codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Via delle Terme di Caracalla 00100 ROME Tel.: 39.06.57051 Telex: 625852-625853 FAO I E-mail: Codex@fao.org Facsimile: 39.06.5705.4593

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POSITION PAPER ON ZEARALENONE

Background

1. The 31st Session of the Codex Committee on Food Additives and Contaminants (CCFAC) requested Norway to finalize the position paper for consideration at its next session (ALINORM 99/12A, para. 112).

Introduction

2. Zearalenone is an important mycotoxin in temperate and warm regions of the world. It is produced by fungi of the genus *Fusarium*. The toxin occurs in maize and small grains like barley, wheat, sorghum, millet and rice, but has also been found in soybeans. In maize the toxin is common, but very high levels (11-15 mg/kg) has also been detected in barley samples from Japan (19).

3. In addition, the toxin has been detected in cereal products like flour, malt, beer, soybeans and products thereof (1, 3, 4). While samples of African beer have been found to contain high levels of zearalenone, very few samples of beer from Europe and Canada contained a low level of the mycotoxin (1, 2, 18). Occurrences in mixed feeds associated with hyperoestrogenism and other problems in swine and cattle in various countries have been reported.

4. Fusarium taxonomy is a complex matter and classification a difficult task. Because of this complexity, many isolates have been misidentified. Many earlier reports have been reviewed and most errors corrected. Zearalenone is now considered being produced by *F. graminearum, F. culmorum, F. cerealis, F. equiseti*, and *F. semitectum.* Reports of production of zearalenone by other species have been questioned (5, 6, 7).

5. Fungi of the genus *Fusarium* infect cereals in the field. Toxin production mainly takes place before harvesting, but may also occur post harvest if the crop is not handled and dried properly.

6. Zearalenone is a resorcyclic acid lactone chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)- β -resorcyclic acid lactone. In mammals, the keto group at C-6 is reduced to two stereoisomeric metabolites of zearalenone (α and β isomers). These metabolites are also produced by the fungi, but at much lower concentrations than for zearalenone. Another compound with structural similarity is zearalanol, which is used as a growth promoter. This compound is only distinguished from zearalenone by its lack of a C1-C2 double bond and a hydroxy group at C6 instead of a keto group (3, 4).

7. Various analytical methods for the identification and quantification of zearalenone have been developed. Early methods were generally based on thin-layer chromatography (TLC). Today, methods using HPLC with fluorescence detection are most common, although UV and electrochemical detection are also used (3, 7, 8). ELISA kits for the determination of zearalenone are also available.

Toxicological Evaluations

8. The International Agency for Research on Cancer (IARC) evaluated the carcinogenic potential of zearalenone and concluded that there was limited evidence of carcinogenicity of zearalenone in experimental animals and that zearalenone was not classifiable according to their human carcinogenicity criteria (Group 3) (4). In a 2 year US NTP-study, a statistical significant increase in hepatocellular adenomas was seen in female mice. Furthermore, a statistically significant increase in pituitary adenomas and a small non-statistically significant increase in pituitary carcinomas, were found in mice, but not in rats (9). An increase in the incidence of pituitary adenomas in rats would, however, be difficult to detect due to a high natural incidence in this species. No carcinogenic effects were found in another 2 year study with rats (10). Subsequently, formation of DNA-adducts has been found in mice, but not in rats, after a single exposure to zearalenone (11). Evidence of DNA-damage has also been found using the ^{32}P - postlabelling method (12). This corresponds well with the results from the two-year US NTP study showing a carcinogenic effect of zearalenone in mice, but not in rats. Altogether, this indicates that zearalenone may have a species-dependent carcinogenic effect possibly secondary to the hormonal effect. No information about formation of DNA-adducts in humans is available.

9. An extensive review of the occurrence and toxicity of zearalenone and risk assessment was made in Canada in 1987 (3). The risk assessment concluded that oestrogenic and possible carcinogenic effects are the critical effects of zearalenone. Deriving a TDI from the possible carcinogenic effect using a mathematical linear extrapolation with a risk level of $1:10^{-6}$ would lead to a virtually safe dose of 0.05 µg/kg b.w. per day. In a study exposing monkeys to zearalanol, which has a higher oestrogenic activity than zearalenone, a
no-hormone-effect level of 50 µg/kg b.w per day was found. Deriving a TDI from this study, using a safety factor of 500 due to the uncertainties in the animal model, lead to an estimated safe intake of 0.10 µg/kg b.w per day. After an overall evaluation, a temporary TDI of 0.1 µg/kg b.w. per day was proposed, based on an estimated no-hormonal-effect level and a virtually safe dose with respect to carcinogenicity estimated with a conservative model with a risk level of $1:10^{-6}$.

10. A Nordic expert group considered in 1998 the Canadian TDI as still valid since no relevant additional *in vivo* information on the dose-effect relations of hormonal effects and no more data concerning the possible carcinogenic effect of zearalenone were available (7).

11. Zearalenone or the similar growth promotor zearalanol was suspected to be the causative agent in an epidemic of precocious pubertal changes in young children in Puerto Rico (13, 14). Zearalenone or metabolites were detected in blood plasma. The authors reported high levels of the growth promotor zearalanol in locally produced meat, but later studies by FDA failed to detect any oestrogenic growth promotor. Natural sources of oestrogen-acting compounds, like plant metabolites or mycotoxins as a cause of the epidemic have not been ruled out.

12. Zearalenone has recently been evaluated by JECFA, but no final report is yet available. The committee observed that the test for genotoxicity in a variety of test systems covering several endpoints, including point mutations, unscheduled DNA synthesis and chromosomal aberrations were negative, except for the induction of chromosomal aberrations after exposure of mammalian cells *in vitro* to very high concentrations. In a ^{32}P-postlabelling assay, evidence for DNA modification by zearalenone was reported. However, the Committee concluded that these results do not unequivocally demonstrate covalent binding to DNA by zearalenone and/or its metabolites and most likely reflect oxidative damage to DNA, since the DNA damage was greatly reduced by co-administration of the antioxidant α-tocopherol. Hepatocellular adenomas and pituitary tumours observed in long-term studies of carcinogenicity in mice were observed only at doses greatly in excess of the concentrations that have hormonal effects i.e. at levels 8-9 mg/kg of body weight or more. The Committee concluded that these tumours are a consequence of the oestrogenic

effects of zearalenone. A similar conclusion was drawn by the Committee at its thirty-second meeting in the evaluation of α-zearalanol. In rats, there was no treatment-related increase in the incidence of tumours at doses of 1-3 mg/kg of body weight per day. The Committee concluded that the safety of zearalenone could be evaluated on the basis of the dose that had no hormonal effects in pigs, the most sensitive species. Using a safety factor of about 100, the Committee established a provisional maximum tolerable daily intake (PMTDI) for zearalenone of 0.5 μg/kg of body weight. This decision was based on the NOEL of 40 μg/kg of body weight per day obtained in a 15-day study in pigs. The Committee also took into account the lowest observed effect level of 200 μg/kg body weight per day in this pig study and the previous established ADI of 0-0.5 μg/kg 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.

Dietary Intakes

13. Due to the rapid biotransformation and excretion of zearalenone in animals, the dietary intake from meat and products thereof is probably of little significance (3,4,17). Only minimal transmission of zearalenone to milk of dairy cows has been found after exposure to low doses of zearalenone (15), and there is no evidence of zearalenone in milk intended for human consumption. Nor has zearalenone been reported in eggs from commercial production. It is therefore assumed that the main dietary sources of zearalenone are cereals and products thereof, while meat, egg and milk probably are of less significance.

14. The Canadian daily intake of zearalenone from maize, and maize-based cereals has been estimated to be 0.005 - 0.087 μg/kg b.w. for 12 - 19 year-old males, the highest consumption group. An additional intake from popcorn was estimated to be 0.001 - 0.023 μg/kg b.w. (3). A theoretical intake of zearalenone of 0.027 - 0.066 μg/kg b.w. from milk was alsoestimated. These estimates were based on estimated concentrations of zearalenone and not analytical data. Later studies have demonstrated that only minimal transmission of zearalenone to milk of dairy cows occur under exposure to realistic levels of zearalenone (15). Intake from cereals other than maize was not estimated. Official 1999 data obtained from Canada based on intake estimates of 6 cereal products for 60 kg adults, show a mean zearalenone intake of 0,985 μg/day or 0,016 μg/kg/body weight/day. Intake estimates of infant cereal, infant formula and creamed maize for Canadian infants, 6-9 months of age, show a daily estimate of 0.521 μg/kg or 0,060 μg/kg body weight.

15. The dietary intake of zearalenone from cereals and products thereof in the Scandinavian countries was preliminarily estimated to be 0.02 - 0.04 μg/kg b.w. per day (7). However, the data used in these estimations are rather old, and no detailed intake calculations were made.

16. Estimates of average dietary intakes of zearalenone presented by JECFA are based on the five FAO regional diets range from 1.5 to 3.5 μg/day (for «European» and «Middle Eastern» diets, respectively). The zearalenone values were only based on analytical values from Canada. Assuming a mean body mass of 60 kg, these intakes correspond to 0.03 and 0.06 μg/kg of body weight per day respectively. Estimates of average dietary intakes of zearalenone based on individual diet records are <0.98 μg/day (0.02 μg/kg bw per day) for Canady, 1.2 μg/day (0.02 μg/kg bw per day) for Denmark, 1.1 μg/day (0.02 μg/kg bw per day) for Norway and <2.1 μg/day (0.03 μg/kg bw per day) for the US. For α-zearalanol used as a veterinary drug, a theoretical maximum daily intake is calculated to be 1.6 μg per day (0.02 μg/kg bw per day) on the basis of the recommended maximum residue limits of 10 μg/kg in bovine liver and 2 μg/kg in bovine muscle. All these values are well below the PMTDI set by JECFA.

Maximum Limits for Zearalenone

17. No international harmonised maximum limit for zearalenone in foodstuff exists. Eight countries have specific regulations, ranging from 30 to 1000 μg/kg, for zearalenone. The limits apply to either specific foodstuffs or all food (16). No barriers to international trade have been reported.

Conclusions and Recommendations

18. JECFA has established a provisional maximum tolerable daily intake (PMTDI) for zearalenone and its metabolites (including α-zearalanol) of 0.5 μg/kg of body weight per day.

19. Although preliminary intake calculations indicate values well below the PMTDI, more analytical data are required. Intake calculations for the FAO regional diets should be based on representative analytical values on zearalenone on food from each region. Some recent RAPEX messages in Europe on zearalenone indicate high intakes from maize in baby food with intake calculations exceeding the JECFA PMTDI values. Further action thus seems required to reduce the levels of zearalenone in risk products for especially children with a high intake of these products.

20. The present position paper on zearalenone in food leads to the following recommendations:

1) The best way to protect consumers from the toxic effects of zearalenone is to reduce the fungal infection of cereals and toxin production as much as possible by:

   a) identifying the critical points where the fungi infect the cereals and produce zearalenone during the production and storage of cereals
   b) including quality control programmes in agricultural production
   c) improving training of all persons involved in production of cereals
   d) supporting research on methods and techniques to prevent fungal contamination in the field and during storage

2) Codex is working on a code of practice for zearalenone aimed at reducing the levels of this mycotoxin and other mycotoxins in cereals.

3) It still seems required to establish maximum levels of zearalenone in risk products intended for high risk consumers.

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