhalation exposure to *tert*-butanol were increased kidney weights and associated increased severity of chronic nephropathy in male F344 rats. No treatment-related histopathological lesions were noted in the female F344 rats or in the male and female B6C3F1 mice. However, the liver weights of female mice in the two highest dose groups (1080 and 2100 ppm) in the 13-week studies were significantly increased.

3.2.3 Reproductive and developmental toxicity

In a developmental toxicity study by Yang et al. (2007), female Sprague-Dawley rats (22 animals per dose) were administered *tert*-butyl acetate via oral gavage at doses of 0, 400, 800 or 1600 mg/kg bw per day during gestational day (GD) 6 to 19 (corn oil was used as the vehicle). In a preliminary dose range finding study, significant systemic toxicity (maternal death, decreased maternal body weight gain and decreased fetal weight) was observed at doses greater than 1000 mg/kg bw per day. In the main experiment, 2 out of 22 dams in the high-dose group died 1 to 3 days following initiation of dosing. Animals in the high-dose group also exhibited decreased body weight gain, piloerection, abnormal gait, decreased locomotor activity, loss of fur, vocalization, reddish tears from the eyes, reddish vaginal discharge, nasal haemorrhage and coma throughout the study. At terminal necropsy (gestational day 20), surviving dams from the high-dose group showed one case each of congestion/haemorrhage of the duodenum and atrophy of the spleen. The terminal organ weights of the adrenal gland (absolute and relative) and liver (relative) were increased and that of the thymus (absolute) decreased in animals from the high-dose group. The two dams that died prior to gestational day 10 showed expansion of the stomach, hypertrophy of the liver, and congestion/haemorrhage of the small intestine. Although a dose-dependent tendency towards reduced body weights was apparent (not statistically significant), dams exposed to 400 or 800 mg/kg bw per day appeared normal throughout the study period and no significant treatment-related effects were noted during terminal necropsy. Statistically significant effects on the fetus were observed in offspring of dams from the middle and high-dose groups. Reduced body weight was seen in the fetuses of females in the high-dose group and increases in the incidence of skeletal variations (supernumerary rib and retarded ossification) in fetuses of females in both the middle and high-dose groups. Based on the effects summarized above, the NOAEL for maternal toxicity was 800 mg/kg bw per day. For embryo-fetal development, the NOAEL was 400 mg/kg bw per day (Yang et al., 2007).

In a non-guideline study, female Sprague-Dawley rats (Crl:CD(SD); 22 per group) were exposed to 0 (corn oil), 400, 800, 1000 or 1600 mg/kg bw per day *tert*-butyl acetate via oral gavage from GD 6 to 20 (Faber et al., 2014). Effects related to irritation and central nervous system alteration (for example, rocking, lurching or swaying while ambulating, walking on tiptoes, splayed hindlimbs, circling and/or retropulsion) were observed shortly after dosing in dams exposed to doses ≥800 mg/kg bw per day. Additionally, one female from the high-dose group was found dead shortly after exposure on GD 8 and other animals given the high dose showed hypoactivity, prostration, impaired use of right and/or left hindlimb, dragging body and hunched posture. Lacrimation

and salivation were also noted before and after dosing in all treatment groups. Slight and transient decreases in feed consumption and body weight gains were observed in dams exposed to doses ≥800 mg/kg bw per day. Following gross necropsy of the surviving animals, findings were restricted to the dams from the high-dose group, namely, increased weights of the adrenal gland (absolute and relative), liver (relative) and kidney (relative), and decreased weight of the thymus (absolute and relative). Except for severely distended ureters and a severely dilated renal pelvis in the moribund female from the high-dose group, no adverse histopathological findings were noted in the treated dams. Indicators of reproductive toxicity such as mean gravid uterine weights, mean number of viable fetuses per litter, intrauterine growth and survival, mean numbers of corpora lutea and implantation sites, and the mean litter proportions of preimplantation loss were similar across all groups. Small but statistically significant reductions in body weight were observed in fetuses from all treated groups, with a dose-related trend. The authors of the study commented, based on historical control data, that the unusually small average litter size in the controls resulted in higher average fetal body weights in this group and concluded that there was no effect on fetal body weight attributable to the treatment. The NOAEL for maternal toxicity from this study was 400 mg/kg bw per day. Since the effect on fetal weight is unclear and the fetuses were not examined for morphological abnormalities, the Committee concluded that a NOAEL for developmental toxicity could not be identified from this study.

In the reproductive and developmental inhalation toxicity study by Faber et al. (2014), male and female Sprague-Dawley rats (Crl:CD(SD); 10 per sex and dose) were exposed to 0, 100, 400 or 1600 ppm of *tert*-butyl acetate via whole-body exposure, for 70 consecutive days prior to mating, throughout mating, and until GD 20 (109–110 days for males and 108–119 days for females). Following this period, inhalation exposure of the females was discontinued and the males were euthanized. Inhalation exposure of the dams was re-initiated on PND 5 and continued until euthanasia postweaning. F_1 offspring were euthanized, apart from one pup per sex from each litter, on PND 24. A selected group of pups were exposed to the same whole-body inhalation concentration as their parents for an additional 6 days postweaning and euthanized on PND 27. Except for transient decreases in body weight gain and food consumption, no evidence of reproductive or systemic toxicity was observed in the parental generation. Furthermore, apart from slightly decreased body weight and body weight gain from PNDs 22 to 23 in pups from the high-dose group, there was no evidence of developmental toxicity in the F_1 generation (Faber et al., 2014).

Tert-butanol (metabolite)

Daniel & Evans (1982) reported that *tert*-butanol produced developmental delay in postnatal physiological and psychomotor performance in Swiss-Webster mice whose mothers were fed a liquid diet containing 0, 0.5, 0.75 or 1.0% *tert*-butanol (equal to doses between 2830 and 8721 mg/kg bw per day) during GD 6 to 20. Grant & Samson (1982) reported decreased absolute and relative brain weights in neonatal Long-Evans rats exposed to *tert*-butanol in milk formula during PNDs 4 to 7 (onset of the "brain growth spurt"), via a feeding tube at doses between 600 and 2690 mg/kg bw. However, in contrast to ethanol, *tert*-butanol did not induce alterations in myelin formation and protein production (Grant & Samson, 1982). Faulkner et al. (1989) observed an increased number of resorptions per litter in CBA/J and C57ABL/6J mice exposed to 10.5 mmol/kg bw (equal to ~780 mg/kg bw) twice daily during GD 6 to 18. Nelson et al. (1989) reported reduced fetal weight in Sprague-Dawley rats exposed to *tert*-butanol via whole-body inhalation of 0, 2000, 3500 and 5000 ppm during GD 1 to 19 (statistically significant reductions in male and female fetal weights were recorded at all concentrations; LOAEC = 2000 ppm).

The NTP (1995) reported that mice exposed to 11 620 mg/kg bw per day *tert*-butanol in drinking water for 13 weeks showed a significantly increased estrous cycle length. In contrast, no significant effect on estrous cycles was observed in rats and no significant differences in sperm morphology or motility were observed in mice or rats following 13 weeks of exposure via drinking water (NTP 1995; study summaries previously provided). Additionally, the NTP (1997) did not observe any adverse effects on reproductive parameters in male or female rats or mice exposed to *tert*-butanol via inhalation for 18 days and 13 weeks (studies briefly summarized previously).

The Committee concluded that developmental toxicity occurs at high doses of *tert*-butanol and that other more sensitive end-points have been identified.

3.2.4 Genotoxicity

According to the available information, *tert*-butyl acetate is non-genotoxic in vitro and in vivo. The genotoxicity studies on *tert*-butyl acetate are summarized in Table 13.

Tert-butanol (metabolite)

Using the standard battery of genotoxicity tests, *tert*-butanol shows negative results (NTP, 1995). Notably, *tert*-butanol did not induce micronuclei in erythrocytes of male and female mice exposed to extremely high doses (up to 11 620 mg/kg bw per day) in drinking water for 13 weeks.

Table 13
Summary of some in vitro and in vivo genotoxicity studies on *tert*-butyl acetate

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537, TA102) and *Escherichia coli* (WP2uvrA/pKM 101)	50–5000 µg/plate	Negative (S9– and S9+)	OEHHA (2018)
Chromosome aberration in human lymphocytes	290–1160 µg/mL	Negative (S9– and S9+)	OEHHA (2018)
Studies based on in vivo systems			
Bone marrow micronucleus test in Sprague-Dawley rats	100–1600 ppm via nose-only inhalation	Negative	OEHHA (2018)

Positive results have been reported for *tert*-butanol from genotoxicity tests using non-standard testing methodology. Sgambato et al. (2009) suggested that the positive results in the Comet assay are due to indirect mechanisms (i.e. oxidative stress) as indicated by substantial increases in the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), an important marker of DNA oxidative damage. The positive results reported for *S.* Typhimurium strain TA102 provide further indications of an oxidative stress mechanism (Levin et al., 1982; Williams-Hill et al., 1999; McGregor et al., 2005). *Tert*-butanol has been shown to bind to DNA in the liver, lungs and kidneys of mice following oral gavage exposure to single doses of between 0.1 and 997 µg/kg bw, using a sensitive analytical technique (Yuan et al., 2007). In view of the negative results in a range of genotoxicity tests in vitro and in vivo, the Committee considered these findings to have questionable relevance for the genotoxicity of *tert*-butanol. Table 14 summarizes the available information on genotoxicity.

3.2.5 Allergenicity

No studies regarding the allergenic effects of *tert*-butyl acetate on humans following oral exposure were identified. The German MAK Commission (2016) briefly mentioned negative results from a Buehler skin sensitization test in guinea-pigs. No other relevant information was identified.

Tert-butanol (metabolite)

Tert-butanol is generally considered a mild skin irritant and there is one case report of allergic contact dermatitis from its use in sunscreen (Edwards & Edwards, 1982).

3.2.6 Impurities

No impurities of concern were identified for *tert*-butyl acetate.

Table 14
Summary of some in vitro and in vivo genotoxicity studies on *tert*-butanol

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	100–10 000 µg/plate	Negative (S9– and S9+)	NTP (1995)
Gene mutation in *Salmonella* Typhimurium (TA102) (non-GLP)	5–5 000 µg/plate	Equivocal (S9–) Positive (S9+)	McGregor et al. (2005); Williams-Hill et al. (1999)
Gene mutation in mouse lymphoma cells (*L5178Y TK+/–*)	625–5 000 µg/mL	Negative (S9– and S9+)	NTP (1995)
Sister chromatid exchange in Chinese hamster ovary cells	160–5 000 µg/mL	Negative (S9– and S9+)	NTP (1995)
Chromosome aberration in Chinese hamster ovary cells	160–5 000 µg/mL	Negative (S9– and S9+)	NTP (1995)
DNA damage (modified Comet assay) in rat fibroblasts	0.44 mM (~33 µg/mL)	Positive (S9–)	Sgambato et al. (2009)
Studies based on in vivo systems			
Micronucleus test in B6CF1 mouse peripheral blood cells	350–11 620 mg/kg bw per day for 13 weeks via drinking water	Negative	NTP (1995)
Bone marrow micronucleus test in F344 rats	39–2 500 mg/kg bw via intraperitoneal injection	Negative[a]	NTP[b]
DNA binding in mouse (male Kunning) liver, lung and kidney	~0.1–997 µg/kg bw via oral gavage	Positive	Yuan et al. (2007)

GLP, good laboratory practice.
[a] Summary results published by NTP [online] (https://ntp.niehs.nih.gov/whatwestudy/testpgm/status/ts-10402-n.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=ts-10402-n.)
[b] https://tools.niehs.nih.gov/cebs3/views/index.cfm?action=main.download&bin_id=10265&library_id=13672&fileIdsSelected=1de240ff6604d3e201660c33ea8a0308

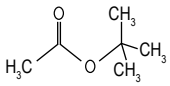
3.3 Observations in humans

The German MAK Commission (2016) described a study in humans investigating the odour threshold (0.008 mL/m^3) and irritation potential (eye; 177 mL/m^3) of airborne *tert*-butyl acetate. It concluded that *tert*-butyl acetate is about as or possibly somewhat less irritating to the eyes than *n*-butyl acetate.

4. Occurrence and 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 A4.3).

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.

5. Comments

5.1 Chemical and technical considerations

The chemical and technical considerations for *tert*-butyl acetate are summarized in Table 15.

5.2 Biochemical aspects

There are no 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 (WHO/IPCS, 2005). 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 (Cruzan & Kirkpatrick, 2006), 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.

5.3 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 (WHO/IPCS, 2005). 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. (2007), *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.

Due 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 (NTP, 1995), *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 α-2u-globulin in the kidneys (NTP, 1995; US EPA IRIS, 2017; Hard et al., 2019) and the Committee considered this mechanism to be male rat-specific and not relevant to humans. The Committee considered that although chronic progressive nephropathy (CPN) is a commonly diagnosed rat-specific condition of questionable relevance to humans (WHO, 2015), the lesions associated with chronic progressive nephropathy in the female rats (tubular degeneration, glomerular sclerosis, etc.) also occur in the human kidney (US EPA, 2017). 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 (NTP, 1995). At significantly higher concentrations in drinking-water, and 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) (NTP, 1995). 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 in vitro and in vivo information on *tert*-butyl acetate 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 (NTP, 1995).

5.4 Allergenicity

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

5.5 Impurities

No impurities of concern were identified.

5.6 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 A4.3).

6. 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 (NTP, 1995) 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.

IV. *n*-Pentane

1. 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 in contact with foodstuffs (SCF, 1995). 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) 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 health-based guidance value (HBGV).

Table 16
Physical and chemical characteristics of pentane

Chemical name	Pentane
Synonyms	*n*-pentane; pentan; amyl hydride
CAS number	109-66-0
Chemical structure	
Molecular formula; molar mass	C_5H_{12}; 72.15 g/mol
Description	Clear colourless liquid with a petroleum-like odour. Forms highly flammable vapour–air mixtures
Melting point	−129.7 °
Boiling point	36 °C
Flash point	−49 °C (closed cup)
Solubility	Insoluble in water, soluble in most organic solvents

For the present assessment, previous assessments (monographs) completed 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 in regard to the oral route of exposure. In an effort to supplement the 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.

2. Chemical and technical considerations

The physicochemical characteristics of pentane are listed in Table 16.

2.1 Manufacture and uses of *n*-pentane

Pentane is produced by distillation from natural gasoline or naphtha. Natural gasoline contains approximately 7% and catalytic cracker naphtha contains 1% pentanes (Mears & Eastman, 2005). Pentane is also produced by dehydration and subsequent hydrogenation of 2- and 3-pentanol and from 2-bromopentane by Grignard reaction (The Merck Index, 2000).

Pentane is used as an additive in motor and aviation fuel, as a propellent and solvent in cosmetics, and as a blowing agent to make foamed food packaging materials (Mears & Eastman, 2005; PubChem online database).

2.2 Impurities and secondary contaminants

Pentane may contain residues of sulfur (< 0.5 mg/kg), naphthenes (< 1 mg/kg), benzene (< 3 mg/kg), toluene (< 3 mg/kg), aromatics (benzene, toluene and xylene) (< 5 mg/kg) and *n*-hexane (0.1 mg/kg) (Shell Chemicals Online). Technical grade pentane may contain branched and cyclic hydrocarbons of similar molecular mass, which are not expected to be of concern (EFSA, 2012a).

The major route of exposure to benzene is inhalation rather than diet (WHO, 2000; Duarte-Davidson et al., 2001). Estimated absorbed exposures of inhaled benzene in the United Kingdom have been estimated to range from 0.71 µg/kg bw per day for rural children to 14.12 µg/kg bw per day for urban adult smokers who work adjacent to busy roads (Duarte-Davidson et al., 2001). Estimates of dietary exposure range from 1.4–2.8 µg per day (WHO, 2000), 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, 0.0009 µg/kg bw per day, is minimal compared to total exposure to benzene.

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 (Vinci et al., 2015). 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 (Ingenbleek & Kimura, 2013).

Naphthenes (< 1%) and *n*-hexane (< 0.1%) may also be present in *n*-pentane (Shell Chemicals Online). Maximum naphthene and hexane exposures associated with exposure to *n*-pentane at a concentration 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 other sources (for example see Environment and Climate Change Canada, 2009). Upper bound estimates of total exposure to *n*-hexane from all sources in Canada ranged from 31.6 µg/kg bw per day for people aged 60+ years to 95.5 µg/kg bw per day for children aged 0.5–4 years (Environment and Climate Change Canada, 2009).

2.3 Reactivity and reactions with fats and oils

No reaction products of concern are expected with edible fats and oils (EFSA, 2012a). The fundamental chemical reactions for pentane are sulfonation to form sulfonic acids, chlorination to form chlorides, nitration to form nitropentanes, oxidation to form various compounds, and cracking to form free radicals (Mears & Eastman, 2005).

2.4 Methods of analysis

No test methods for analysing pentane in fats and oils have been found in the literature. Residues of pentane may be analysed by GC-FID or GC-MS (PubChem online database).

3. Biological data

3.1 Biochemical aspects

No studies on the biochemical aspects of *n*-pentane following oral exposure were identified.

Using an enclosed chamber system, male Sprague-Dawley rats were exposed to *n*-pentane via inhalation (approximately 0.34 µmol of [l,5-^{14}C] *n*-pentane plus 1.0 mL of 10.1 µmol/mL unlabelled pentane gas; 99.0% purity), and chamber air concentrations of *n*-pentane were measured at 5-minute intervals for up to 8 hours. In the first experiment, radioactivity in whole blood and various tissues (liver, kidney, lung, testes, brain, muscle, heart, small and large intestine, spleen and fat) of six rats were measured at necropsy (8 hours), whereas in the second experiment, urine and blood samples were collected from four rats. At the end of the experiment, approximately 4.7% of the *n*-pentane remained in the chamber system, and approximately 50% of the total [^{14}C] activity added to the chamber system was recovered as CO_2 (in both experiments). Results from the first experiment showed that liver, small intestine and kidneys contained the highest concentration of radioactivity. However, the muscle and liver showed the largest proportion of total [^{14}C] activity recovered (6.98 and 3.37%, respectively with a total recovery of 14.92% in all tissues). In the second experiment, recovery of [^{14}C] activity in blood was 2.1%, whereas the recovery in urine was 7.6%. When the authors combined the results from both experiments, approximately 78.9% of the administered [^{14}C]-pentane was accounted for, predominantly as CO_2 (Daugherty, Ludden & Burk, 1988).

Chiba & Oshida (1991) reported that in rats exposed by inhalation to 5% *n*-pentane for 1 hour, *n*-pentane is metabolized to 2-pentanol, 3-pentanol and 2-pentanone. Similar results have been observed in vitro. For example,

2-pentanol (major metabolite, 83–89%) and 3-pentanol (minor metabolite, 11–16%) were detected when rat and rabbit liver microsomes were incubated with *n*-pentane; and 2-pentanol, 3-pentanol and 2-pentanone were detected following incubation with mouse liver microsomes (Frommer, Ulrich & Staudinger, 1970). Chiba & Oshida (1991) also demonstrated that pretreatment of rats with phenobarbital increased the concentration of 2-pentanol, 3-pentanol and 2-pentanone in the blood of exposed rats, providing evidence that hepatic microsomes play a critical role in the metabolism of *n*-pentane. There is also evidence that alcohol dehydrogenase may be involved in pentane metabolism in rats, since administration of ethanol or 4-methyl-pyrazole diminished pentane clearance (Burk, Ludden & Lane, 1983; Allerheiligen, Ludden & Burk, 1987).

Filser et al. (1983) studied the pharmacokinetics of *n*-pentane (99.7% purity) following inhalation exposure of rats in closed desiccator jar chambers to a wide range of *n*-pentane concentrations. According to Filser et al. (1983), *n*-pentane showed first order kinetics at low exposure concentrations (<100 ppm) and saturation kinetics at high concentrations (>100 ppm). In this study, an elimination half-life for *n*-pentane of approximately 8 minutes was estimated. The rapid metabolism and excretion of *n*-pentane offers little potential for tissue accumulation (McKee et al., 1998).

Perbellini et al. (1985) studied the partitioning of *n*-pentane in human tissues/air and human blood/air ex-vivo. The tissues were collected from two men (aged 30 and 40 years) who had died suddenly from a heart attack, and stored human blood was obtained from a hospital blood bank. The tissue/air partitions were: liver – 2.1; kidney – 0.6; brain – 2.2; fat – 39.6; muscle – 0.7; heart – 0.2; and lung – 0.5. The blood/air partition was 0.38. Based on the estimated partitions, *n*-pentane preferentially partitions to fat.

3.2 Toxicological studies

3.2.1 Acute toxicity

n-Pentane exhibits low acute oral toxicity in rats, with an estimated LD_{50} of > 2000 mg/kg bw (PubChem[1]). McKee et al. (1998) indicated that following oral exposure to 2000 mg/kg bw, Crl:CDBR rats (five per sex) exhibited no treatment-related mortality or persistent signs of systemic toxicity 14 days after exposure. On the day of treatment, Mckee et al. (1998) observed that all of the exposed rats showed oral and nasal discharge, swollen abdomen, anogenital staining and/or soft or mucoidal stool. Based on the available animal data, inhalation of *n*-pentane at high concentrations (>30 000 ppm) may also cause neurobehavioural and neurotoxic effects (ECB, 2003; German MAK Commission, 2015).

[1] https://pubchem.ncbi.nlm.nih.gov/compound/8003#section=NIOSH-Toxicity-Data

3.2.2 Short-term and long-term studies of toxicity and carcinogenicity

Halder et al. (1985) and, more extensively, the US EPA (2009), summarized the results of a repeated-dose oral toxicity screening study that specifically investigated the nephrotoxicity potential of various hydrocarbon solvents, including *n*-pentane. In this study, male Fischer 344 rats (n = 10 per group) were administered *n*-pentane via oral gavage at doses of 0, 500 or 2000 mg/kg bw per day (5 days per week) for 4 weeks. Although gross examination of tissues and organs from all animals was conducted, histopathological observations were restricted to the kidneys and consisted of grading for:

- hyaline droplet accumulation in the proximal convoluted tubules;
- regenerative epithelium in the renal cortex; and
- intratubular cast formation in the renal medulla.

No treatment-related clinical signs were noted; however, mortality (incidence rates of 2/10 and 4/10, respectively; cause uncertain), weight loss (>14%) and decreased absolute kidney weights (8% and 9%, respectively) were observed in animals given doses of 500 and 2000 mg/kg bw per day. Histopathological examination of the kidneys did not reveal any effects commonly associated with hydrocarbon-induced nephropathy (for example, hyaline droplet changes, regenerative epithelium formation or tubular dilatation). No other significant changes were noted, except for lesions in the forestomach consisting of raised, pale, white or dark foci, most notably among rats from the high-dose group.

Halder et al. (1985) also investigated the effects of isopentane (a structural analogue of *n*-pentane). According to the limited results reported, isopentane was lethal in 90% of the rats administered a dose of 2000 mg/kg bw per day and 10% of the animals that received 500 mg/kg bw per day. No histopathological evidence of neuropathy was observed following exposure to isopentane. Given the limitations in study design (for example, males only; limited clinical observations; no results on haematology or clinical chemistry; histopathology restricted to kidneys) and reporting, the results of these assays are considered inadequate for hazard characterization.

In a 90-day guideline-compliant (OECD 413) inhalation study, Sprague-Dawley rats (10 per sex and concentration) were exposed via whole-body inhalation to *n*-pentane vapours (>95% purity) with nominal concentrations of 0, 5000, 10 000 or 20 000 mg/m^3 for 13 weeks (6 hours/day, 5 days/week). There were no clinical signs of toxicity, and mortality was not evident in either controls or treated animals. There were no treatment-related changes in haematology or clinical chemistry among treated rats (data not available) and body weight was similar among all groups. Ophthalmological, gross and histological examinations

did not reveal any adverse effects of *n*-pentane exposure. A statistically significant increase in the absolute liver weight was noted in animals in the low-dose group; however, it was not concentration-dependent. Based on the absence of evidence for systemic effects, a NOAEC of 20 483 mg/m^3 (measured value), the highest concentration tested, was identified (McKee et al., 1998; ECHA, 2020d).

In an inhalation study, Crl:CD rats (10 males per group) were exposed to 0, 1000, 3000 or 10 000 ppm of *n*-pentane (or 3003, 9009 and 30 030 mg/m^3) for 2 weeks (6 hours/day, 5 days/week). Five rats per group were killed at day 14 and the remaining five rats per group were killed after a 14-day recovery period. Overall, no clinical signs of toxicity were evident in animals in any of the treatment groups; body weight was similar to the controls, and clinical pathological and histopathological findings were unremarkable. The functional observation battery test did not reveal any abnormal behavioural responses to pentane exposure. Statistically significant increases in serum calcium (11.6 and 12.1 mg/L; versus 11.1 mg/L in controls) and phosphorus concentrations (10.4 and 11.0 mg/L; versus 9.8 mg/L in controls) were seen in rats exposed to 3000 or 10 000 ppm, respectively. However, these changes were reversible during the 2-week recovery period. The authors of the study proposed a conservative NOAEC of 1000 ppm based on reversible clinical chemistry changes occurring at 3000 and 10 000 ppm (Stadler et al., 2001).

Takeuchi et al. (1981) studied the neurotoxicity of *n*-pentane (purity over 99%), *n*-hexane and *n*-heptane in male Wistar rats (7 per group) exposed to 3000 ppm in an exposure chamber for 12 hours/day, 7 days/week for 16 weeks. The conduction velocity of the tail nerves was measured as an indicator of peripheral neurotoxicity. Rats exposed to *n*-pentane behaved normally throughout the experiment, but transiently decreased body weights were observed after 4 weeks. No significant differences from controls in nerve conduction were observed in animals in the *n*-pentane- and *n*-heptane-treated groups whereas *n*-hexane significantly disturbed nerve conduction. The results reported by Takeuchi et al. (1981) are supported by the lack of neurotoxicity observed by Frontali et al. (1981) who exposed male Sprague-Dawley rats (six to nine) to 3000 ppm *n*-pentane (purity 99%) for 9 hours/day, 5 days/week for 30 weeks.

3.2.3 Reproductive and developmental toxicity

In a range-finding developmental toxicity study, pregnant Sprague-Dawley rats (7 per group) were administered *n*-pentane in corn oil via gavage at a dose of 0, 250, 500, 750 or 1000 mg/kg bw per day, once daily from GD 6 to 15. Animals were sacrificed at GD 21. Decreased body weight gain of animals in the group exposed to 1000 mg/kg bw per day (in relation to controls) was the only maternal effect that was considered attributable to *n*-pentane exposure. Treatment with *n*-pentane did

not cause any significant changes in fetal body weight or sex ratio. No biologically significant external malformations were observed (McKee et al., 1998).

In the follow-up study, pregnant rats (25 per group) were administered *n*-pentane in corn oil via gavage at doses of 0, 100, 500 and 1000 mg/kg bw per day during GD 6 to 15. Maternal toxicity was not evident at any dose level. Most of the dams survived until scheduled terminal sacrifice on GD 21 and were free of clinical or postmortem effects that could be attributed to treatment. Mean body weight, body weight change, uterine weight, corrected body weight and uterine implantation data were similar between treated and control dams. Among the fetuses there were no statistically significant differences in mean fetal body weight or mean skeletal ossification sites between treated and control groups. Furthermore, there were no statistically significant differences in total or individual variations or malformations (external, visceral or skeletal) between treated and control groups. Based on these results, a NOAEL of 1000 mg/kg bw per day, the highest dose tested, was identified for maternal and developmental toxicity (McKee et al., 1998).

In a developmental toxicity study summarized in Hurtt & Kennedy (1999), female Crl:CD®BR rats were exposed to *n*-pentane vapours at target concentrations of 0, 1000, 3000 or 10 000 ppm for 6 hours/day during GD 6 to 15. No evidence of developmental or maternal toxicity was observed in the developmental toxicity study and a NOAEC of at least 10 000 ppm was identified.

Overall, the results described here suggest that exposure to *n*-pentane via the oral and inhalation routes is not hazardous to the developing rat fetus.

Isopentane

In the ECHA (2020d) registration dossier for *n*-pentane, toxicity data on *n*-pentane's isomer (isopentane) was considered as read-across. According to the German MAK Commission (2015) for pentane (all isomers) "the toxic effects and mode of action of isopentane and *tert*-pentane are similar to those of *n*-pentane". Consequently, for the purposes of assessing the oral toxicity of *n*-pentane, the results of the one-generation reproductive toxicity study with isopentane are summarized below.

Yu et al. (2011) summarized the results of a guideline-compliant (OECD 415; GLP) one-generation reproductive toxicity study with isopentane. In this study, isopentane was administered via oral gavage in corn oil to male and female Sprague-Dawley rats (24 per sex and treatment group) at doses of 0, 100, 300 or 1000 mg/kg bw per day. F_0 generation males were treated for 10 weeks prior to mating and during mating (total 12 weeks of exposure). F_0 generation females were treated starting from 2 weeks prior to mating to PND 21 of the F_1 generation (10 weeks total exposure). No treatment-related evidence of reproductive or

developmental toxicity was observed at any dose. Effects were limited to the F_0 generation and consisted of transient salivation immediately following dosing in males administered 300 and 1000 mg/kg bw per day and in females administered a dose of 1000 mg/kg bw per day (probably due to the irritating properties of isopentane). Male F_0 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 treated with 1000 mg/kg bw per day. Male rats treated with 1000 mg/kg bw per day also exhibited increased relative brain, liver, kidney and testes weights. 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/regeneration (the incidence rates in the 0, 100, 300 and 1000 mg/kg bw per day dose groups were 13/24, 10/24, 11/24 and 18/23, respectively). No other effects were reported in the other F_0 generation males or females. Based on effects observed in the F_0 generation rats exposed to 1000 mg/kg bw per day, the Committee identified a NOAEL of 300 mg/kg bw per day.

3.2.4 Genotoxicity

In an in vitro clastogenicity test in Chinese hamster ovary (CHO) cells, *n*-pentane produced a dose-related increase in the percentage of aberrant cells following 20 hours harvest in the presence of S9 with statistically significant increases being noted at 1200 and 1500 µg/mL. However, no increases were observed in the initial 20 hours harvest treatment with and without S9 and no increases were observed in either the initial or repeat 44 hours harvest in the presence and absence of S9. Additionally, *n*-pentane has produced negative genotoxicity results in vitro and in vivo (Table 17) and lacks structural alerts for mutagenicity/genotoxicity. Consequently, the weight of evidence suggests that *n*-pentane does not raise concerns for genotoxicity.

3.2.5 Allergenicity

n-Pentane did not induce skin sensitization reactions in the guinea-pig maximization test (Mckee et al., 1998).

3.2.6 Impurities

Some of the impurities identified in section 2.2 are associated with carcinogenicity (for example, benzene (IARC, 2018)). However, the estimated maximum dietary exposure to benzene present in fats and oils when *n*-pentane is carried as a previous cargo, 0.0009 µg/kg bw per day, is minimal compared to total benzene exposure.

Table 17
Summary of some in vitro and in vivo genotoxicity studies on *n*-pentane

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	1 to 10% (v/v%) Cytotoxic at vapour concentrations of 25 and 50%	Negative (S9– and S9+)	Kirwin, Thomas & Simmon (1980)
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	0.2 mL	Negative (S9– and S9+)	ECB (2003)
Chromosomal aberrations in Chinese hamster ovary cells (using vials with limited headspace)	600 to 1 500 µg/mL	Negative (S9–) Equivocal (S9+)	McKee et al. (1998)
Studies based on in vivo systems			
Bone marrow micronucleus test in Sprague-Dawley rats following 13 weeks of inhalation exposure (6 hours/day)	5 000 to 20 000 mg/m^3	Negative	McKee et al. (1998)

3.3 Observations in humans

There are no published reports on the toxicity of *n*-pentane after oral administration to humans. However, human volunteers (3 to 6) exposed to 5000 ppm *n*-pentane (76.5% *n*-pentane, 20.8% isopentane, 1.4% hexane and 1.3% butane) for 10 minutes did not exhibit any adverse symptoms and, in particular, did not demonstrate mucous membrane irritation or vertigo (Galvin & Marashi, 1999; Carreón & Herrick, 2012; German MAK Commission, 2015). Galvin & Marashi (1999) reported neurotoxic effects associated with exposure to *n*-pentane as a component of a solvent mixture used in the occupational environment; however, these results are confounded by the presence of other hydrocarbons (for example, *n*-hexane) and consequently, are not considered relevant for this assessment.

4. Occurrence and exposure

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

No data were found on concentrations of 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

Table 18
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.

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 A4.3).

5. Comments

5.1 Chemical and technical considerations

The chemical and technical considerations for *n*-pentane are summarized in Table 18.

5.2 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 to fat. It is rapidly metabolized to pentanols and pentanone, and exhaled as carbon dioxide (Filser et al. 1983; Daughtery et al. 1988; Chiba & Oshida, 1991). Considering the rapid metabolism and excretion of *n*-pentane, there is little potential for tissue accumulation (McKee et al., 1998).

5.3 Toxicological studies

n-Pentane exhibits low acute oral toxicity in rats, with an estimated LD_{50} of > 2000 mg/kg bw (McKee et al., 1998). 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 during GD 6 to GD 15 (McKee et al., 1998). 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 (Yu et al., 2011). No evidence of treatment-related reproductive or developmental toxicity was observed at any dose. Effects were limited to the F_0 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 F_0 generation rats treated with 1000 mg/kg 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 F_0 generation males or females. Based on effects observed in the F_0 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.

5.4 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.

5.5 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 a pentane exposure of 0.3 mg/kg bw per day in oil as carryover from previous cargoes (see ssection A4.3) 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 (WHO, 2000; Duarte-Davidson et al., 2001). 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 (Duarte-Davidson et al., 2001). Estimates of dietary exposure range from 1.4 to 2.8 µg per day (WHO, 2000), 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, 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 (Vinci et al., 2015). 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 (Ingenbleek & Kimura, 2013).

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, 2009).

5.6 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 (section A4.3).

6. 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.

V. Cyclohexane

1. 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 haemopoietic injury described in the literature may be attributed to benzene contamination, specifications were prepared (Annex 1, reference *50*). SCF (1981) 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 (SCF, 1997). EFSA (2012a) 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) completed 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.

2. Chemical and technical considerations

The physicochemical characteristics of cyclohexane are listed in Table 19.

Table 19
Physical and chemical characteristics of cyclohexane

Chemical name	Cyclohexane
Synonyms	Hexamethylene; hexanaphthene; hexahydrobenzene
CAS number	110-82-7
Chemical structure	
Molecular formula; molar mass	C_6H_{12}; 84.16 g/mol
Description	Colourless, clear, flammable liquid, with characteristic odour
Melting point	4–7 °C
Boiling point	80.7 °C
Flash point	−18 °C (closed cup)
Solubility	Insoluble in water; soluble or miscible with ethanol, methanol, diethyl ether, acetone, benzene and carbon tetrachloride

2.1 Manufacture and uses of cyclohexane

Cyclohexane is produced by hydrogenation of benzene in either the liquid or the vapour phase in the presence of hydrogen. Several cyclohexane processes requiring nickel, platinum or palladium catalysts have been developed. Usually, the catalyst is supported, for example, on aluminium oxide, but at least one commercial process utilizes Raney nickel (PubChem online database). Cyclohexane is also obtained by fractional distillation of petroleum where it occurs in amounts between 0.5 and 1.0%. In distillation of petroleum, the C_4 boiling range naphthas are fractionated to obtain C_5-naphtha containing 10–14% cyclohexane which, upon further fractionation yields an 85% concentrate (which is sold as such). Further purification of 85% concentrate cyclohexane necessitates isomerization of pentanes to cyclohexane, heat cracking to remove open chain hydrocarbons, and sulfuric acid treatment to remove aromatic compounds (The Merck Index, 2000).

Cyclohexane is used as a solvent for lacquers and resins, as a paint and varnish remover and for extraction of essential oils. In the EU, cyclohexane is approved as an extraction solvent in the preparation of flavourings from natural flavouring materials with the maximum residue limit in food of 1 mg/kg (Directive 2009/32/EC). In the United States, cyclohexane is approved for use as a diluent in colour additive mixtures (21 CFR 73.1). It is also used in industrial recrystallization of steroids and in the manufacture of adipic acid (INS No. 355) and caprolactam, which is used in the production of polyamide (nylon) for food packaging materials.

2.2 Impurities and secondary contaminants

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. According to FOSFA, the cyclohexane transported as a previous cargo is unlikely to contain more than 0.1% benzene (EFSA, 2012a). Cyclohexane may contain residues of sulfur and polycyclic aromatic hydrocarbons (PAH) (JECFA specifications).

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 (WHO, 2000; Duarte-Davidson et al., 2001). Estimated quantities of inhaled benzene absorbed by people in the United Kingdom have been estimated to range from 0.71 µg/kg bw per day for rural children to 14.12 µg/kg bw per day for urban adult smokers who work adjacent to busy roads (Duarte-Davidson et al., 2001). Estimates of dietary exposure range from 1.4–2.8 µg/day (WHO, 2000), equivalent to 0.02–0.05 µg/kg bw per day for adults weighing 60 kg. The estimated maximum dietary exposure to benzene if present in fats and oils when cyclohexane is carried as a previous cargo, 0.3 µg/kg bw per day, is below the range of inhalation exposures, although it is high compared to estimated total dietary exposures.

Cyclohexane may also contain PAH residues, 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.

2.3 Reactivity and reactions with fats and oils

Cyclohexane is not expected to react with edible fats and oils (EFSA, 2012a).

2.4 Methods of analysis

Residues of cyclohexane can be analysed by GC-FID or GC-MS. Yousefi & Hosseini (2017) analysed the hexane content in 40 samples of different types of edible vegetable oils using solid phase microextraction-gas chromatography (SPME)-GC-FID. The LOD and the LOQ were 3 µg/kg and 5 µg/kg, respectively.

3. Biological data

3.1 Biochemical aspects

Cyclohexane and methylcyclohexane share a 6-membered saturated aliphatic ring structure and exhibit similar physicochemical properties.[1] Based on the toxicokinetic information presented for rabbits following oral exposure (Elliott, Parke & Williams, 1959; Elliott, Tao & Williams, 1965), both compounds undergo hydroxylation and excretion in the urine as glucuronide conjugates and both compounds are expired as CO_2 or unchanged parent compound. Potentially due to a lower vapour pressure, it is estimated that, compared to cyclohexane, significantly less unchanged methylcyclohexane is excreted in the expired air following ingestion (Elliott, Tao & Williams, 1965). In all species, the amount of cyclohexanone produced following exposure to cyclohexane is limited (ECB, 2004). Based on the physicochemical properties of cyclohexane, crossing of the blood–brain and placental barriers is possible (ECB, 2004).

Female Chinchilla rabbits (four per sex and dose) were administered single oral doses by gavage of 0.3, 100, 330, 360 or 390 mg [^{14}C]-cyclohexane/kg bw. Total residues in tissue at termination of the experiments (3 to 6 days after administration) showed that only 2.5% of the initial dose was present. Unchanged [^{14}C]-cyclohexane in exhaled air accounted for 0, 38 and 24.5% at doses of 0.3, 360 and 390 mg/kg bw, respectively; and exhaled [^{14}C]O_2 accounted for an additional 5.5, 8.5 and 10% of the dose, respectively. Cyclohexanone and cyclohexanol were not detected in exhaled air. Urinary excretion accounted for 98, 59, 33, 41 and 56% of the radioactivity at doses of 0.3, 100, 330, 360 and 390 mg [^{14}C]-cyclohexane/kg bw, respectively. The major urinary metabolites were glucuronic acid conjugates of cyclohexanol and (±) *trans*-cyclohexane-1,2-diol. Faecal excretion of total [^{14}C] was minimal. Total radioactivity accounted for in this study ranged from 93.3 to 96.3% (Elliott, Parke & Williams, 1959).

Methylcyclohexane was excreted mainly via the urine (65%) and expired air (15%) following oral administration of approximately 200 to 245 mg/kg bw methylcyclohexane to rabbits. The main urinary metabolites were glucuronide conjugates of *trans*-4-methylcyclohexanol, *cis*-3-methylcyclohexanol and *trans*-3-methylcyclohexanol, whereas the main components in exhaled air were unchanged methylcyclohexane (~10%) and CO_2 (~5%). The percentage of the

[1] Methylcyclohexane: molecular weight = 98.2 g/mol; water solubility = 14 mg/L at 25 °C; and Log K_{ow} = 3.61; vapour pressure = 5.73 kPa at 25 °C
https://pubchem.ncbi.nlm.nih.gov/compound/Methylcyclohexane
Cyclohexane: molecular weight = 84.16 g/mol, water solubility = 55 mg/L at 25 °C; and Log K_{ow} = 3.44; vapour pressure = 10.3 kPa at 20 °C
https://pubchem.ncbi.nlm.nih.gov/compound/Cyclohexane

radioactivity found in the urine ranged between 54.2 and 77.4%. The pattern of hydroxylation suggested that steric factors influenced metabolism (for example, hydroxylation occurred to the greatest extent at the carbon furthest away from the methyl group). Total radioactivity accounted for in this study ranged between 65.2 and 93.9% (Elliott, Tao & Williams, 1965).

In an unpublished study summarized in an ECHA (2020f) registration dossier, male Fisher 344 rats were administered single oral doses by gavage of 100 (n = 2), 200 (n = 25), 1000 (n = 3) or 2000 mg [^{14}C]-cyclohexane/kg bw (n = 3), and an additional group of three rats received a single intravenous dose of 10 mg [^{14}C]-cyclohexane/kg bw. Following administration of 200 mg/kg bw, peak concentrations of total [^{14}C] were attained by 6–12 hours post-dosing in the whole blood and plasma and were significantly reduced after 72 hours (approximately 0.4% of the administered 200 mg/kg bw dose). Elimination half-lives for total [^{14}C] from plasma and tissues were 10–15 hours. The ratio of total [^{14}C] in adipose tissue and blood ranged from 16:1 (low dose) to 47:1 (high dose). Following oral administration of 100, 200 or 1000 mg/kg, unchanged cyclohexane exhaled during the 72-hour period following dosing accounted for 59.4, 59.8 and 92.1% of the doses respectively. According to the ECHA (2020f) dossier, the high value for cyclohexane after a dose of 1000 mg/kg may have been due to an error in estimating the dose. The urinary metabolite profile was similar for all test groups (both intravenous and oral exposure), with only trace quantities of cyclohexane, cyclohexanone and cyclohexanol being present. Most of the [^{14}C] was present as four unidentified polar metabolites (probably glucuronic acid conjugates of hydroxylated metabolites). A preliminary experiment showed that only minor amounts of [^{14}C] (<0.3% of the dose) were excreted in faeces. Following intravenous exposure to 10 mg/kg bw, 79.5% of the dose was exhaled unchanged during the initial 24 hours with a further 1.27% and 1.43% being exhaled during the 24–48- and 48–72-hour periods, respectively. [^{14}C] exhaled as either cyclohexanone or cyclohexanol only accounted for 0.22% of the dose over the 0–72-hour period.

In a human volunteer study, four men and four women (ages 31–55 years) were exposed to cyclohexane in a closed exposure chamber for 8 hours at a vapour concentration of 1010 mg/m^3 cyclohexane (Mráz et al., 1998). Following exposure, the urine was collected (18 samples per person for 72 hours) and analysed for cyclohexanol, 1,2-cyclohexanediol and 1,4-cyclohexanediol following acidic hydrolysis of the glucuronide conjugates. The amount of each compound determined represents the sum of its conjugated and unconjugated forms in the urine. According to the results reported, 0.5% was excreted in the urine as cyclohexanol, 23.4% as 1,2-cyclohexanediol and 11.3% as 1,4-cyclohexanediol. The authors estimated the elimination half-lives of the 1,2- and 1,4-cyclohexanediol metabolites in humans as 14 to 18 hours.

3.2 Toxicological studies

3.2.1 Acute toxicity

Based on the available information, cyclohexane exhibits low acute oral toxicity with reported LD_{50} values in rats and rabbits being greater than 5000 mg/kg bw (ECB, 2004; Gad, 2014). Clinical symptoms reported following oral exposure to high doses of cyclohexane include diarrhoea/soft faeces, weight loss, increased respiration rate, central nervous system effects (for example, transient depression/narcosis and convulsions) and salivation. Like many other organic solvents, the suspected mechanism of action of central nervous system depression is the disruption of membrane enzymes and the corresponding alterations in cell function (Gad, 2014). Kimura, Ebert & Dodge (1971) suggested that older adult rats are more sensitive to the acute toxicity of cyclohexane administered via the oral route than younger adult rats.

Methylcyclohexane

In rats, mice and rabbits, LD_{50} values of greater than 1200 mg/kg bw have been reported for methylcyclohexane (PubChem[1]). Clinical symptoms associated with acute exposure to high doses are similar to those reported following exposure to high doses of cyclohexane. Based on oral LD_{50} values in rabbits, Treon, Crutchfield & Kitzmiller (1943) suggested that toxicity decreases from ketone (for example, cyclohexanone) to alcohol (for example, cyclohexanol) to hydrocarbon (cyclohexane) and that the methylated compounds (i.e. methylcyclohexane, methylcyclohexanol and methylcyclohexanone) are more hazardous than the corresponding non-methylated compounds (i.e. cyclohexane, cyclohexanol and cyclohexanone). Treon, Crutchfield & Kitzmiller (1943) reported oral LD_{50} values in rabbits of 5500 to 6000 mg/kg bw for cyclohexane versus 1000 to 1250 mg/kg bw for methylcyclohexane.

3.2.2 Short-term and long-term studies of toxicity and carcinogenicity

No short-term or long-term oral toxicity studies on cyclohexane were identified. The following paragraph briefly summarizes the toxicity observed following exposure by the inhalation and intraperitoneal routes.

According to the ECB's (2004) assessment of toxicity of cyclohexane following exposure via inhalation, a NOAEC of 400 ppm (1400 mg/m^3) was identified in rats based on slight neurobehavioural effects observed at 2000 ppm (6880 mg/m^3) following three consecutive acute exposures. Similarly, a NOAEC of 500 ppm (1720 mg/m^3) was identified based on transient sedative effects being observed at 2000 ppm (6880 mg/m^3) in both rats and mice following 90 days of

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

inhalation exposure. Another NOAEC of 435 ppm (1500 mg/m^3) was identified based on an older inhalation study in rabbits showing systemic effects (slight histopathological lesions in the liver and kidney) at 786 ppm (2700 mg/m^3). In one monkey exposed to cyclohexane at a concentration of 1243 ppm for 6 hours/day, 5 days/week, for 10 weeks, no clinical signs of toxicity or histopathological effects were noted (ECB, 2004). Bernard et al. (1989) observed evidence of nephrotoxicity in female Sprague-Dawley 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 probably due to cyclohexanol.

Cyclohexane is a volatile organic compound and limited toxicological information is available on exposure via the oral route. Therefore, critical information from repeated-dose studies on oral toxicity of methylcyclohexane and cyclohexanone is briefly summarized below.

Methylcyclohexane

In a 28-day repeated dose oral toxicity study (OECD 407; GLP), methylcyclohexane (99.8% pure) was administered by gavage to groups of Crj:CD(SD) rats (five per sex and group) at 0, 100, 300 and 1000 mg/kg bw per day. Animals in the recovery group (sacrificed 14 days after termination of treatment) were also included in the control and high-dose groups (five per sex and group). Male and female animals that received 1000 mg/kg bw per day showed transient salivation after dosing. Additionally, males from the high-dose groups showed increased total protein and decreased alkaline phosphatase immediately after treatment and following recovery. Clinical biochemistry changes in females given the highest dose included increased total cholesterol and decreased alanine aminotransferase (not observed following recovery). Following terminal necropsy, males and females from the high-dose group showed increased absolute and relative liver weights (recovery not mentioned), hepatocellular hypertrophy (not observed following recovery) and hyaline droplet formation or degeneration in the kidneys (observed following recovery). Males from the high-dose group also showed increased body weight and food consumption and females showed slightly increased absolute and relative ovary weights (only after a recovery period). 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. Except for slight hyaline droplet degeneration observed in one male from the low-dose group, no other toxicologically relevant effects were noted. A NOAEL of 100 mg/kg bw per day was identified (ECHA, 2020e).

Cyclohexanone

In a pair of guideline-compliant (OECD 453) 2-year studies on rats and mice, cyclohexanone was administered via drinking water. F344 rats (52 per sex and treatment) were given concentrations of 0, 3300 and 6500 ppm (equivalent to ~165 and 325 mg/kg bw per day[1]). B6C3F1 mice (controls both sexes, n = 52; low- and middle dose females, n = 50; middle dose males, n = 52; high dose n = 47 males and n = 41 females) were given cyclohexanone at concentrations of 0, 6500 and 13 000 ppm in males (equivalent to ~585 and 1170 mg/kg bw per day) and 0, 6500, 13 000 and 25 000 ppm in females (equivalent to ~585, 1170 and 2250 mg/kg bw per day[2]). Increased incidences of cancer were observed sporadically in treated rats and mice. In male rats, the incidences of adenoma of the adrenal cortex were 1/52, 7/52 and 1/50 in rats exposed to 0, 3300 and 6500 ppm, respectively. In male mice, the incidences of combined benign and malignant hepatocellular neoplasms were 16/52, 25/51 and 13/46 in mice exposed to 0, 6500 and 13 000 ppm, respectively. In female mice, the incidences of malignant lymphoma were 8/52, 17/52, 4/50 and 0/41 in mice exposed to 0, 6500, 13 000 and 25 000 ppm, respectively. Given the lack of a clear dose-response, the increased incidences of cancer observed in rats and mice were not considered toxicologically significant (Litjinsky & Kovatch, 1986).

3.2.3 Reproductive and developmental toxicity

No relevant studies of the effects of oral exposure to cyclohexane on reproductive or developmental toxicity were identified. However, reproductive and developmental toxicity testing of cyclohexane administered via the inhalation route has been undertaken in rats and rabbits, and there is reproductive and developmental toxicity information available for methylcyclohexane and cyclohexanol administered via the oral route. Kreckmann et al. (2000), the US EPA (2003b) and ECHA (2020f), extensively summarize the results of reproductive and developmental toxicity testing in rats and rabbits following inhalation exposure to cyclohexane. Based on the summaries available in the published literature, the results of these inhalation studies are outlined below.

In a developmental toxicity study in Sprague-Dawley rats (25 per concentration), dams were exposed to cyclohexane by whole-body inhalation for 6 hours per day during GD 6 to 15 at concentrations of 0, 500, 2000 or 7000 ppm (equivalent to 0, 1721, 6886 or 24 101 mg/m^3). Dams were sacrificed on GD

[1] Dose conversion used the default values for chronic drinking water studies in rats proposed by EFSA (i.e. 0.05 mg/kg bw per day per mg/L).

[2] Dose conversion used the default values for chronic drinking water studies in mice proposed by EFSA (i.e. 0.09 mg/kg bw per day per mg/L). (https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2012.2579 accessed 16 December 2020).

21 and fetuses were examined for soft tissue and skeletal abnormalities. Dams exposed to 2000 and 7000 ppm cyclohexane showed maternal toxicity in the form of reduced body weight and reduced response to voice stimuli. Necropsy of the dams and offspring did not reveal any gross lesions and there were no histopathological changes noted in the soft or skeletal tissues of the offspring. No evidence of developmental toxicity was observed at any dose. A NOAEC for maternal toxicity of 500 ppm (1721 mg/m^3) was identified.

Pregnant New Zealand white rabbits (20 per concentration) were exposed via whole body inhalation to 0, 500, 2000 or 7000 ppm (equivalent to 0, 1721, 6886 or 24 101 mg/m^3) of cyclohexane for 6 hours/day on GD 6 to 18. This study revealed a statistically significant decrease in the mean number of corpora lutea of females in the groups treated with 2000 ppm and 7000 ppm. There was also a significant trend towards an increased ratio of male pups to total number of pups in the 2000 ppm and 7000 ppm groups. There were no significant differences in fetal malformation between control and treatment groups. A NOAEC of 500 ppm (1721 mg/m^3) was identified (Kreckmann et al., 2000; ECHA, 2020f).

In a two-generation reproduction study, male and female Crl:CD BR rats (F_0; 30 per sex and concentration) were exposed to cyclohexane (99.9%) by inhalation, through whole-body exposure, at concentrations of 0, 500, 2000 or 7000 ppm (equivalent to 0, 1721, 6886 or 24 101 mg/m^3) for 10 weeks prior to mating for 6 hours/day and 5 days/week. F_0 dams were continuously exposed throughout GD 0–20. Exposure of the F_0 dams paused on GD 21 but resumed on PND 5 and continued until weaning. A selected group of F_1 offspring (30 per sex and group) was also exposed to the same dosing regimen as the F_0 generation beginning at least 11 weeks after weaning and continuing until parturition of the F_2 litters. Clinical signs of toxicity included transient sedation, salivation, stained perioral area and wet chin in F_0 males and females following exposure to 2000 ppm and 7000 ppm. In addition, females exposed to 2000 ppm and 7000 ppm showed a transient diminished or absent response to a sound stimulus during each exposure session. Although no alterations in body weight or food consumption were observed in F_0 males, F_0 females exposed to 7000 ppm exhibited a significant reduction in mean body weight during gestation and lactation. F_0 dams and F_1 parents (males and females) exposed to 7000 ppm showed significant reductions in body weights and corresponding decreases in F_1 and F_2 pups were observed from lactation days 7 to 25. Exposure to cyclohexane did not significantly alter fertility or gestational indices, implantation efficiency or gestational length in either the F_0 or F_1 parents. Although a significant reduction in the percentage of F_1 pups born alive was observed at 7000 ppm, a normal percentage of F_2 pups born alive was observed at this dose. No toxicologically relevant effects were noted during gross necropsy and histopathology in any of the generations. A NOAEC of 2000 ppm (6886 mg/m^3) was identified for developmental effects (decreased body

weight in F_1 and F_2 pups) and a NOAEC of 500 ppm (1721 mg/m^3) was identified for maternal toxicity (decreased body weight and transient diminished or absent response to a sound stimulus). Considering the decreased pup weights in the F_1 and F_2 generations, the US EPA (2003) calculated a benchmark concentration with 95% lower confidence limit ($BMCL_{1SD}$) of 1822.48 mg/m^3 and proposed an inhalation reference concentration (RfC) of 6 mg/m^3 for cyclohexane, using an overall uncertainty factor of 300.

Methylcyclohexane

ECHA (2020e) described the results of a combined repeated dose toxicity study with the Reproduction/Developmental Toxicity Screening Test (OECD 422; GLP) in Crj:CD(SD) rats. According to the summary provided in the ECHA (2020e) dossier, the rats (six males and five females per group) were administered methylcyclohexane (99.9% pure) daily (7 days/week) via oral gavage at doses of 0, 62.5, 250 and 1000 mg/kg bw per day. Satellite control and treatment groups (six males and five females per group) were included for a 14-day recovery period following the cessation of treatment. Treatment started 14 days prior to mating and continued until the end of the 14-day mating period (males) or until day 4 of lactation (females). Reproductive parameters assessed included: estrous cycles, pairing numbers with successful copulation and conceiving days, fertility index, length of gestation, number of corpora lutea, implantation scars and implantation index. The developmental parameters analysed were: number of pups born, stillbirths, sex ratio at birth, birth index, live birth index, number of live pups on day 4 of lactation, sex ratio on day 4 of lactation, viability index, external abnormalities and pup body weight. Treatment appeared to be well tolerated in animals given 62.5 and 250 mg/kg bw per day. However, following administration of 1000 mg/kg bw per day, male and female animals showed transient salivation. Changes in haematology and clinical biochemistry were limited to animals treated with 1000 mg/kg bw per day and consisted of increased reticulocytes and monocytes in females following treatment but not following recovery, and increased alanine aminotransferase and cholesterol in males after treatment and recovery. Following terminal necropsy, males given 1000 mg/kg bw per day showed increased absolute and relative liver and kidney weights (not observed following recovery) and increased relative testes weights (after recovery only). Females given the high dose also showed increased relative liver and kidney weights (absolute liver weights were only measured following recovery) and increased relative and absolute adrenal weights (not observed following recovery). Following histopathological examination, an increased incidence of hyaline droplets in the renal tubules was observed in males dosed with 250 and 1000 mg/kg bw per day following treatment (0/6, 0/6, 4/6 and 6/6 in the 0, 62.5,

250 and 1000 mg/kg bw per day dose groups, respectively; not observed following recovery). No effects were observed in females that received 250 mg/kg bw per day. However, males dosed with 62.5 and 250 mg/kg bw per day also showed a slight increase in cholesterol following treatment (not statistically significant following recovery and within historical control limits reported by Lee et al., 2012). No significant effects on reproduction or development were noted at any dose. Despite being exposed for longer, female rats appeared to be less sensitive to the effects of methylcyclohexane exposure than male rats.

According to the OECD (2014) summary of this study, immunohistochemical examination for α-2u-globulin in the kidneys of rats in the control and high dose (1000 mg/kg bw per day) group, revealed similar levels of α-2u-globulin (positive controls confirmed assay function). Although α-2u-globulin-related nephropathy in male rats is a common effect associated with repeated exposures to hydrocarbon solvents (McKee, Adenuga & Carrillo, 2015), the Committee concluded that the evidence of α-2u-globulin -related effects in rats exposed to methylcyclohexane is incomplete. In the absence of definitive evidence for α-2u-globulin-associated effects, and considering that hyaline droplet accumulation was also observed in female rats given the high dose, the renal effects observed in male rats are considered relevant for the current evaluation. A NOAEL of 62.5 mg/kg bw per day was identified.

3.2.4 Genotoxicity

According to the available information, cyclohexane is not genotoxic in vitro or in vivo. Similarly, methylcyclohexane and cyclohexanol are not genotoxic in vitro. To add to the weight of evidence for the negative in vivo genotoxicity of cyclohexane, it is also noted that cyclohexanone is nongenotoxic in vitro and in vivo, and all four substances lack structural alerts for mutagenicity/genotoxicity. The genotoxicity data that form the basis of these conclusions are summarized in Tables 20 to 23.

3.2.5 Allergenicity

Repeated exposure to cyclohexane did not produce delayed contact hypersensitivity in guinea-pigs assessed by the modified Buehler method (ECHA, 2020f). No data on the allergenic potential of methylcyclohexane were available.

3.2.6 Impurities

Some of the impurities identified in section 2.2 are associated with carcinogenicity (for example, benzene and PAH (IARC, 2018)).

Table 20
Summary of in vitro and in vivo genotoxicity studies on cyclohexane

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537)	0, 10, 33, 100, 333, 1000, 3333, 10 000 µg/plate	Negative (S9+) Negative (S9–)	ECHA (2020f); Mortelmans et al. (1986)
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537 and TA1538)	7 graded doses 7.1 to 5200 µg/plate	Negative (not specified if with or without S9)	US EPA (2003)
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537)	Up to 1000 µg/plate	Negative (S9+) Negative (S9–)	McCann et al. (1975)
Gene mutation in *Salmonella* Typhimurium TA100	25, 50, 100, 200, 500 µL/plate	Negative	Maron, Katzenellenbogen & Ames (1981)
Gene mutation in mouse lymphoma cells	Up to 100 µg/mL	Negative (S9+) Negative (S9–)	US EPA (2003)
Gene mutation in mouse lymphoma cells (*L5178Y*)	313, 625, 1250, 2500, 3000, 4000, 5000, 6000, 7000 and 8000 nL/mL	Negative (S9+) Negative (S9–)	ECHA (2020f)
Sister chromatid exchange test in Chinese hamster ovary cells	Up to 25 µg/mL	Negative (S9+) Negative (S9–)	US EPA (2003)
Unscheduled DNA synthesis in human lymphocytes	0, 0.01, 0.001 and 0.0001 mol/L	Negative (S9+) Negative (S9–)	ECHA (2020f); Perocco, Bolognesi & Alberghini (1983)
DNA binding assay in *Escherichia coli* Q13	10 and 100 µM	Equivocal	Kubinski, Gutzke & Kubinski (1981)
Studies based on in vivo systems			
Bone marrow chromosome aberration test in male and female CRL:COBS CD(SD) BR rats	0, 100, 300, 1000 ppm via inhalation route for 5 days (6 hours per day)	Negative	ECHA (2020f)

Table 21
Summary of in vitro and in vivo genotoxicity studies on methylcyclohexane

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537)	0.0977 to 25 µg/plate (S9–) 0.781 to 200 µg/plate (S9+)	Negative (S9–) Negative (S9+)	ECHA (2020e)
Gene mutation in *Escherichia coli* WP2 uvrA	0.0977 to 25 µg/plate (S9–) 0.781 to 200 µg/plate (S9+)	Negative (S9–) Negative (S9+)	ECHA (2020e)
Chromosome aberration test in Chinese hamster lung (CHL/IU) cells	0, 245, 490 and 980 µg/L	Negative after short-term treatment (6 hours) (S9– and S9+) Negative after continuous treatment (24 and 48 hours) (S9–)	ECHA (2020e)

Table 22
Summary of in vitro and in vivo genotoxicity studies on cyclohexanol

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA1535, TA1537 and TA1538)	0, 500, 1000, 2500, 5000, 7500, 10 000, 15 000 µg/plate	Negative (S9+) Negative (S9–)	ECHA (2020g)
Gene mutation in mouse lymphoma cells (*L5178Y*)	0, 62.5, 125, 250, 500, 1000 µg/mL	Negative (S9+) Negative (S9–)	ECHA (2020g)
Studies based on in vivo systems			
Bone marrow chromosome aberration test in male and female CRL:COBS CD(SD) BR rats	0, 500, 1000, 1500 mg/kg bw via gavage (single dose)	Negative at all sacrifice intervals (16, 24, 48 hours)	ECHA (2020g)

Table 23
Summary of in vitro and in vivo genotoxicity studies on cyclohexanone

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537)	At least five concentrations tested (not specified); highest concentration was 10 mg/plate in absence of toxicity	Negative (S9–) Negative (S9+)	ECHA (2020h); Haworth et al. (1983)
Gene mutation in *Salmonella* Typhimurium (TA98 and TA100)	0 and 3 µmol/plate	Negative (S9–) Negative (S9+)	ECHA (2020h); Florin et al. (1980)
Gene mutation in *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537)	10 to 5000 µg/plate (standard plate test) 10 to 1000 µg/plate (pre-incubation test)	Negative (S9–) Negative (S9+)	ECHA (2020h)
Gene mutation in *Escherichia coli* WP2 uvrA	10 to 5000 µg/plate (standard plate test) 10 to 1000 µg/plate (pre-incubation test)	Negative (S9–) Negative (S9+)	ECHA (2020h)
Unscheduled DNA synthesis in human fibroblasts	Up to 9.48 mg/mL	Negative (S9–) Negative (S9+)	ECHA (2020h)
Gene mutation in Chinese hamster ovary cells	Experiment 1: 0, 122.5, 245, 490, 980 µg/mL (4-hour exposure with and without S9) Experiment 2: 0, 61.3, 122.5, 245, 490, 980 µg/mL (24-hour exposure without S9) and 0, 100, 200, 400, 980 µg/mL (4-hour exposure with S9)	Negative in both experiments (S9–) Negative in both experiments (S9+)	ECHA (2020h)
Comet assay in human epidermal skin model	Up to 1600 µg/cm^2	Negative	ECHA (2020h); Reus et al. (2013)
Gene mutation in mouse lymphoma cells (*L5178Y*)	0, 312.5, 625, 1250, 2500, 500 µg/mL	Negative (S9–) Negative (S9+)	ECHA (2020h)
[^{3}H]TdR uptake study in human lymphocytes	0, 0.01, 0.001, 0.0001 mol/L	Negative (S9–) Negative (S9+)	ECHA (2020h); Perocco, Bolognesi & Alberghini (1983)
HPRT assay in Chinese hamster ovary cells	2.5 to 12.5 µL/mL	Negative (S9–) Negative (S9+)	ECHA (2020h)

Table 23 (continued)

Test system and testing conditions	Treatment	Result	Reference
Studies based on in vivo systems			
Bone marrow chromosome aberration test in male and female CD rats	0, 50, 400 ppm via inhalation route for 1 or 5 days (7 hours per day)	Negative	ECHA (2020h)
Rodent dominant lethal test in male and female CD rats	0, 50, 400 ppm via inhalation route for 1 or 5 days (7 hours per day)	Negative	ECHA (2020h)
Sex-linked recessive lethal test in male and female *Drosophila melanogaster*	0 or 36% saturation (~1900 ppm) via inhalation route for 1 day (4 hours per day)	Negative	ECHA (2020h)
Sex-linked recessive lethal test in male and female *Drosophila melanogaster*	50 ppm via inhalation route for 1 day (1, 3 or 6.75 hours per day) 400 ppm via inhalation route for 1 day (10, 20 or 70 minutes per day)	Negative	ECHA (2020h)

3.3 Observations in humans

Very limited information on exposure to cyclohexane via the oral route is available. According to a brief entry in the *Encyclopedia of toxicology* (Gad, 2014), acute ingestion of cyclohexane may cause sore throat, nausea, diarrhoea or vomiting, whereas prolonged exposure may produce liver and kidney damage. The basis for this statement was not provided. However, owing to its use in the occupational environment, the effects of cyclohexane following inhalation exposure in humans have been studied.

In the ECB (2004) assessment, the results of a double-blind, two-way cross-over study in human volunteers (summary results later published in Hissink et al., 2009 and Lammers et al., 2009) were used to identify an inhalation NOAEC of 250 ppm (860 mg/m^3) cyclohexane for neurobehavioural effects in humans. Twelve male volunteers (ages ranging from 20 to 40 years; weight 66 to 95 kg) were exposed to 0, 25 or 250 ppm (equivalent to 0, 86 or 860 mg/m^3) cyclohexane for 4 hours in a specially constructed temperature and relative humidity-controlled exposure room. Automated neurobehavioural tests and questionnaires were administered prior to exposure, during, and approximately 60 minutes after the exposure. Mean blood concentrations of cyclohexane, sampled near the end of the exposure period, were 55 and 618 ng/ml, respectively, for the 25 and 250 ppm dose groups. No adverse neurobehavioural effects were reported; however, headaches and eye and throat irritation were reported more frequently after exposure to 250 ppm (860 mg/m^3) cyclohexane.

Yasugi et al. (1994) investigated the effects of cyclohexane exposure in female workers (n = 33 exposed; mean age ~25 years) who were in close proximity to an automated spraying system used for glue application. The glues used in this factory were reported to contain at least 75% cyclohexane and up

to 15.8% toluene. According to Yasugi et al. (1994) the workers were exposed to mean concentrations of approximately 27 ppm cyclohexane (maximum concentration 274 ppm). Cyclohexane exposure was correlated with "end of shift" (~8 hours) cyclohexanol and cyclohexanone concentrations in the workers' blood and urine. The workers did not report any significant subjective symptoms apart from "dimmed vision" and "unusual smell". Haematology appeared normal; serum biochemistry was within the normal limits for liver and kidney function; and no significant increases in sister chromatid exchange were observed. Yuasa et al. (1996) reported that following 1.2 years of exposure to cyclohexane, no adverse effects on the peripheral nervous system were evident in 18 women (ages 19 to 56 years) exposed to glue containing 75% cyclohexane, 12% toluene and 0.9% *n*-hexane. Twelve of these women were reported to have been exposed to *n*-hexane for a median of 2.8 years previously.

4. Occurrence and exposure

Cyclohexane may be used as an extraction solvent in the preparation of flavourings 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 A4.3).

5. Comments

5.1 Chemical and technical considerations

The chemical and technical considerations for cyclohexane are summarized in Table 24.

Table 24
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.

5.2 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 (Elliott, Parke & Williams, 1959). Cyclohexane administered orally to rats is eliminated from the plasma and tissues with a measured elimination half-life of 10–15 hours (ECHA, 2020f). 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 (Elliott, Tao & Williams, 1965). However, due to its lower volatility, it is estimated that significantly less unchanged methylcyclohexane is excreted in the expired air following ingestion compared to cyclohexane (Elliott, Tao & Williams, 1965)

and greater systemic exposure to methylcyclohexane may be expected compared to cyclohexane. Both cyclohexane and methylcyclohexane have a high affinity for adipose tissues.

5.3 Toxicological studies

Cyclohexane exhibits low acute oral toxicity in rodents, with an estimated LD_{50} of > 5000 mg/kg bw (ECB, 2004). Methylcyclohexane exhibits a slightly higher acute oral toxicity with reported oral LD_{50} values of ≥1200 mg/kg bw (PubChem[1]). Based on oral LD_{50} values in rabbits Treon, Crutchfield & Kitzmiller (1943) suggested that toxicity decreases from ketone (for example, cyclohexanone) to alcohol (for example, cyclohexanol) to hydrocarbon (cyclohexane) and that the methylated compounds (i.e. methylcyclohexane, methylcyclohexanol 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 (US EPA, 2003). Bernard et al. (1989) 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; GLP) methylcyclohexane was administered via gavage to groups of rats at doses of 0, 100, 300 and 1000 mg/kg bw per day (ECHA, 2020e). At 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; GLP), rats were administered methylcyclohexane daily via oral gavage at doses of 0, 62.5, 250 and 1000 mg/kg bw per day (ECHA, 2020e). Once again, male and female animals exposed to

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

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 (2014) summary of this study, 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 (McKee, Adenuga & Carrillo, 2015), 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.

5.4 Allergenicity

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

5.5 Impurities

Cyclohexane transported as a previous cargo may contain benzene, but is unlikely to contain more than 0.1% benzene (EFSA, 2012a). 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 (WHO, 2000; Duarte-Davidson et al., 2001). 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 (Duarte-Davidson et al., 2001). Estimates of dietary exposure range from 1.4 to 2.8 μg/day (WHO, 2000), 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 PAH residues, 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.

5.6 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 A4.3).

6. 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 an MOE of 208. The Committee noted that this MOE is based on a potentially more toxic compound (Treon, Crutchfield & Kitzmiller, 1943) 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 have been clarified.

7. 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.

8. References

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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 antioxidant, 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.

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ANNEX 2

Abbreviations and acronyms used in the monographs

24HDR	24-hour dietary recall
ADI	acceptable daily intake
bw	body weight
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CCFO	Codex Committee on Fats and Oils
CIFOCOss	Chronic Individual Food Consumption Database – Summary statistics
CHO	Chinese hamster ovary
C_{max}	maximum concentration
CONTAM	European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain
CPSC	Consumer Product Safety Commission
DMBA	7,12-dimethybenz[a]anthracene
EAT	ergotamine tartrate
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
ELSO	epoxidized linseed oil
EPA	(United States) Environmental Protection Agency
ESBO	epoxidized soybean oil
ETBE	ethyl tertiary butyl ether
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GC-FID	gas chromatography with flame ionization detection
GC-MS	gas chromatography–mass spectrometry
FCID	Food Commodity Intake Database (US Environmental Protection Agency)
FFQ	food frequency questionnaire
GEMS/Food	Global Environment Monitoring System, Food Contamination Monitoring and Assessment Programme
GF-AAS	graphite furnace atomic absorption spectrometry
HBGV	health-based guidance value
HPLC	high-performance liquid chromatography
IMO	International Maritime Organization

JECFA	Joint FAO/WHO Expert Committee on Food Additives
LB	lower bound
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
LD_{50}	median lethal dose
LOD	limit of detection
LOQ	limit of quantification
LOR	limits of reporting
MTBE	methyl tertiary butyl ether
MOAH	mineral oil aromatic hydrocarbons
MOE	margin of exposure
MOH	mineral oil hydrocarbons
MOSH	mineral oil saturated hydrocarbons
MTDI	maximum tolerable daily intake
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
P95	95th percentile
PBTK	physiologically based toxicokinetic
PCB	polychlorinated biphenyl
PTMI	provisional tolerable monthly intake
QSAR	quantitative structure-activity relationship
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
TLC	thin layer chromatography
T_{max}	time to maximum concentration
TRS	Technical Report Series
UB	upper bound
UL	upper intake level
USA	United States of America
WHO	World Health Organization

ANNEX 3

Participants in the ninetieth meeting of the Joint FAO/ WHO Expert Committee on Food Additives

Virtual meeting 1 to 12 February 2021

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This volume contains monographs prepared at the ninety-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met virtually online from 1 to 12 February 2021.

The detailed monographs in this volume summarize data on specific contaminants in food. Individual monographs present the assessment of exposure to cadmium from all food sources, the technical, analytical, dietary exposure and toxicological data on ergot alkaloids, an assessment of five substances that may occur as previous cargoes, and a revision of the specifications for steviol glycosides.

This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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