numerous incidents of mycotoxicosis in farm animals, especially in pigs. It is found worldwide in a number of cereal crops. Zearalenone has not been evaluated previously by the Committee. However, a mammalian metabolite of zearalenone, α-zearalanol (zeranol), was considered by the Committee at its twenty-sixth, twenty-seventh and thirty-second meetings (Annex 1, references *59, 62* and *80*) for use as a veterinary drug; at the latter meeting, the Committee allocated an ADI of 0–0.5μg/kg of body weight.

6.3.1 *Intake*

The average dietary intakes of zearalenone from cereals and legumes in two of the five GEMS/Food regional diets were estimated to be 1.5μg/day in the European diet and 3.5μg/day in the Middle Eastern diet. If a mean body mass of 60kg is assumed, these intakes correspond to 0.03 and 0.06μg/kg of body weight per day, respectively. The average dietary intakes of zearalenone estimated from individual dietary records are 0.98μg/day (0.02μg/kg of body weight per day) in Canada, 1.2μg/day (0.02μg/kg of body weight per day) in Denmark, 1.1μg/day (0.02μg/kg of body weight per day) in Norway and 2.1μg/day (0.03μg/kg of body weight per day) in the USA.

The theoretical maximum daily intake of α-zearalanol when used as a veterinary drug was calculated to be 1.6μg/day (0.02μg/kg of body weight per day) on the basis of the recommended maximum residue limits of 10μg/kg in cattle liver and 2μg/kg in cattle muscle (Annex 1, reference *80*).

6.3.2 *Pharmacokinetic data*

Studies of the pharmacokinetics and metabolism of zearalenone indicate that it is extensively metabolized by intestinal tissue in pigs (and possibly in humans) during its absorption, with the formation of α- and β-zearalenol and α- and β-zearalanol, which are subsequently conjugated with glucuronic acid. The existence of this pathway limits the value of studies conducted using parenteral administration for assessing the risk associated with dietary intake. Biliary excretion with enterohepatic circulation occurs in rats and mice, while urinary excretion predominates in rabbits. Urinary excretion is also the main route of elimination in pigs in spite of the demonstrated enterohepatic circulation of zearalenone, owing to a high degree of reabsorption in the gut. The very limited data in humans (one individual) suggest that urinary excretion is also significant. Differences between species in the metabolism of zearalenone were found: a higher proportion of the administered zearalenone was metabolized to α-zearalenol in pigs than in rats or cattle. In the one human subject, as in pigs, zearalenone

was found mainly in urine as glucuronide conjugates of the parent compound and α-zearalenol.

6.3.3 *Toxicity data*

Zearalenone has little toxicity after administration as single oral or intraperitoneal doses. In studies in which the drug was administered orally for up to 90 days, the effects appeared to be dependent on the estrogenic activity of zearalenone and/or its metabolites. Pigs and sheep were more sensitive than rodents; in controlled studies with well-defined exposure to multiple doses, the NOEL in pigs was 40μg/kg of body weight per day on the basis of estrogenic effects in responsive tissues and reproductive performance, while the NOEL in rats was 3mg/kg of body weight per day.

Zearalenone has been tested for genotoxicity in a variety of test systems covering several end-points, including point mutations, unscheduled DNA synthesis and chromosomal aberrations. The results were negative, except for the induction of chromosomal aberrations after exposure of mammalian cells in vitro to very high concentrations of zearalenone. Evidence that zearalenone modifies DNA was obtained with a [^{32}P]-postlabelling assay. However, the Committee concluded that these results do not unequivocally demonstrate covalent binding of zearalenone and/or its metabolites to DNA but probably reflect oxidative damage to DNA, since the DNA damage was greatly reduced by coadministration of the antioxidant α-tocopherol.

Hepatocellular adenomas and pituitary tumours were observed in a long-term toxicity and carcinogenicity study in mice given zearalenone at 8–9mg/kg of body weight per day, which is greatly in excess of the concentration that has hormonal effects. The Committee concluded that these tumours were a consequence of the estrogenic effects of zearalenone. A similar conclusion was drawn by the Committee in evaluating α-zearalanol at its thirty-second meeting (Annex 1, reference *80*). No treatment-related increase in the incidence of tumours was seen in rats given zearalenone at 1–3mg/kg of body weight per day.

6.3.4 *Conclusions*

The Committee concluded that the safety of zearalenone could be evaluated on the basis of the dose that had no hormonal effect in pigs, the most sensitive species. Using a safety factor of approximately 100, the Committee established a provisional maximum tolerable daily intake (PMTDI) for zearalenone of 0.5μg/kg of body weight. This decision was based on the NOEL of 40μg/kg of body weight per day in a 15-day study in pigs. The Committee also took into account the

lowest-observed-effect level of 200μg/kg of body weight per day in this study and the previously established ADI of 0–0.5μg/kg of body weight for the metabolite α-zearalanol, evaluated as a veterinary drug. The Committee recommended that the total intake of zearalenone and its metabolites (including α-zearalanol) should not exceed this value.

A toxicological monograph was prepared.

7. Intake assessments of specific food additives

In response to a request by the Codex Committee on Food Additives and Contaminants at its Thirtieth Session (*14*), the Expert Committee assessed the intake of four food additives. The Expert Committee's conclusions are summarized in Annex 2.

7.1 Annatto extracts

Annatto extracts, which are food additives used to impart a yellow colour to food, were previously evaluated by the Committee at its twenty-sixth meeting (Annex 1, reference *59*), when it allocated an ADI of 0–0.065 mg/kg of body weight, expressed as bixin (the primary chemical colouring agent). At its present meeting, the Committee assessed the intake of annatto extracts, although national authorizations are also generally expressed in terms of bixin. Maximum limits have been proposed for use in a wide range of solid foods in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants.

Seven Member States provided information on the intake of annatto extracts: Australia, Brazil, Canada, France, the Netherlands, the United Kingdom and the USA. The assessments were conducted on the basis of a variety of assumptions about the potential concentrations of annatto extracts and for various patterns of consumption. The Expert Committee concluded that the intake of annatto extracts would exceed the ADI for bixin if all foods contained annatto extracts at the maximum levels proposed in the draft General Standard for Food Additives. However, intake assessments based on national permitted levels would not exceed the ADI for most population groups.

Since estimates of intake based on the assumption that all foods in a specific category are coloured by the same additive at the maximum level are overestimates, the Expert Committee recognized that the ADI for bixin is unlikely to be exceeded as a result of the use of annatto extracts. Information from Brazil indicated, however, that

about 44 million people (28% of the population) consume annatto seeds directly as a condiment and have done so for many years, at a level of consumption that is approximately 150% of the ADI.

Although the results of studies in humans normally take precedence over those in experimental animals, the submitted reports were of only limited value. In order to ensure that all of the relevant data on annatto extracts have been reviewed, the Expert Committee recommended their re-evaluation in 2001. The Expert Committee also recommended that populations that have a high intake of annatto extracts continue to be monitored.

7.2 Canthaxanthin

Canthaxanthin, a food additive used to colour foods both directly and also indirectly through its use in animal feeds, was previously evaluated by the Committee at its tenth, eighteenth, thirty-first, thirty-fifth and forty-fourth meetings (Annex 1, references *13*, *35*, *77*, *88* and *116*). The Committee established an ADI of 0–25 mg/kg of body weight at its eighteenth meeting, which it changed to a temporary ADI of 0–0.05 mg/kg of body weight at its thirty-first meeting. This temporary ADI was not extended by the Committee at its thirty-fifth meeting. At its forty-fourth meeting, the Committee established an ADI of 0–0.03 mg/kg of body weight. At its present meeting, the Committee assessed the intake of canthaxanthin. Maximum limits have been proposed for its use in a variety of solid foods and beverages in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants.

Five Member States provided information on the intake of canthaxanthin: Australia, France, New Zealand, the United Kingdom and the USA. A joint assessment was submitted by Australia and New Zealand. Information was also provided by a manufacturer of canthaxanthin. The intake assessments were based on "poundage" data, model diets and individual dietary records.

The Expert Committee noted that estimates of intake based on the assumption that all foods contain canthaxanthin at the maximum levels proposed in the draft General Standard for Food Additives greatly exceed the ADI, as the range of foods in which its use is proposed is much broader than in those countries in which canthaxanthin is used. Intake assessed on the basis of national permitted levels did not exceed the ADI. The data submitted by the manufacturer indicated that indirect exposure through the use of canthaxanthin as a colourant in animal feeds is the major source of canthaxanthin in food.

The Expert Committee concluded that long-term intake of canthaxanthin is unlikely to exceed the ADI.

7.3 Erythrosine

Erythrosine, a food additive used to impart a red colour to food, was previously evaluated by the Committee at its thirty-seventh meeting (Annex 1, reference *94*), when it established an ADI of 0–0.1 mg/kg of body weight. At its present meeting, the Committee assessed the intake of erythrosine. Maximum limits have been proposed for its use in a wide range of solid foods and non-alcoholic and alcoholic beverages in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants.

Information on the intake of erythrosine was received from seven Member States: Australia, Brazil, Canada, Japan, New Zealand, the United Kingdom and the USA. All of the national estimates of erythrosine intake were below the ADI. In assessing the risk of exceeding the ADI, the Expert Committee noted that non-food sources of erythrosine, such as pharmaceutical products, should also be considered as they may make a significant contribution to the total intake if consumed over a long period. The Expert Committee also noted that the ADI could be exceeded if the maximum limits proposed in the draft General Standard for Food Additives are widely adopted at the national level. However, models based on the maximum limits proposed in the draft General Standard give overestimates of actual intake, because erythrosine would be used in only a limited number of foods. The Expert Committee therefore concluded that it is unlikely that long-term intake of erythrosine would exceed the ADI.

7.4 Iron oxides

Iron oxides were previously evaluated by the Committee at its eighteenth, twenty-second and twenty-third meetings (Annex 1, references *35*, *47* and *50*); at the latter meeting, the Committee established an ADI of 0–0.5 mg/kg of body weight. At its present meeting, the Committee assessed the intake of iron oxides.

Iron oxides are permitted for use in foods in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants, their use being limited only by good manufacturing practice. At its present meeting, the Expert Committee assessed national estimates of intake of iron oxides used as additives for colouring food. Use of iron oxides is permitted in most countries. Data were submitted by four Member States: Australia, Canada, the United Kingdom and the USA.

The current use of iron oxides as a food colour is limited, and the estimated intakes based on national permitted levels do not exceed the ADI. The Committee therefore concluded that it is unlikely that intake of iron oxides would exceed the ADI.

8. Specifications for certain food additives

A total of 36 substances were examined for specifications only (see Annex 2). New specifications were prepared for four substances and the existing specifications for 29 were revised. The existing specifications for two substances were maintained and the specifications for one were deleted.

New specifications were prepared for argon, helium and oxygen gas.

No information was received on actual uses of calcium hydrogen sulfite in food, and all existing data indicated that the substance is not used as a food additive. The Committee therefore decided to withdraw the existing specifications.

The information on the level of selenium in potassium metabisulfite, potassium sulfite, sodium hydrogen sulfite, sodium metabisulfite, sodium sulfite and sodium thiosulfate that had been requested at the fifty-first meeting (Annex 1, reference *137*) was received. The existing tentative specifications were revised and the "tentative" designation was removed.

The existing tentative specifications for ferrous sulfate were revised in the light of new information about the presence of mercury as a contaminant and the "tentative" designation was deleted. The Committee was informed of the existence of a dried product of ferrous sulfate and prepared new specifications for "ferrous sulfate, dried".

The existing tentative specifications for citric acid were revised on the basis of new data on the need for a test for oxalate and a suitable limit for this impurity and the "tentative" designation was deleted.

The existing tentative specifications for ferrous gluconate were revised and the "tentative" designation was deleted. The limits for mercury and oxalate were considered unnecessary and were therefore deleted.

The existing tentative specifications for magnesium gluconate were revised and the "tentative" designation was deleted. Microbiological criteria were considered unnecessary, and the requirement was therefore deleted.

The existing tentative specifications for thaumatin were revised. As the specific identification test that had been requested at the fifty-first

meeting (Annex 1, reference *137*) had been submitted, this test was included and the "tentative" designation was deleted.

The Committee revised the specifications for two enzyme preparations produced from genetically modified organisms: α-acetolactate decarboxylase from *Bacillus brevis* expressed in *B. subtilis* and maltogenic amylase from *B. stearothermophilus* expressed in *B. subtilis.* The source descriptions of the specifications were amended in accordance with the Committee's decision on the citation of microbial strains (see section 2.6.3).

The existing tentative specifications for five other enzyme preparations prepared from genetically modified microorganisms (α-amylase from *B. stearothermophilus* expressed in *B. subtilis*, α-amylase from *B. megaterium* expressed in *B. subtilis*, chymosin A from *Escherichia coli* K-12 containing the prochymosin A gene, chymosin B from *Aspergillus niger* var. *awamori* containing the prochymosin B gene and chymosin B from *Kluyveromyces lactis* containing the prochymosin B gene) were also revised to align the descriptions of the source strains with Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*; see section 2.6.3). The "tentative" designation was deleted since the Committee deleted the "tentative" designation in Appendix B to Annex 1 at its fifty-first meeting (Annex 1, reference *137*).

The existing specifications for carob bean gum were revised. The methods for microbiological criteria were changed in the light of comments that the existing procedures were inadequate.

The existing specifications for carotenes (algae) and carotenes (vegetable) were reviewed in response to a request to consider whether a limit for residual ethanol was required (see section 2.6.1). The Committee decided to maintain the existing specifications.

The existing specifications for xanthan gum were revised to include a limit for residual ethanol.

The existing specifications for sucrose esters of fatty acids were revised, with minor changes.

The existing specifications for guar gum were revised to reflect the use of ethanol and isopropanol in the manufacturing process, and the methods for meeting microbiological criteria were changed in the light of comments that the existing procedures were inadequate.

The existing specifications for nitrogen were revised to include improved methods for determining oxygen, carbon monoxide and nitrogen.

The existing specifications for riboflavin derived from *B. subtilis* were revised to reflect the existence of new products on the market.

The existing specifications for adipic acid, fumaric acid, DL-malic acid, DL-tartaric acid and L-tartaric acid were revised to include uses other than as flavouring agents.

9. Future work

1. The Committee considered that Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*) should be reviewed and revised at a future meeting. Annex 1 requires updating in the light of technological developments and to ensure consistency and coherence with the appendices, including Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms).
2. The Committee noted that the data available on some of the flavouring agents were inadequate to allow the appropriate specifications to be established. The Committee considered that this could have implications for the safety evaluation of these substances, which should be clarified at a future meeting.

10. Recommendations

1. In view of the large number of food additives, food ingredients and contaminants requiring evaluation or re-evaluation, the important role that the recommendations of the Committee play in the development of international food standards and of regulations in many countries, and the need for maintaining consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.
2. The usefulness of the Committee's recommendations depends heavily on its understanding of the needs of those who request and will use its recommendations. The Committee found that a lack of clear understanding of the task to be performed hindered its work in some instances. The Committee therefore recommended that the Codex Alimentarius Commission and other bodies that seek advice ensure that their requests are clearly formulated and are placed in the appropriate context.

3. Assessments of dietary intake are an important component of the evaluations performed by the Committee of the risk posed by food additives and contaminants. As the necessary expertise and capacity to perform such assessments has not yet been developed in many countries, the Committee recommended that FAO and WHO assist countries and regions to develop national capacity and expertise in conducting food consumption surveys and in determining the concentrations of food additives and contaminants in food products.
4. In view of the international importance of its evaluations, the Committee recommended that FAO and WHO take steps to improve communication with all parties interested in its work. Improvement in communication will allow for more timely responses to calls for data and dissemination of its reports and evaluations. The suggested actions include:
   - publication of reports and evaluations in searchable CD-ROM format;
   - development of a searchable electronic database of the work of organizations interested in the Committee's work, including Member States, commercial enterprises, trade organizations and consumer groups; and
   - continued use of the FAO and WHO web sites (www.fao.org and www.who.int) as a means of disseminating current information in a timely fashion. The existence of the two sites should be publicized more widely.

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