

SCIENTIFIC OPINION

Scientific Opinion on the risks for public health related to the presence of zearalenone in food¹

EFSA Panel on Contaminants in the Food Chain^{2,3}

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ABSTRACT

Zearalenone is a mycotoxin produced by several *Fusarium* species. It is commonly found in maize but can be found also in other crops such as wheat, barley, sorghum and rye. The European Commission asked the European Food Safety Authority to review the safety of zearalenone and the risk to consumers of a possible increase of the maximum level (ML) for zearalenone in breakfast cereals. A total of 13,075 analytical results obtained on food samples and 9,877 results on unprocessed grains sampled by 19 European countries in 2005-2010 were used in the evaluation. The highest concentrations of zearalenone were reported for wheat bran, corn and products thereof (e.g. corn flour, cornflakes). Grains and grain-based foods, in particular grains and grain milling products, bread and fine bakery wares, made the largest contribution to the estimated zearalenone exposures. Vegetable oils also made an important contribution to the zearalenone exposure. The critical effects of zearalenone result from its oestrogenic activity. Based on recent data in the most sensitive animal species, the pig, and taking into account comparisons between pigs and humans, the Panel on Contaminants in the Food Chain established a tolerable daily intake (TDI) for zearalenone of 0.25 µg/kg b.w. Estimates of chronic dietary exposure to zearalenone based on the available occurrence data are below or in the region of the TDI for all age groups and not a health concern. A potential increase in the ML for zearalenone in breakfast cereals from 50 µg/kg to 75, 100, 125 or 150 µg/kg is unlikely to result in a chronic dietary exposure exceeding the TDI. In a worst case scenario it is possible that an individual could consume the same batch of breakfast cereal containing zearalenone at the ML every day for 2 to 4 weeks, in which case exposures may exceed the TDI.

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KEY WORDS

Mycotoxins, *Fusarium*, zearalenone, food, wheat bran, breakfast cereals, analysis, occurrence, dietary exposure, risk assessment, toxicity, tolerable daily intake (TDI).

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SUMMARY

Zearalenone is a phenolic resorcylic acid lactone mycotoxin produced by several *Fusarium* species, particularly *F. graminearum* (formerly called *F. roseum*) and also *F. culmorum*, *F. equiseti* and *F. verticillioides*. It is commonly found in maize but can be found also in other crops such as wheat, barley, sorghum and rye throughout various countries of the world. Generally, the *Fusarium* species grow and invade crops in moist cool field conditions. *F. graminearum* also produces trichothecenes, such as deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, 4-acetylnivalenol and fusarenon-X. Whilst zearalenone is primarily a field contaminant, toxin production may also occur under poor storage conditions.

Zearalenone has previously been evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) which established a provisional maximum tolerable daily intake (PMTDI) of 0.5 µg/kg bodyweight (b.w.) in 2000, based on the oestrogenic activity of zearalenone and its metabolites, in the most sensitive animal species, the pig. Also, in 2000, the Scientific Committee on Food (SCF) established a temporary TDI (t-TDI) of 0.2 µg/kg b.w. This TDI was designated as temporary and included an additional uncertainty factor because of some 'deficiencies in the data base (e.g. the question of a higher sensitivity of prepubertal vs adult pigs raised by new information from the study of Bauer et al., 1987)'. The SCF recommended that additional studies were needed to determine the no-hormonal-effect level in pre-pubertal pigs, on the potential genotoxicity of zearalenone, on species differences in metabolism, and on blood levels of zearalenone in humans in order to help clarify the toxicokinetic behaviour.

The European Commission (EC), in considering if changes are needed to the current legal provisions for the presence of zearalenone in bran and breakfast cereals, asked the European Food Safety Authority (EFSA) to provide a scientific opinion on the effects on consumer health risk of a possible increase of the maximum level (ML) for zearalenone in breakfast cereals. The request also specified a review of the opinion of the SCF in the light of results of more recent toxicological studies.

Methods for analysis of zearalenone in food and feed are well established and are also suitable for analysis of biological samples with appropriate clean-up procedures, such as use of immunoaffinity columns. Analysis mostly uses high performance liquid chromatography coupled to fluorescence detection or triple quadrupole mass spectrometers. Quantification can be achieved via matrix calibration or by using stable isotope labelled standards.

Following a call for data issued in July 2010, a total of 13,075 analytical results obtained on food samples and 9,877 results on unprocessed grains sampled by 19 European countries in 2005-2010 were used in the evaluation. Zearalenone was reported at quantifiable levels in 15 % of the samples. The highest concentrations of zearalenone were reported for wheat bran, corn and products thereof (e.g. corn flour, cornflakes). Notably high levels have been found in corn germ oil and wheat germ oil. There were indications that soy can be contaminated with zearalenone but there were insufficient data to draw conclusions. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) used a lower bound-upper bound (LB-UB) approach in its assessment of the occurrence data. The lower bound assigns a value of zero to non-detects; the upper bound assigns the value of the limit of detection (LOD) or limit of quantification (LOQ) to results below the LOD and LOQ, respectively.

Levels of zearalenone in the group 'Unprocessed grains' were considerably higher than in the group 'Grains for human consumption'. This suggests that cleaning and selection steps applied to grains after harvesting result in lower zearalenone concentrations in grains intended for human consumption. In general, zearalenone is redistributed between the milling fractions. The by-products from cleaning the raw cereal grains (dust, hulls and others) were characterised by 3- to 30-fold higher zearalenone concentrations than the cleaned cereal grains while bran contained up to 2-fold higher concentrations.

Zearalenone is generally stable during cooking, except under alkaline conditions or during extrusion cooking (heating under a high degree of pressure).

The CONTAM Panel estimated total chronic dietary exposures to zearalenone across 19 European countries, using LB and UB mean concentrations of zearalenone in foods, and consumption data for different age groups. For adults the minimum LB to maximum UB was 2.4 to 29 ng/kg body weight (b.w.) per day for average consumers (average consumption in total population), and 4.7 to 54 ng/kg b.w. for high consumers (95th percentile consumption in total population). The highest exposure estimates are for toddlers (aged ≥ 12 months to < 36 months), at 9.3 to 100 ng/kg b.w. per day for average consumers, and 23 to 277 ng/kg b.w. for high consumers.

Grains and grain-based foods, in particular grains and grain milling products, bread and fine bakery wares, made the largest contribution to the zearalenone exposure in all age classes. Vegetable oils, especially corn germ oil and wheat germ oil, make an important contribution to the zearalenone exposure.

From the average values across the European countries, breakfast cereals provide a contribution of 0.4-17 % to total dietary exposure of zearalenone in adults. Increasing the ML from 50 $\mu\text{g}/\text{kg}$ up to 150 $\mu\text{g}/\text{kg}$ has the potential to increase chronic total dietary exposure to zearalenone by up to 35 %. From the average values across the European countries, breakfast cereals provide a contribution of 0.1-5.1 % to total dietary exposure of zearalenone in toddlers (age ≥ 12 months to < 36 months). Increasing the ML from 50 $\mu\text{g}/\text{kg}$ up to 150 $\mu\text{g}/\text{kg}$ has the potential to increase chronic total dietary exposure to zearalenone by up to 16 %. Short-term mean dietary exposure in consumers of breakfast cereals could increase to up to 357 ng/kg b.w. per day and the 95th percentile exposure up to 1029 ng/kg b.w. per day if the ML for zearalenone were to be increased from 50 $\mu\text{g}/\text{kg}$ up to 150 $\mu\text{g}/\text{kg}$.

Limited data indicate that dietary exposure of vegetarians to zearalenone could be up to 2-fold higher than for the general population.

Zearalenone is extensively absorbed and metabolised by three major routes. Reduction results in formation of α -zearalenol, which is more oestrogenic, and β -zearalenol, which is less oestrogenic than zearalenone. Efficient glucuronidation of zearalenone in the small intestine and liver significantly reduces the amounts of unconjugated (i.e. receptor-active) parent compound that reaches the circulation. It is possible that fetuses and neonates could be more susceptible than adults to the oestrogenic effects of zearalenone, based on higher internal exposures due to metabolic and physiological immaturity. Cytochrome P450-mediated oxidation produces catechol metabolites that are subject to redox cycling to reactive quinones.

Of the laboratory and domestic animals studied, pigs are the most sensitive species for oestrogenic effects of zearalenone, with females being more sensitive than males. The greater formation of α -zearalenol in the pig, relative to other animal species studied, may contribute to this sensitivity.

In well-conducted carcinogenicity bioassays, no increase in tumors was observed in two rat studies, while in mice, significant increases in pituitary and liver adenomas, but not carcinomas, were observed in one study, providing limited evidence of carcinogenicity. Zearalenone does not cause gene mutations in bacterial test systems but is clastogenic and aneugenic *in vitro* and has been confirmed as an *in vivo* clastogen in the mouse. A plausible mechanism for the clastogenic effects has been proposed, namely formation of catechols that can be oxidised to quinones that undergo redox-cycling.

The oestrogenic effects of zearalenone in pigs are observed at doses around three orders of magnitude lower than doses reported to cause clastogenicity and increases in adenomas in mice. The CONTAM Panel therefore decided to establish a TDI for zearalenone based on its oestrogenic effects. Adverse effects of zearalenone and its metabolites on testosterone synthesis, sexual behaviour, sex organ

weights, testicular histology and spermatogenesis have been observed in male animals. In females, adverse effects of zearalenone on the reproductive tract, fertility and embryo survival have been reported. The female pig is the most sensitive, with immature pigs possibly more sensitive than mature pigs. Effects include disturbance of the oestrous cycle, ovulation, conception and implantation, embryonic death, reduced fetal weight, reduced litter size and impaired neonatal survival. In female pigs, the tissues that are most sensitive to the oestrogenic effect of zearalenone and its metabolites are the ovary, uterus and vulva. Lowest-observed-effect-levels (LOELs) for these tissues in mature and immature gilts range from 17 to 200 µg/kg b.w. per day, with an overall no-observed-effect-level (NOEL) of 10 µg/kg b.w. per day.

Toxicodynamic information indicates that it is likely that the human female would not be more sensitive to zearalenone and its metabolites than the female pig. For derivation of a TDI, it is therefore not necessary to include an uncertainty factor of 2.5 for toxicodynamic differences between pigs and humans. Using the NOEL of 10 µg/kg b.w. per day and an uncertainty factor of 40 (4 for interspecies differences in toxicokinetics and 10 for interhuman variability), a TDI of 0.25 µg/kg b.w. can be derived. As a number of relevant studies, including in the pig, have become available since the previous t-TDI was established by the SCF in 2000, the CONTAM Panel concluded that a full TDI of 0.25 µg/kg b.w. can now be established.

Estimates of chronic dietary exposure to zearalenone based on the available occurrence data are below or in the region of the TDI for all age groups and not a health concern. A potential increase in the ML for zearalenone in breakfast cereals from 50 µg/kg to 75, 100, 125 or 150 µg/kg is unlikely to result in a chronic dietary exposure exceeding the TDI.

In a worst case scenario it is possible that an individual could consume the same batch of breakfast cereal containing zearalenone at the ML every day for 2 to 4 weeks. The highest estimated short-term exposure is for children aged ≥ 3 to < 10 years old and, at the current ML, is 60 % above the TDI. Increasing the ML from 50 µg/kg up to 150 µg/kg has the potential to increase the short-term exposure of children aged ≥ 3 to < 10 years old to up to approximately 1 µg/kg b.w. per day. High fibre breakfast cereals, which are the most likely to be contaminated with zearalenone due to the high content of wheat bran, are more likely to be consumed by adults than by children. For adults the highest estimates of short-term exposure are below the TDI for ML scenarios up to a concentration of 100 µg/kg zearalenone in breakfast cereals.

The mycotoxins that usually co-occur with zearalenone do not have oestrogenic effects. Combined effects of zearalenone and other mycotoxins are not expected to arise in humans at dietary exposures below the respective health-based guidance values of the individual toxins. The possible impact of combined exposure to zearalenone with other oestrogenic substances in food (such as phytoestrogens in soya) or the environment could be additive or antagonistic.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Zearalenone is a mycotoxin that can be produced by several field fungi including *Fusarium graminearum* (*Gibberella zae*), *F. culmorum*, *F. cerealis*, *F. equiseti* and *F. semitectum*. Fungi of the genus *Fusarium* infect cereals pre-harvest in the field during blooming, but growth and toxin production may also occur post-harvest under poor storage conditions. The toxin is common in maize, but because the spores of *Fusarium* are ubiquitous, cereal crops such as barley, oats, wheat, rice, sorghum and soy beans are also susceptible to contamination with zearalenone, both in the temperate and warmer climate zones.

The opinion from the Scientific Committee for Food and the JECFA

The Scientific Committee on Food issued on 22 June 2000 an opinion on *Fusarium* toxins Part 2: Zearalenone (ZEA).⁴

The Scientific Committee on Food 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, and established a temporary tolerable daily intake (t-TDI) for zearalenone of 0.2 µg/kg of body weight (b.w.). This decision was based on the no-observed-effect-level (NOEL) of 40 µg/kg of b.w. per day obtained in a 15-day study in pigs and the lowest observed effect level of 200 µg/kg b.w. per day in this study. A safety factor of 200 was used because it is a temporary TDI due to some deficiencies in the data base.

Also the JECFA concluded in its assessment in 2000⁵ 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, but used a safety factor of about 100, for the establishment of a provisional maximum tolerable daily intake (PMTDI) for zearalenone of 0.5 µg/kg b.w.

The problem:

Commission Regulation (EC) No 1881/2006 of 19 December 2006⁶ lays down maximum levels for certain contaminants in foodstuffs, including maximum levels for zearalenone.

In spring 2009, the Commission was informed by the professional stakeholder organisation CEEREAL (the European Breakfast Cereal Association) of serious supply problems experienced for wheat bran from the harvest 2008 to be used in high fibre breakfast cereals due to increased levels of zearalenone found in wheat bran

Although no maximum level for wheat bran used as ingredient has been established, the wheat bran for use in the high fibre breakfast cereals should not have a level of higher than 75 µg/kg in order to enable high fibre breakfast cereal producers to comply with the existing maximum level of 50 µg/kg for breakfast cereals other than maize based breakfast cereals.

Therefore CEEREAL requested a temporary increase of the level of zearalenone in high-fibre breakfast cereals from currently 50 µg/kg to 135 µg/kg. Based on a risk assessment performed by the Food Standards Agency (FSA) UK, it could be concluded that there is unlikely to be a health risk from the increase of the zearalenone limit.

⁴ Opinion of the Scientific Committee on Food on *Fusarium* toxins – Part 2: Zearalenone (ZEA), adopted on 22 June 2000. (SCF/CS/CNTM/MYC/22 Rev 3 Final) http://ec.europa.eu/food/fs/sc/scf/out65_en.pdf

⁵ FAO/WHO, 2000. Zearalenone. Safety evaluation of certain food additives and contaminants Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series 44, World Health Organization, Geneva, Switzerland, p. 393-482. <http://www.inchem.org/documents/jecfa/jecmono/v44jec14.htm>

⁶ OJ L 364, 20.12.2006, p. 5-17.

Based on this assessment and to ensure the supply and availability of high-fibre breakfast cereals, given their beneficial health effects, it was recommended to the Member States to apply on a temporary basis for a limited period of time (i.e. high-fibre breakfast cereals with production date before 31 October 2009) a level of 100 µg/kg of zearalenone for high-fibre breakfast cereals other than maize based breakfast cereals (the current maximum level of zearalenone in maize-based breakfast cereals is 100 µg/kg).

Although no maximum level for wheat bran used as ingredient has been established, the wheat bran for use in the high fibre breakfast cereals should not have a level of higher than 125 µg/kg in order to enable high fibre breakfast cereal producers to comply with the proposed temporary level of 100 µg/kg for high fibre breakfast cereals other than maize based breakfast cereals

It cannot be excluded that, due to the change in weather conditions, also in the future increased levels of zearalenone could be observed more frequently in wheat bran and cereal bran in general. If this would be the case, it would then be appropriate to consider if changes are needed to the current legal provisions as regards the presence of zearalenone in bran and breakfast cereals. Therefore, EFSA is requested to provide a scientific opinion on the effects on consumer health risk of a possible increase of the maximum level for zearalenone in breakfast cereals.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority to provide a scientific opinion on the potential consumer health risk by

- a) reviewing the opinion of the Scientific Committee on Food of 22 June 2000 on zearalenone in food in the light of results of toxicological studies published since that time.
- b) assessing the potential increase of consumer health risk by a possible increase of the currently existing maximum level of 50 µg/kg to 75 µg/kg, 100 µg/kg, 125 µg/kg or 150 µg/kg for zearalenone in breakfast cereals taking into account the exposure to zearalenone from other food sources considering
 - occurrence data provided;
 - specific consumption patterns of breakfast cereals in the different Member States;
 - specific (vulnerable) groups of the population, including children, and high level consumers.

ASSESSMENT

1. Introduction

Zearalenone is a phenolic resorcylic acid lactone mycotoxin produced by several *Fusarium* species, particularly *F. graminearum* (formerly called *F. roseum*) and also *F. culmorum*, *F. equiseti* and *F. verticillioides*. It is commonly found in maize but can be found also in other crops such as wheat, barley, sorghum and rye throughout various countries of the world. Generally, the *Fusarium* species grow and invade crops in moist cool field conditions during blooming, but growth and toxin production may also occur post-harvest under poor storage conditions. *F. graminearum* also produces trichothecenes, such as deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, 4-acetylnivalenol and fusarenon-X.

Zearalenone exhibits oestrogenic activity and has been implicated in numerous mycotoxicoses in farm animals, especially pigs, resulting in alterations in the reproductive tract, decreased fertility, increased number of resorptions and reduced litter size.

1.1. Previous assessments

Zearalenone was evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) at its 53rd meeting (FAO/WHO, 2000). The JECFA noted that the toxic effects appeared to be dependent on the oestrogenic activity of zearalenone and its metabolites, and that pigs and sheep were more sensitive than rodents. The no-observed-effect-level (NOEL) in pigs was 40 µg/kg bodyweight (b.w.) per day on the basis of oestrogenic effects in responsive tissues, compared with NOEL of 3 mg/kg b.w. per day in rats. The JECFA concluded that hepatocellular adenomas and pituitary tumours observed in mice were a consequence of the oestrogenic effects of zearalenone, and that the safety could be evaluated on the basis of the dose having no hormonal effect in the most sensitive species, pigs. A provisional maximum tolerable daily intake (PMTDI) of 0.5 µg/kg b.w. was established based on the NOEL of 40 µg/kg b.w. per day in a 15-day study in pigs, using a safety factor of “about 100”. This was based on the study of Edwards et al. (1987), in which young sexually mature non-pregnant sows (gilts) were given 2 kg of feed containing 0, 1, 5 or 10 mg/kg zearalenone between day 5 and day 20 of oestrus (equivalent to 0, 40, 200 and 400 µg/kg b.w. per day). In the gilts given 5 or 10 mg/kg zearalenone in the diet, the inter-oestrous interval was significantly increased, accompanied by increased plasma concentrations of progesterone and prolonged maintenance of corpora lutea. The PMTDI also took into account that the lowest-observed-effect-level (LOEL) in the pig study was 200 µg/kg b.w. per day and that an acceptable daily intake (ADI) of 0-0.5 µg/kg b.w. had been previously established for the metabolite α -zearalanol (FAO/WHO, 1988). The PMTDI applied to the total intake of zearalenone and its metabolites including α -zearalanol.

In 2000, the Scientific Committee on Food (SCF) similarly concluded that the safety of zearalenone could be evaluated on the basis of the dose that had no hormonal effects in pigs (SCF, 2000). The SCF also used the NOEL of 40 µg/kg b.w. per day from the pig study of Edwards et al. (1987), but applied a safety factor of 200 to establish a temporary TDI (t-TDI) of 0.2 µg/kg b.w. This was due to some “deficiencies in the data base (e.g. the question of a higher sensitivity of prepubertal vs adult pigs raised by new information from the study of Bauer et al., 1987)”. The SCF recommended that additional studies were needed to determine the no-hormonal-effect level in prepubertal pigs, on the potential genotoxicity of zearalenone, on species differences in metabolism, and on blood levels of zearalenone in humans in order to help clarify the toxicokinetic behaviour.

The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) has previously evaluated zearalenone as an undesirable substance in animal feed (EFSA, 2004a). The CONTAM Panel confirmed that pigs are the most sensitive animal species, and that it can be expected that animal-derived foods contribute only marginally to total human dietary exposure, as compared to cereals and grain products. The t-TDI was not reviewed at that time. Similarly, other authorities that have conducted risk assessments of zearalenone have cited the SCF t-TDI and/or the JECFA PMTDI (e.g. Cressey and Thomson, 2006 (New Zealand Food Safety Authority (NZFSA)); AFSSA, 2006; 2009)

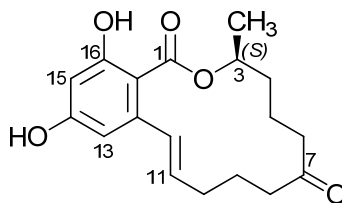
α -Zearalanol (Zeranol[®]), a metabolite of zearalenone, has been evaluated by the JECFA as a veterinary drug for use as a growth promoter (FAO/WHO, 1988). It increases liveweight gain in food animals following implantation. α -Zearalanol was shown to be a weak oestrogen in mice, rats, dogs, and monkeys. Tumours of the pituitary gland were considered to result from the oestrogenic properties, and the JECFA concluded that the safety assessment could be based on determination of a no-hormonal-effect level. In ovariectomized female cynomolgus monkeys the no-hormonal-effect level was 0.05 mg/kg b.w. per day. The JECFA concluded that this model could be relevant to the human population and, since the ovariectomized female cynomolgus monkey is highly sensitive to oestrogenic substances, considered that using this study as a basis for establishing an ADI was a conservative approach. Applying a safety factor of 100, an ADI of 0-0.5 μ g/kg b.w. was proposed.

The use of α -zearalanol for growth promotion in food animals was banned in the European Union (EU) in 1985.

The United Kingdom (UK) Food Standards Agency (FSA) submitted a risk assessment to the European Commission (EC) in 2009 in support of a proposed temporary revised maximum level (ML) of 100 μ g/kg for high-fibre breakfast cereals, which has been forward to EFSA by the EC. The FSA assessment was based on UK data for consumption of high-fibre breakfast cereals with wheat, and scenarios in which zearalenone was present at the current EU ML of 50 μ g/kg, at 135 μ g/kg, as proposed by industry for the ML, or at 100 μ g/kg. Estimated 97.5th percentile exposures of adults were below the SCF t-TDI of 0.2 μ g/kg b.w. The FSA noted that for toddlers aged 1.5-4.5 years, high level (97.5th percentile) consumption of high-fibre breakfast cereals containing zearalenone at 135 μ g/kg would result in an exposure of 0.35 μ g/kg b.w. per day and zearalenone at 100 μ g/kg would result in an exposure of 0.26 μ g/kg b.w. per day. Since in reality not all the breakfast cereal would be at the ML, these toddlers would only potentially exceed the t-TDI on some but not all days.

1.2. Chemistry

Zearalenone (3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1,7(8*H*)-dione; C₁₈H₂₂O₅; molecular weight: 318.36; Chemical Abstracts Service (CAS) Registry Number 17924-92-4) is a macrocyclic β -resorcylic acid lactone. It is a non-steroidal mycotoxin produced by *Fusarium* spp. (reviewed in Chełkowski, 1998). Different abbreviations for zearalenone, such as ZON or ZEA, are used in the literature. It is biosynthesized via the polyketide pathway (see review by Huffmann et al., 2010). Zearalenone was first isolated in 1962 from *Fusarium*-infected corn by Stob et al. (1962) and its chemical structure including the stereochemistry at position 3 was elucidated by Urry et al. (1966) and Kuo et al. (1967) (Figure 1).



Zearalenone

Figure 1: Chemical structure of zearalenone.

Zearalenone is heat stable up to 150°C. Degradation has been observed only at higher temperatures or under alkaline conditions (see Section 4.3. for details). Besides zearalenone there are several other closely related metabolites formed in fungal cultures e.g. α -zearalenol, β -zearalenol, α -zearalanol and β -zearalanol. However, there is only limited evidence that these metabolites occur in food (see Section 4.1.) (Figure 2).

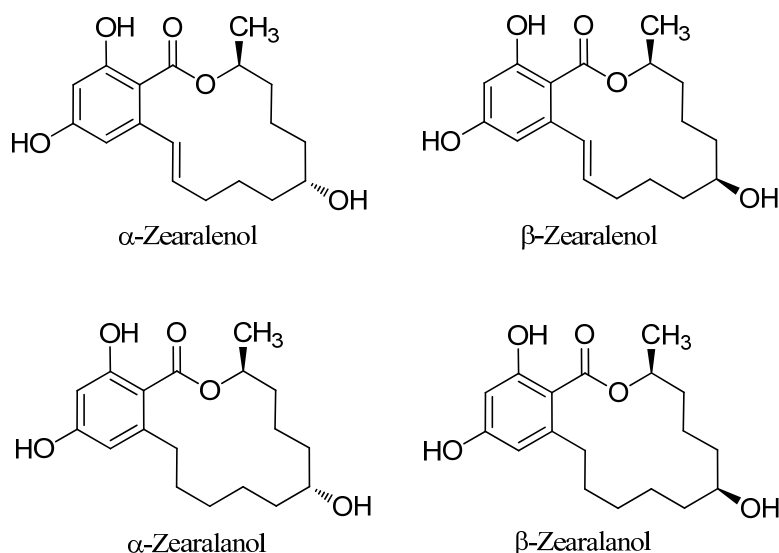


Figure 2: Chemical structures of α -zearalenol, β -zearalenol, α -zearalanol and β -zearalanol.

2. Legislation

Currently the Commission Regulation (EC) No 1881/2006 of 19 December 2006⁷ lays down MLs for certain contaminants in foodstuffs, including MLs for zearalenone (Table 1). The MLs apply to the edible part of the foodstuffs unless it is otherwise specified. The MLs set for unprocessed cereals and unprocessed maize apply to unprocessed cereals placed on the market for first-stage processing. The Regulation specifies that ‘ ‘First-stage processing” shall mean any physical or thermal treatment, other than drying, of or on the grain. Cleaning, sorting and drying procedures are not considered to be “first-stage processing” insofar no physical action is exerted on the grain kernel itself and the whole grain remains intact after cleaning and sorting. In integrated production and processing systems, the ML applies to the unprocessed cereals in case they are intended for “first-stage processing” ’. Because of the low concentration levels of *Fusarium* toxins found in rice, no MLs are set for rice or rice products. Therefore for the application of MLs for zearalenone, rice is not included in ‘cereals’ and rice products are not included in ‘cereal products’.

⁷ OJ L 364, 20.12.2006, p. 5-17, and its amendment Commission Regulation (EC) No 1126/2007 of 28 September 2007. OJ L 255, 29.9.2007, p. 14-17.

The Article 2 of the Regulation (EC) No 1881/2006 specifies that when ‘applying the MLs to foodstuffs which are dried, diluted, processed or composed of more than one ingredient, the following shall be taken into account:

- (a) changes of the concentration of the contaminant caused by drying or dilution processes;
- (b) changes of the concentration of the contaminant caused by processing;
- (c) the relative proportions of the ingredients in the product;
- (d) the analytical limit of quantification.’

For the zearalenone content in food colours, the Commission Directive 2008/128/EC of 22 December 2008⁸ laying down specific purity criteria concerning colours for use in foodstuffs requires an absence of zearalenone in the crystallised product of beta-carotene (food orange) obtained by a fermentation process.

Table 1: The maximum levels (MLs) for zearalenone as laid down in Commission Regulation (EC) No 1881/2006. See the original regulations for further definitions and explanations of individual food commodities.

	Foodstuffs	ML (µg/kg)
1	Unprocessed cereals other than maize	100
2	Unprocessed maize with the exception of unprocessed maize intended to be processed by wet milling ^(a)	350
3	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, with the exception of foodstuffs listed below in 6, 7, 8, 9 and 10	75
4	Refined maize oil	400
5	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize snacks and maize based breakfast cereals	50
6	Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals	100
7	Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children ^{(b), (c)}	20
8	Processed maize-based foods for infants and young children ^{(b), (c)}	20
9	Milling fractions of maize with particle size >500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size >500 micron not used for direct human consumption falling within CN code 1904 10 10	200
10	Milling fractions of maize with particles size <500 micron falling within CN code 1102 20 and other maize milling products with particle size <500 micron not used for direct human consumption falling within CN code 1904 10 10	300

ML: maximum level; CN: Combined Nomenclature (Maize milling fractions are classified according to the particle size in different headings in the Combined Nomenclature based upon a rate of passage through a sieve with an aperture of 500 microns).

(a): The exemption applies only for maize for which it is evident e.g. through labelling, destination, that it is intended for use in a wet milling process only (starch production);

(b): The maximum levels refer to dry matter;

(c): Foodstuffs listed in this category as defined in Commission Directive 96/5/EC of 16 February 1996 on processed cereal-based foods and baby foods for infants and young children⁹ as last amended by Directive 2003/13/EC.¹⁰

⁸ OJ L 6, 10.1.2009, p. 20-63.

⁹ OJ L 49, 28.2.1996, p. 17-28.

¹⁰ OJ L 41, 14.2.2003, p. 33-36.

3. Methods of analysis

3.1. Sample collection and storage

The methods of sampling and analysis for concentrations of zearalenone in foodstuffs are stipulated in the Commission Regulation (EC) No 401/2006 of 23 February 2006¹¹ which lays down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs, and as regards groundnuts (peanuts), other oilseeds, tree nuts, apricot kernels, liquorice and vegetable oil. The foodstuffs for which the regulation stipulates the sampling and analytical methods include cereals and cereal products, baby foods and processed cereal based foods for infants and young children and vegetable oils.

The Annex 1 of the Regulation (EC) No 401/2006 defines the methods of sampling e.g. how to take samples from different types of lots. The regulation also stipulates the requirements for the analytical results for accepting and rejecting the lots and sub-lots. For the acceptance, the result of the laboratory sample needs to conform to the ML when the recovery correction and measurement uncertainty have been taken into account. The lot or sub-lot is rejected if the result of the laboratory sample exceeds the ML beyond reasonable doubt taking into account the correction for recovery and measurement uncertainty.

For official control the samples should be collected as described in the legislation. Only a few studies published in recent years included comprehensive sample analysis of wheat or wheat based products. Collected samples have been taken from the retail market or directly from mills (Schollenberger et al., 1999; Cramer et al., 2007; Ghali et al., 2008; Bankole et al., 2010; Giraud et al., 2010). Breakfast cereals have not been subject to a specialized examination, but have been used for method development (Cunha and Fernandes, 2010). Some surveys compared the occurrence of mycotoxins in organic and conventional foodstuff (Hoogenboom et al., 2008; Bernhoft et al., 2010). As the products were recommended to be kept cool and dry by the manufacturers, analysts take this as guidance for storage before the analysis.

3.2. Methods of analysis of zearalenone in food

The Annex 2 of the Regulation (EC) No 401/2006 stipulates the criteria for sample preparation and for analytical methods used for the official control. The treatment of the laboratory sample, the replicate samples, method of analysis to be used and laboratory control requirements are prescribed. The control requirements define the performance criteria of the analytical methods for zearalenone setting the recoveries, relative standard deviation under repeatability conditions and under reproducibility conditions. Also a fitness-for-purpose approach can be applied if a limited number of fully validated methods are available. Estimation of measurement uncertainty, recovery calculation and reporting of the results are also specified. The analytical results corrected for recovery shall be used for controlling compliance and the analytical results must be reported together with the expended measurement uncertainty.

Methods used for the analysis of mycotoxins vary widely. There are hyphenated chromatographic-mass spectrometric (MS) approaches (Tanaka et al., 2000; Sulyok et al., 2007; Spanjer et al., 2008; Vendl et al., 2009; Martos et al., 2010; Tanaka et al., 2010) besides more conventional coupling of high performance-liquid chromatography (HPLC) with optical detection (Schollenberger et al., 1999; Arranz et al., 2007; CEN, 2010). Gas chromatography (GC) is also coupled to flame ionization or electron capture detectors based on the derivatization used (Jimenez et al., 1997; Tanaka et al., 2000). Fast on-site testing can be done by antibody-based techniques (Shim et al., 2009), and is also used in the laboratory (Suzuki et al., 2007; Thongrussamee et al., 2008).

¹¹ OJ L 70, 9.3.2006, p. 12-70 and its amendment Commission Regulation (EU) No 178/2010 of 2 March 2010. OJ L 52, 3.3.2010, p. 32-43.

The coupling of HPLC and MS clearly dominates the spectrum of used techniques. In general, it has to be differentiated between methods focusing on a single mycotoxin like zearalenone or a group of toxins, like the trichothecenes or the *Fusarium* toxins (Tanaka et al., 2006; Cramer et al., 2007). These methods are often combined with a sample clean-up via immunoaffinity columns and differ from multi-contaminant analyses which lack a clean-up (Sulyok et al., 2007; Spanjer et al., 2008). However the first LC-MS/MS multi-mycotoxin method using multi-mycotoxin immunoaffinity columns have been published recently (Romagnoli et al., 2010).

A reference method, standardised and validated by the European Committee for Standardization (CEN), for the determination of zearalenone in maize-based baby food, barley flour, maize flour, polenta, wheat flour and cereal based foods for infants and young children using HPLC with immunoaffinity clean-up and fluorescence detection (FLD) is available (CEN, 2010). This method has been validated in two interlaboratory studies. The first study was for the analysis of samples of maize based baby food, barley flour, maize flour, polenta and wheat flour with zearalenone concentrations ranging from 10 µg/kg to 335 µg/kg, and the second study was for samples of cereal based foods for infants and young children with zearalenone concentrations ranging from 9 µg/kg to 44 µg/kg.

A standard for zearalenone is commercially available. In addition, certified reference material for zearalenone in maize is available from the Institute for Reference Materials and Measurements (IRMM)¹² and quality control materials for breakfast cereals, baby food and animal feed from FAPAS[®].¹³ Also proficiency tests for zearalenone are organized by FAPAS.¹³

Non-chromatographic methods such as enzyme linked immunosorbent assays (ELISA) or bioassays are of minor relevance. The former have proven value as a fast screening technique on-site (Shim et al., 2009) or as a preselection tool (Krska et al., 2001; Thongrussamee et al., 2008).

In addition to zearalenone and its mammalian metabolites, other plant-specific conjugated metabolites, e.g. zearalenone glucosides, have been described. For the analysis of these conjugated metabolites LC-MS is the method of choice (Schneweis et al., 2002; Vendl et al., 2009).

3.2.1. Sample preparation

The extraction of the mycotoxins from foodstuff is done by liquid extraction using acetonitrile or methanol and water or buffer. Vigorous mixing is performed by ultra turrax, horizontal shakers or stomacher (Shephard et al., 2009; Martos et al., 2010). Afterwards centrifugation steps separate the phases. For methods using column clean-up before injection, the amount of organic solvent is reduced in a nitrogen stream under gentle heating or with a rotary evaporator (Shephard et al., 2009).

Columns for clean-up and concentration may be immunoaffinity columns, more or less specific for zearalenone and its metabolites α -zearalenol, β -zearalenol, α -zearalanol or β -zearalanol. These columns are quite expensive as the antibodies are denaturated during elution of the analyte. Therefore, endeavours are made for the development of molecular imprinted materials that combine ruggedness of conventional solid phase extraction columns with a better selectivity (Urraca et al., 2006; Choi et al., 2009; Lucci et al., 2010). Besides this, experimental bead-based extraction is under study, possibly offering alternatives in the future (Hervas et al., 2009; Siegel et al., 2010).

A proper quantification of zearalenone can effectively be done by matrix matched calibration with an internal standard. Often zearalanone or α -zearalanol have been used, as these do not usually occur naturally in samples (Berthiller et al., 2005; Klötzel et al., 2006). The more sophisticated way is the usage of stable isotope labelled standards (Cramer et al., 2007; Sulyok et al., 2007) if they are available

¹² European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (EC-JRC-IRMM), <http://www.irmm.jrc.be>.

¹³ FAPAS[®], Food Analysis Performance Assessment Scheme, The Food and Environment Research Agency. <http://www.fapas.com/proficiency-testing-schemes/fapas>.

in sufficient amounts and purity. As these underlie the same conditions during clean-up, chromatography and ionisation a reliable quantification can be achieved.

Multi-mycotoxin methods are being developed using solid phase extraction columns or multifunctional columns (Tanaka et al., 2010). Although the matrix is not so far removed effectively by these methods, they can be used to control the MLs listed in Table 1) (Sulyok et al., 2007; Spanjer et al., 2008). However, these methods are not useful for foodstuffs for special dietary needs (baby food, infants and young children) as the MLs are much lower for these products.

3.2.2. Instrumental techniques

As GC requires derivatisation, which inevitably causes a labour intensive sample clean-up, its importance for the determination of zearalenone in foods is decreasing (Josephs et al., 2001).

One of the standard methods for the analysis of zearalenone in foods is HPLC-FLD. This technique in combination with immunoaffinity clean-up is also used in the CEN reference method (CEN, 2010).

Recently mostly HPLC coupled with triple quadrupole MS is used, since it is most applicable based on quantification and qualification. The chromatographic separation is usually done on C18-columns with water or ammonium-based buffers and acetonitrile or methanol as organic solvents. Details are described in the literature (Driffield et al., 2003; Biselli and Hummert, 2005; Klötzel et al., 2006; Cramer et al., 2007; Sulyok et al., 2007; Driehuis et al., 2008; Spanjer et al., 2008; Jin et al., 2010; Romagnoli et al., 2010). MS techniques other than triple quadrupole are used more seldom (Tanaka et al., 2006; Herebian et al., 2009). Ionisation is mostly done by electrospray ionization (ESI). The negative mode has advantages of better sensitivity and signal-to-noise ratio as there are only $[M - H]^-$ ions for zearalenone in contrast to competing $[M + H]^+$ ions and ammonium or alkaline metal adducts in the positive mode. ESI mode is for example used in the multi-mycotoxin method of Spanjer et al. (2008) as the number of analytes does not allow permanent switching between polarities, and positive mode is required for other mycotoxins. Even if small molecules like mycotoxins would be good targets for atmospheric pressure chemical ionisation, especially if matrices are complex, this is not common.

With the currently available methods, limits of detection (LODs) and limits of quantification (LOQs) below 1-5 and 5-20 $\mu\text{g}/\text{kg}$, respectively, can easily be reached (see Section 4).

3.2.3. Quantitative analysis of zearalenone and its metabolites for toxicokinetic studies

Extraction of parent zearalenone and its unconjugated reduced metabolites is often performed using liquid-liquid extraction (Shin et al., 2009). Sometimes an enzymatic deconjugation step involving β -glucuronidase and sulphatase activities is used in order to distinguish between unconjugated species, which are active in oestrogen receptor (ER) binding, and Phase II metabolites, which are inactive (Biehl et al., 1993; Dänicke et al., 2005a). The same analytical methods used for foods have also been applied for the quantification of zearalenone and its major metabolites for toxicokinetic studies. Biehl et al. (1993) used LC with radiochemical detection. The applicability of available HPLC-FLD methods to a pharmacokinetic study is limited, requiring relatively large biological sample volumes and time-consuming sample clean-up procedures (Trenholm et al., 1981; Olsen et al., 1981; Prelusky et al., 1989; Dänicke et al., 2005a). ELISA offers greater sample throughput and high sensitivity but, due to cross reactivity, a complementary method such as HPLC needs to be used for analyte identification (Warner et al., 1986; Nuryono et al., 2005). GC-MS has been used to simultaneously determine zearalenone and its metabolites, but the major drawbacks include the need for derivatization and extra sample preparation procedures (Tanaka et al., 2000; Blokland et al., 2006). LC-MS/MS methods have been used to simultaneously determine zearalenone and related metabolites in urine (Jodlbauer et al., 2000; Zöllner et al., 2002; Launay et al., 2004; Schmidt et al., 2008), milk (Sørensen and Elbæk, 2005) and tissues (Jodlbauer et al., 2000; Zöllner et al., 2002; Mallis et al., 2003; Songsermsakul et al., 2006;

Shin et al., 2009). In general, LC-MS/MS in the multiple-reaction monitoring mode provides a rapid, simple, and highly sensitive method for the quantification of zearalenone and its metabolites using small volumes of serum for pharmacokinetic studies (Shin et al., 2009).

3.2.4. Conclusions

Analysis of zearalenone in food and feed as well as biological samples is mostly done with HPLC coupled to triple quadrupole MS. Quantification can be achieved via matrix calibration or by using stable isotope labelled-standards. The application of clean-up steps is required for samples for special dietary purposes (baby food, infants and young children) as the MLs are much lower for these products, and also for biological samples. Further developments are expected in the coming years using multifunctional columns, probably offering opportunities for multiple analytes in screening methods.

4. Occurrence of zearalenone in food

4.1. Previously reported occurrence results

The occurrence data on zearalenone in foods of plant origin have previously been reviewed by JECFA (FAO/WHO, 2000) and of animal origin by EFSA (EFSA, 2004a). The occurrence data on zearalenone in food in Europe published since these reviews are reported below. If the numerical value for the LOD or LOQ are not mentioned in the section below they were not reported in the study.

4.1.1. Foods of plant origin

Zearalenone is a field contaminant of crops, because toxin production takes place before the harvest and to a lesser extent during the storage of the crops. The weather conditions, especially at growing and flowering time, greatly influence the *Fusarium* infection of the plant and the mycotoxin production, and therefore zearalenone concentrations vary from year to year.

In 1999 in Germany, Schollenberger et al. (1999) analysed 20 baby and infant food samples but did not detect zearalenone or its metabolites α - and β -zearalenol. Later the same authors analysed 60 wheat flour samples and reported the total incidence of 38 % for zearalenone with a median concentration of 3 μg zearalenone/kg of the positive samples (Schollenberger et al., 2002). In another German study a total of 219 samples of foodstuffs of plant origin (84 cereal based-samples, 85 vegetables and fruit samples and 35 oilseed and nuts samples) were collected in 2000-2001 and analysed for zearalenone and its metabolites. In the group of 84 cereal-based samples, 32 samples were positive for zearalenone (38 %) with a range of concentration of 2-67 $\mu\text{g}/\text{kg}$. The level of 50 $\mu\text{g}/\text{kg}$ of zearalenone was exceeded in only one wheat bran sample containing 67 $\mu\text{g}/\text{kg}$. Seven of the 85 vegetable and fruit samples were positive for zearalenone with a range of 2-17 μg zearalenone/kg, while in oilseeds and nuts zearalenone was found in 4 out of 35 samples with a range of 2-4 $\mu\text{g}/\text{kg}$. No α - or β -zearalenol was detected in any of the samples (Schollenberger et al., 2005). Kappenstein et al., (2005) analysed in total 77 edible oil samples including wheat germ oil, soy oil, wheat germ oil and 'other oils' for zearalenone in Germany. All of the 38 wheat germ oil samples were positive for zearalenone with the mean concentration of 170 $\mu\text{g}/\text{kg}$ while 70 % of the soy oil samples ($n = 20$) gave a positive result with the mean concentration of 4 μg zearalenone/kg (LOD = 0.3 $\mu\text{g}/\text{kg}$). Of the 11 wheat germ oil samples 10 were positive for zearalenone with the mean concentration of 13 $\mu\text{g}/\text{kg}$. Zearalenone was not found in 'other oil' samples (Kappenstein et al., 2005).

Zearalenone concentrations were investigated in a total of 99 cereal samples (41 samples of wheat, 17 of oats and 41 of corn) in Germany (Schollenberger et al., 2006). In wheat, oats and corn samples the incidences of zearalenone were 63, 24 and 85 %, respectively, and the mean concentrations 15, 21, and 48 $\mu\text{g}/\text{kg}$, respectively. In the later study of Schollenberger et al. (2007) 45 samples of soy food,

purchased from food stores in Germany, were analysed for zearalenone and its metabolites. Zearalenone, α -zearalenol and β -zearalenol were found in 7, 5 and 2 samples, respectively. Zearalenone was found to co-occur with α -zearalenol in 5 samples and 2 of these samples contained also β -zearalenol. The zearalenone levels ranged between 2 and 214 $\mu\text{g}/\text{kg}$. The level of 50 μg zearalenone/kg was found in one soy protein sample and it was exceeded in one soy flour sample (Schollenberger et al., 2007). In 2008 Schollenberger et al. (2008) investigated the presence of zearalenone also in edible oils. Zearalenone was found in 12 samples ($n = 110$). Three samples of soybean oil and 9 samples of corn germ oil were contaminated and the mean and maximum zearalenone concentrations were 385 and 1730 $\mu\text{g}/\text{kg}$, respectively. The zearalenone concentration was lower in refined oils.

In the earlier German study, soybean feedstuffs collected arbitrarily from the local feed industry did not reveal significant contamination with zearalenone, deoxynivalenol or aflatoxin B1 (Valenta et al., 2002). The median and maximum zearalenone concentration of 25 soybean meal samples with hulls amounted to 5 and 18 $\mu\text{g}/\text{kg}$, respectively, while 25 soybean meal samples without hulls (so-called high protein soybean meal) contained < 3 $\mu\text{g}/\text{kg}$ (LOD) and 12 $\mu\text{g}/\text{kg}$, respectively. One soybean hull sample contained 6 μg zearalenone/kg. However, 4 suspicious samples of high protein soybean meal were characterized by rather high median and maximum zearalenone levels of 280 and 363 $\mu\text{g}/\text{kg}$.

In a German survey from 2000-2007, 407 samples of wheat and 514 of rye were tested for zearalenone. Zearalenone was detected in 41 % of the wheat samples with maximum, mean and median values of 451, 72 and 35 $\mu\text{g}/\text{kg}$, respectively (Meister, 2009). The ML of 100 $\mu\text{g}/\text{kg}$ was exceeded in 9 % of the wheat samples. Only 16 % of the rye samples were positive for zearalenone and maximum, mean and median values were 37, 23 and 23 $\mu\text{g}/\text{kg}$, respectively. Cereal samples from the harvest of years 2002 and 2007 had higher zearalenone levels compared to the other years (Meister, 2009). In a two-year survey in Germany, Goertz et al. (2010) found that 27 % of the maize samples analysed ($n = 44$) in 2006 were contaminated with zearalenone with a mean level of 70 $\mu\text{g}/\text{kg}$. In 2007 zearalenone was found in 93 % of the maize samples ($n = 40$), with a mean concentration of 480 $\mu\text{g}/\text{kg}$, and α -zearalenol was detected in 10 % of samples (mean concentration 5 $\mu\text{g}/\text{kg}$, $n = 40$).

In a UK study on raw maize imported mainly from France and Argentina, zearalenone was detected in almost all of the samples analysed ($n = 139$) (Scudamore and Patel, 2000). Only in four samples were the zearalenone levels $< \text{LOD}$ (4 $\mu\text{g}/\text{kg}$). In 42 % of samples the zearalenone concentration exceeded 100 $\mu\text{g}/\text{kg}$ (maximum level 584 $\mu\text{g}/\text{kg}$) (Scudamore and Patel, 2000). Later the same authors reported that 33 maize samples originating from France were contaminated with zearalenone at a mean level of 26 $\mu\text{g}/\text{kg}$ ($n = 56$) (Scudamore and Patel, 2009). In a recent study (Vendl et al., 2010) a total of 84 cereal-based food products were collected in the UK and Austria. For every product three to four different commercial brands were purchased and combined into one composite sample. A total of 25 composite samples were analysed for zearalenone and its metabolites. The most abundant zearalenone metabolite was zearalenone-4- sulphate found in 13 composite samples but with low concentrations (1.3-6.1 $\mu\text{g}/\text{kg}$). Zearalenone was detected in seven composite samples, while none of the other metabolites analysed (zearalenol-4-glucopyranoside, α - and β -zearalenol, α - and β -zearalenol-4-glucopyranoside) was detected. The composite sample of bran flakes showed the highest levels with 6.1 $\mu\text{g}/\text{kg}$ of zearalenone-4-sulphate and 44.2 $\mu\text{g}/\text{kg}$ of zearalenone. The 3 bran flake samples used for the composite sample were then analysed individually. All the three samples contained zearalenone-4-sulphate and zearalenone with concentrations similar to those reported for the composite sample.

In a French total diet study, 176 food types were sampled in three different regions and in two different seasons (Leblanc et al., 2005). A total of 2,280 individual samples were collected and 245 composite samples were prepared by combining 5 individual food samples and tested for zearalenone. Only 2 % of the composite samples showed zearalenone levels above the LOD (approximately 10 $\mu\text{g}/\text{kg}$): muesli (200 $\mu\text{g}/\text{kg}$), cornflakes (22 $\mu\text{g}/\text{kg}$), canned soybean (53 $\mu\text{g}/\text{kg}$) and sesame seeds (18 $\mu\text{g}/\text{kg}$).

In 2007-2008 zearalenone was monitored in wheat in Luxemburg and was observed only in 2 wheat samples with 200 and 113 $\mu\text{g}/\text{kg}$ (Giraud et al., 2010). A Belgian survey (Pussemier et al., 2006) on organic and conventional wheat in 2002 and 2003 reported a higher and more frequent zearalenone contamination in conventionally than organically cultivated wheat. In 2002 the incidence rates of zearalenone were 85 and 52 % in conventional and organic samples, respectively. Six samples of conventional wheat showed zearalenone concentrations above 100 $\mu\text{g}/\text{kg}$, while none of the organic samples exceeded this level. Almost all the samples collected in 2003 were below the LOD (1.5 $\mu\text{g}/\text{kg}$) and only three samples had trace levels of zearalenone ($< \text{LOQ}$ of 4 $\mu\text{g}/\text{kg}$) (Pussemier et al., 2006). Hoogenboom et al. (2008) collected organically ($n = 31$) and conventionally ($n = 40$) cultivated summer and winter wheat samples in the Netherlands in 2003 and 2004. In these samples the zearalenone concentrations varied from $< 50 \mu\text{g}/\text{kg}$ (LOD) up to 5700 $\mu\text{g}/\text{kg}$. The high concentrations of zearalenone were detected in wheat after heavy rainfall in the autumn 2004. Overall there was no significant difference in zearalenone concentrations between organic and conventional wheat.

Wheat and rye flour samples were collected in 1998-2001 from mills and retail market in Denmark (Rasmussen et al., 2003). Zearalenone was detected in 10 of 30 wheat flour samples and in 2 of 30 rye flour samples. The concentration levels were close to the LOD of 1 $\mu\text{g}/\text{kg}$. The maximum level did not exceed 2 $\mu\text{g}/\text{kg}$ (Rasmussen et al., 2003). Bernhoft et al. (2010) analysed 248 samples of organic and conventionally produced barley, oats and wheat harvested during 2002 and 2003 in Norway. Zearalenone was detected in only a few samples (not specified) and the maximum concentrations were 9 and 27 $\mu\text{g}/\text{kg}$ in organic and conventional oats, respectively. In Lithuania, Suproniene et al. (2010) detected zearalenone in nearly all the organically cultivated spring and winter cereal (barley, oat, wheat and triticale) samples at low concentrations ranging from 0 to 50.4 $\mu\text{g}/\text{kg}$ in 2005 and 2006 ($n = 36$).

A total of 91 grain samples (54 wheat, 18 barley and 19 maize samples) were collected in Bulgaria during 2007 and tested for zearalenone (Manova and Mladenova, 2009). The incidence of positive samples was higher in maize (21 %) than in barley (11 %) and wheat samples (2 %). The mean levels in the wheat, barley and maize samples were 29.0, 10.0 and 80.6 μg zearalenone/kg, respectively. The highest level was observed in maize (148.0 $\mu\text{g}/\text{kg}$) (Manova and Mladenova, 2009). Zearalenone was determined in 54 corn, 35 wheat and 21 barley samples collected in Romania in 2002-2004. More than 90 % of the samples were contaminated with zearalenone and 33 %, 40 % and 71 % of maize, wheat and barley samples, respectively, had levels above 100 $\mu\text{g}/\text{kg}$ (Tabuc et al., 2009).

A total of 46 maize samples collected in 2002 in Italy was analysed for zearalenone and its metabolites α - and β -zearalenol, α - and β -zearalanol and zearalanone (Cavaliere et al., 2005). Zearalenone was detected in 30 % of the samples with levels ranging between 8 and 969 $\mu\text{g}/\text{kg}$, while α - and β -zearalenol were detected in 28 % of samples with concentrations up to 33 $\mu\text{g}/\text{kg}$. The other metabolites were only rarely detected. β -Zearalanol and zearalanone were found in only two samples while α -zearalanol was not found (Cavaliere et al., 2005). The samples of breakfast cereal ($n = 43$) and baby food ($n = 44$) marketed in Italy were collected under the official monitoring plan and analysed for zearalenone (LOD = 10 $\mu\text{g}/\text{kg}$) (Romagnoli et al., 2010). All the samples were $< \text{EU MLs}$ and only 4 breakfast cereal samples and 1 baby food sample contained zearalenone.

In Portugal, 300 samples including 132 samples of maize and maize-based foods, 82 wheat and wheat-based foods, 25 barley, 53 soybean and 8 sunflower, were analysed for zearalenone. It was detected in 178 samples (56 %). The mean level was 70 $\mu\text{g}/\text{kg}$ and the highest concentration (900 $\mu\text{g}/\text{kg}$) was found in a maize sample (Marques et al., 2008). In Spain, Santos et al. (2010) collected 64 paprika and 35 chilli pepper samples and reported zearalenone incidences of 39 % in paprika and 46 % in chilli pepper samples. The level of 100 $\mu\text{g}/\text{kg}$ was exceeded in 3 % of paprika and 9 % of chilli pepper samples.

Nine European countries provided occurrence data for a total of 4,918 cereal and cereal-based food samples within the Scientific co-operation (SCOOP) task 3.2.10 (collected 1990-2002) (SCOOP, 2003). Zearalenone was detected in 1,591 samples with an incidence of 32 %. The food commodities that

showed the highest incidences and levels of zearalenone were maize, wheat and their respective products. Zearalenone was detected in 70 % out of 1,265 maize and maize product samples (10 % showed concentrations above 200 µg zearalenone/kg). The highest concentration of 6492 µg/kg was reported in a maize grain sample from Italy. Regarding wheat and wheat products (n = 1,900) 25 % of samples were positive for zearalenone but only two samples exceeded the level of 200 µg/kg (the highest concentration of 510 µg/kg was observed in a wheat bran sample from Italy). A high incidence of zearalenone was also found in oat samples from Finland and 44 % of the samples contained more than 200 µg zearalenone/kg with the reported maximum concentration of 1310 µg zearalenone/kg (SCOOP, 2003).

4.1.2. Foods of animal origin

4.1.2.1. Carry over studies

The EC responded to the EFSA opinions on deoxynivalenol, zearalenone, ochratoxin A and fumonisins in feedstuffs (EFSA, 2004a, 2004b, 2005, 2006a) with the release of a recommendation for critical orientation (guidance) values for these toxins in feedstuffs and for monitoring of the simultaneous occurrence of these mycotoxins (Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 toxin and HT-2 toxin and fumonisins in products intended for animal feeding (2006/576/EC)¹⁴). Compliance with these guidance values ensures that no adverse effects on animal health and performance occur under practical production conditions and that no significant transfer of mycotoxins into animal foodstuffs occurs. For all mentioned mycotoxins the lowest guidance values for critical concentrations in feedstuffs were established for pigs because of their special sensitivity among farm animals. For zearalenone complementary and complete feedingstuffs (i.e. the daily ratio) for piglets and gilts (young sows) must not exceed 0.1 mg/kg (88 % dry matter content), while the critical limit for sows and fattening pigs was set at 0.25 mg/kg, and those for calves, dairy cattle, sheep (including lambs) and goats (including kids) at 0.5 mg/kg.

These orientation values for critical mycotoxin concentrations for complete feedingstuffs should be kept in mind when evaluating carry-over studies with farm animals where, often, higher contaminated diets were tested. Thus, the cited residue levels below do not necessarily reflect levels in food on the market (see Section 4.2) but give an indication of transfer and of the possible contribution of mycotoxin residues in animal products to the overall dietary exposure.

Zearalenone and α -zearalenol were detected in livers of laying hens fed a diet containing 1.1 mg zearalenone/kg diet for 112 days at concentrations of < 1-3.2 µg/kg (46 % free, 54 % conjugated with glucuronic acid) 3.5-3.8 µg/kg (36 % free, 28 % conjugated with glucuronic acid, and 36 % with sulphate), respectively. LODs for zearalenone and α -zearalenol in liver were 1 and 0.5 µg/kg, respectively. No residues were detected in yolk, albumen, breast muscle, abdominal fat, ovary and follicles and in the magnum (Dänicke et al., 2002b). The concentrations of the other measured metabolites β -zearalenol, zearalanone, α -zearalanol and β -zearalanol were all < LODs. The LODs were 3-7 µg/kg for β -zearalenol, 20-35 µg/kg for zearalanone and α -zearalanol, and 40-70 µg/kg for β -zearalanol in tissues, yolk, albumen, abdominal fat, ovary, follicles and magnum.

Similarly, residue levels of zearalenone and its metabolites were not detected (LOD of 0.1 µg/kg) in eggs collected from laying hens fed a diet containing 0.5 mg zearalenone/kg (Sypecka et al., 2004).

Dietary zearalenone concentrations of 0.05 to 0.06 mg/kg fed to Peking ducks for 49 days resulted in a dose-dependent increase in the concentration of total zearalenone, α - and β -zearalenol in bile, but concentrations were < LODs for zearalenone, α -zearalenol, β -zearalenol, zearalanone, α -zearalanol and β -zearalanol, respectively, in the liver. LODs were 1, 0.5, 5, 100, 50 and 200 µg/kg in bile and liver for

¹⁴ OJ L 229, 23.8.2006, p. 7-9.

zearalenone, α -zearalenol, β -zearalenol, zearalanone, α -zearalanol and β -zearalanol, respectively (Dänicke et al., 2004).

Zearalenone or its metabolites were not detectable in plasma, liver or breast meat of turkeys fed diets containing up to 0.04 mg zearalenone/kg from day 21 to 56 of age while the concentrations of total zearalenone and α -zearalenol in bile increased with dietary zearalenone concentration. (LODs for all specimens were 1, 0.5, 5, 100, 50 and 200 $\mu\text{g}/\text{kg}$ for zearalenone, α -zearalenol, β -zearalenol, zearalanone, α -zearalanol and β -zearalanol, respectively) (Dänicke et al., 2007b).

In piglets fed diets with increasing zearalenone concentrations of 0.01, 0.06, 0.15, 0.22, 0.42 mg/kg diet for 5 weeks (mean body weight ~ 12-33 kg) the mean total zearalenone and α -zearalenol concentrations in the liver were 1.8 and 0.3, 0.2 and 0.1, 2.1 and 1.1, 2.9 and 1.7, and 5.3 and 2.8 $\mu\text{g}/\text{kg}$, respectively (Döll et al., 2003b). The concentrations of β -zearalenol, zearalanone, α -zearalanol and β -zearalanol were < LODs of 5, 100, 50 and 200 $\mu\text{g}/\text{kg}$ liver, respectively. The LODs for zearalenone and α -zearalenol in liver were 1 and 0.5 $\mu\text{g}/\text{kg}$, respectively (Döll et al., 2003b).

Maximum total zearalenone, α -zearalenol and β -zearalenol concentrations of 3.1, 12 and 4.8 $\mu\text{g}/\text{kg}$ were measured in the liver of fattening pigs fed a diet containing 0.7 mg zearalenone/kg for 18 days while in muscle α -zearalanol and α -zearalenol contents of up to 13.3 and 14.5 $\mu\text{g}/\text{kg}$ were measured. The LODs for zearalenone and its metabolites varied 0.1-0.3 $\mu\text{g}/\text{kg}$ liver (Zöllner et al., 2002). At a much lower dietary zearalenone concentration of 0.05 mg/kg diet only α -zearalenol could be detected in the liver of fattening pigs at a concentration level of 1 $\mu\text{g}/\text{kg}$ (LOD) while zearalenone and β -zearalenol concentrations were lower than the LODs of 1 and 4 $\mu\text{g}/\text{kg}$ liver, respectively) (Goyarts et al., 2007).

For sows fed a diet containing zearalenone (0.358 mg/kg) from gestation days 75 to 110 maximum total zearalenone and α -zearalenol concentrations of 2.3 and 3.4 $\mu\text{g}/\text{kg}$ were determined in their livers while β -zearalenol, zearalanone, α -zearalanol and β -zearalanol concentrations were < LODs of 5, 100, 50 and 200 $\mu\text{g}/\text{kg}$ liver, respectively. The LODs for zearalenone and α -zearalenol in liver were 1 and 0.5 $\mu\text{g}/\text{kg}$. The corresponding zearalenone, α - and β -zearalenol contents in bile were 627, 388 and 22 $\mu\text{g}/\text{kg}$. From regressive evaluation of the corresponding sow and full-term piglet data it was determined that the concentration of these metabolites increased by 0.04 $\mu\text{g}/\text{kg}$ in the bile of the piglets when the corresponding concentrations in the bile of the sows increased by 1 $\mu\text{g}/\text{kg}$ (Dänicke et al., 2007a).

Zearalenone, α -zearalenol, β -zearalenol, zearalanone, α -zearalanol and β -zearalanol concentrations in the milk of Holstein dairy cows were < LODs of 1, 0.5, 5, 100, 50 and 200 $\mu\text{g}/\text{kg}$, respectively, at daily zearalenone intakes between 0.075 and 1.125 mg corresponding to a dietary zearalenone concentration of 0.051 mg/kg dry matter (Seeling et al., 2005). Dry matter intake varied between 5.6 and 20.5 kg/day, and milk yields ranged between 10 and 42 kg fat corrected milk per day in this study.

Total maximum zearalenone, α -zearalenol and β -zearalenol concentrations of 24, 11 and 53 $\mu\text{g}/\text{kg}$ were measured in the bile of growing bulls fed a ration containing 0.11 mg zearalenone/kg dry matter for up to 160 days while the concentrations of zearalenone, α -zearalenol, β -zearalenol, zearalanone, α -zearalanol, β -zearalanol were < LODs of 1, 0.5, 5, 100, 50 and 200 $\mu\text{g}/\text{kg}$, respectively, in muscle, liver, kidney and back fat (Dänicke et al., 2002a).

In a heifer given 1.274 mg zearalenone per day for 84 days the maximum liver zearalenone, α -zearalenol and β -zearalenol concentrations were 1.2, 1.2 and 11.5 $\mu\text{g}/\text{kg}$, respectively (LOD of 1.0 $\mu\text{g}/\text{kg}$ liver), while residues in muscle were < LOD of 0.5 $\mu\text{g}/\text{kg}$ (Kleinova et al., 2002).

For an evaluation of risks to the consumer arising from possible residues of zearalenone and its metabolites in foods of animal origin due to feeding of zearalenone contaminated diets to food-producing farm animals, the ratio between experimentally measured zearalenone residues in food and its concentration in feed (also referred as the 'carry over factor') might provide a rough estimate. Due to

the high entero-hepatic recirculation of zearalenone and its metabolites, the liver is at special risk for possible contamination. Carry over factors for this edible tissue of 0.016, 0.067 and 0.005 have been reported for sows, fattening pigs and laying hens at diet any concentrations of 0.358, 0.009 and 1.1 mg/kg, respectively (Dänicke et al., 2002b, 2007a,b). Neglecting the differences in exposure time, species, diet concentration and other variables potentially influencing the carry over factor, it can be estimated that a 70 kg human would have to consume 18, 4 and 56 kg of such contaminated sow, fattening pig and laying hen liver daily to approach the SCF t-TDI of 0.2 µg/kg b.w. Given the fact that consumption of contaminated foodstuffs of plant origin lacks this dilution by the animal it seems reasonable to conclude that the possible contribution of zearalenone residues in animal products is negligible for the total zearalenone exposure of the consumer.

4.1.2.2. Foods of animal origin that could be consumed

A total of 20 egg samples from laying hens collected from private breeders in Belgium in autumn 2006 and spring 2007 were analysed for zearalenone, α -zearalenol and β -zearalanol (Tangni et al., 2009). None of the samples were reported to have quantifiable concentrations of zearalenone, α -zearalenol and β -zearalanol. However, trace levels ($<$ LOQ) were reported for most of the samples collected during the autumn period. From 6 to 9 samples out of 10 samples contained zearalenone, α -zearalenol and/or β -zearalanol. For most of the 10 samples collected in spring $<$ LOD concentrations for zearalenone, α -zearalenol and β -zearalanol were reported. The reported LODs were 3.0, 1.5 and 6.0 µg/kg for zearalenone, α -zearalenol and β -zearalanol, respectively and the LOQs 10.0, 5.0 and 20.0 µg/kg, respectively (Tangni et al., 2009).

In a French total diet study, 176 food types were sampled in three different regions and in two different seasons (Leblanc et al., 2005). A total of 2,280 individual samples were collected and 245 composite samples were prepared by combining 5 individual food samples and tested for zearalenone. Out of 245 composite samples 30 were egg and egg product samples and 6 offal samples. Zearalenone was not detected in any of these animal product samples (LOD approximately 10 µg/kg) (Leblanc et al., 2005).

In Greece 300 bovine meat samples were analysed for steroids including zearalenone, α -zearalenol, β -zearalenol, α -zearalanol and β -zearalanol during the national residue control (Kaklamanos et al., 2009). The decision limits ($CC\alpha$) were from 0.09 to 0.11 µg/kg and the detection capabilities ($CC\beta$) from 0.15 to 0.24 µg/kg. Zearalenone and its metabolites were not found in any of the samples analysed (Kaklamanos et al., 2009).

4.2. Current occurrence results

4.2.1. Data collection summary

The Dietary and Chemical Monitoring Unit (DCM) (former Data Collection and Exposure Unit, DATEX) call for data on zearalenone in food¹⁵ was launched in July 2010. European national food authorities and similar bodies, research institutions, academia, food business operators and any other stakeholders were invited to submit analytical data on zearalenone in food by November 2010. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a).

Data were received from national food authorities or similar bodies, research laboratories and associations of food and feed business operators. They covered food but also unprocessed grains of undefined end-use (hereafter referred as unprocessed grains) and even feed. Based on the information provided, separate data sets were extracted for food and for unprocessed grains, respectively.

¹⁵ <http://www.efsa.europa.eu/en/dataclosed/call/datex100729.htm>

Analytical results with incomplete or incorrect description of the relevant variables (e.g. parameter type, food classification, result value, LOD or LOQ) were not included in the data set used in this assessment. The data set was checked for duplicates (same samples transmitted twice or repeated analysis of the same sample) and all duplicates were excluded. Results obtained on samples collected before 2005 were not included in the final data set as they may not reflect the zearalenone contamination found in recent years. In this case, five years (2005-2009) were fully covered in the sampling. The year 2010 was not a complete sampling year, as the closing date of the call for data on zearalenone was November 2010. Data on food samples collected before 2005 as well as data on feed are stored in the EFSA database and might be used in the future for other assessments.

After applying the cleaning and validation steps, a total of 13,075 analytical results obtained on food samples and 9,877 results on ‘Unprocessed grains’ were retained for the evaluation. Data on food and ‘Unprocessed grains’ samples were obtained on samples collected in 19 European countries. The sampling country and the country which submitted the data to EFSA was not always the same. The distribution of food samples across the European countries where food samples were collected is illustrated in Figure 3. Most of the results (62 %) were on samples collected in Germany followed by France (12 %) and Slovakia (8 %). The distribution of food samples over the sampling years is presented in Figure 4. The yearly sampling size varied roughly between 1,000 and 3,000 samples.

The distribution of ‘Unprocessed grains’ samples across the European countries and over the sampling years is presented in Figure 5 and Figure 6, respectively.

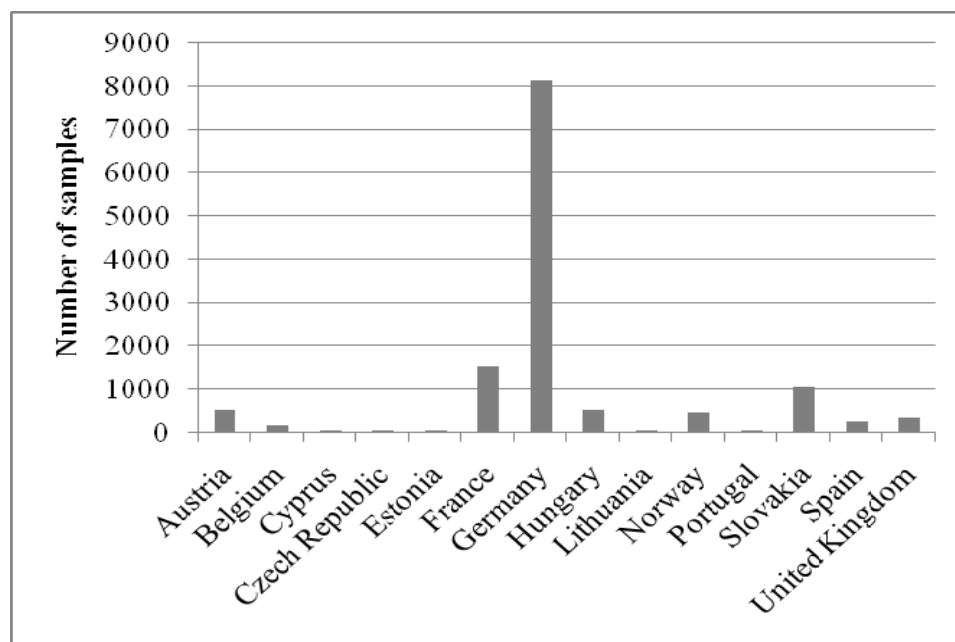


Figure 3: Distribution of food samples across European countries.

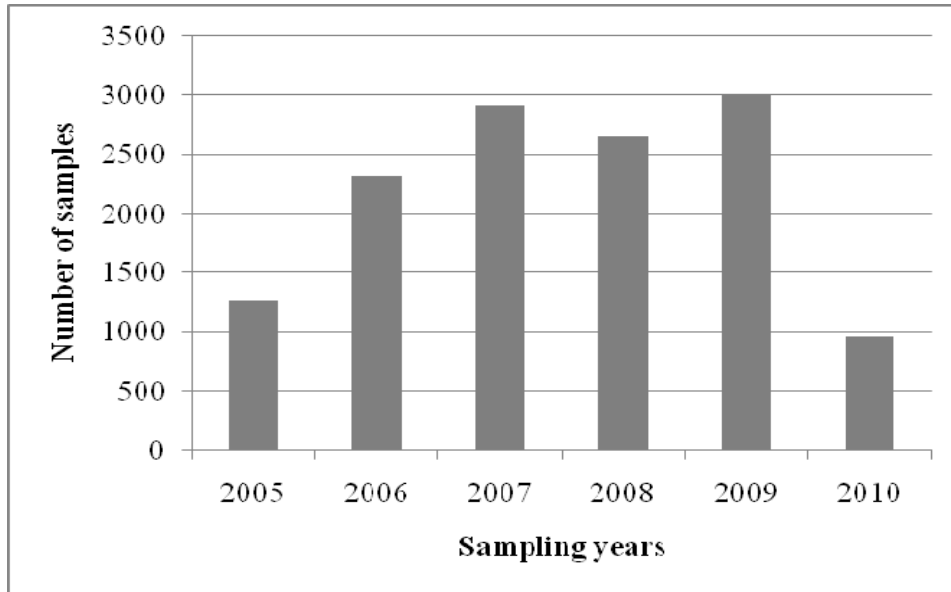


Figure 4: Distribution of food samples over the years of sampling.

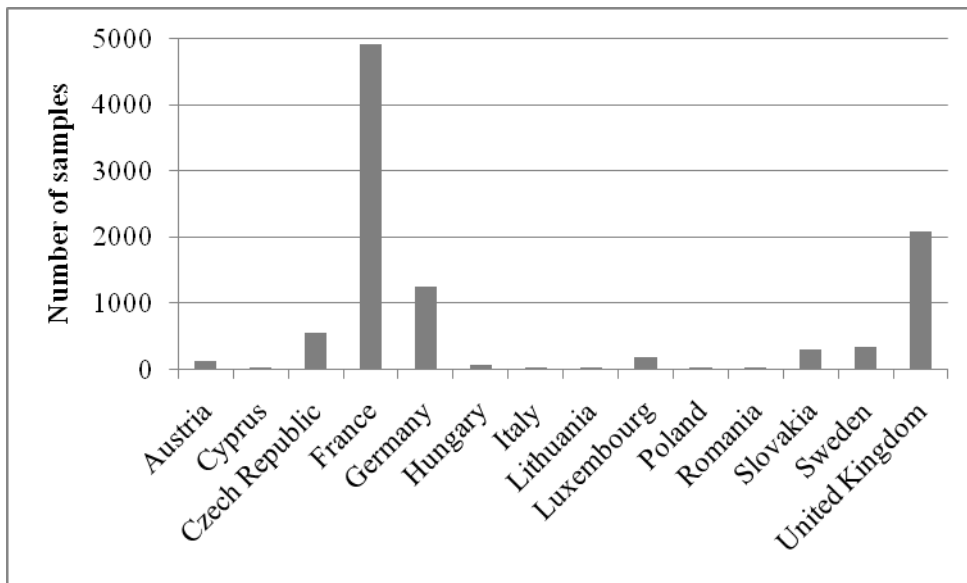


Figure 5: Distribution of 'Unprocessed grains' samples across European countries.

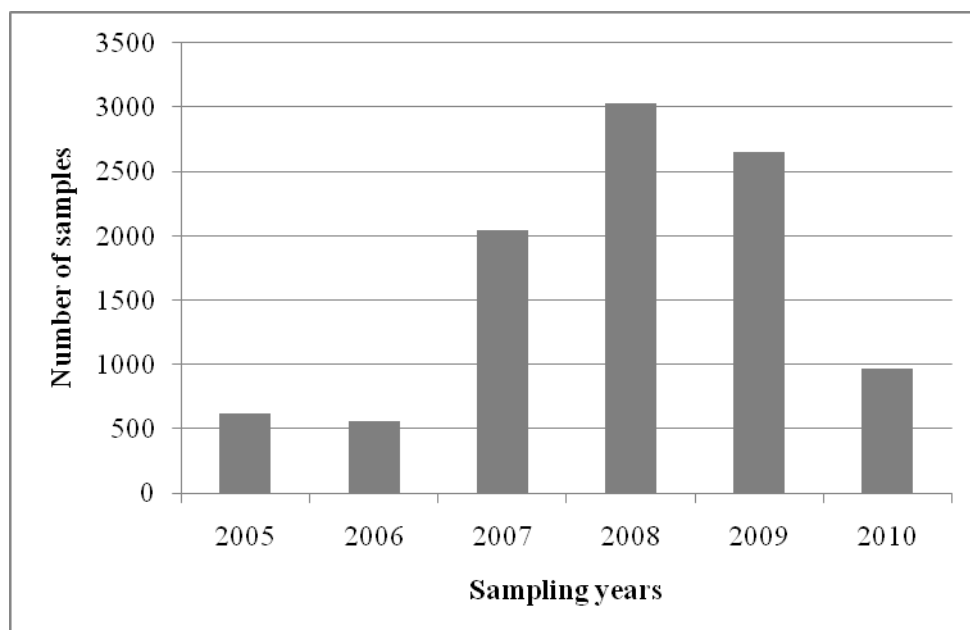


Figure 6: Distribution of ‘Unprocessed grains’ samples over the years of sampling.

4.2.2. Distribution of samples across food groups

The food samples were classified according to the FoodEx classification system (EFSA, 2011a). FoodEx is a flexible food classification system developed by the DCM Unit in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food groups (first level), which are further divided into subgroups having 140 items at the second level, 1,261 items at the third level and reaching about 1,800 end-points (food names or generic food names) at the fourth level. The spread of the analytical results for zearalenone across the several FoodEx groups prevented calculation of summary statistics at a very detailed level of the food classification system. Thus, food items were aggregated to an upper level where the number of samples was sufficiently large to provide robust statistics. Broad food groups with only a limited number of samples ($n < 30$), e.g. ‘Legumes, nuts and oil seeds’ ($n = 20$; 90 % non-detects), ‘Sugar and confectionary’ ($n = 5$; all non-detects), ‘Fish and other seafood’ ($n = 14$; all non-detects), ‘Composite food’ ($n = 27$; all non-detects), or not classifiable foods were all included in the group ‘Other foods’.

The vast majority of data were on grains and grain-based foods. The group ‘Grain milling products’ dominated the product coverage with 37 % of the total samples, followed by ‘Grains for human consumption’ (17 %), ‘Bread and rolls’ (9.5 %), ‘Breakfast cereals’ (11 %), ‘Fine bakery wares’ (6.2 %) and ‘Pasta’ (2.5 %). Other food groups represented were ‘Meat and meat products’ (9.6 %), ‘Food for infants and small children’ (3.2 %), ‘Vegetable oils’ (1.7 %), ‘Vegetables and vegetable products’ (0.8 %), ‘Products for special nutritional use’ (0.4 %), ‘Beer’ (0.3 %) and ‘Snacks’ (0.9 %).

The distribution of samples across the aggregated food groups is shown in Figure 7. A more detailed distribution in less aggregated food groups is presented in Section 4.2.4.

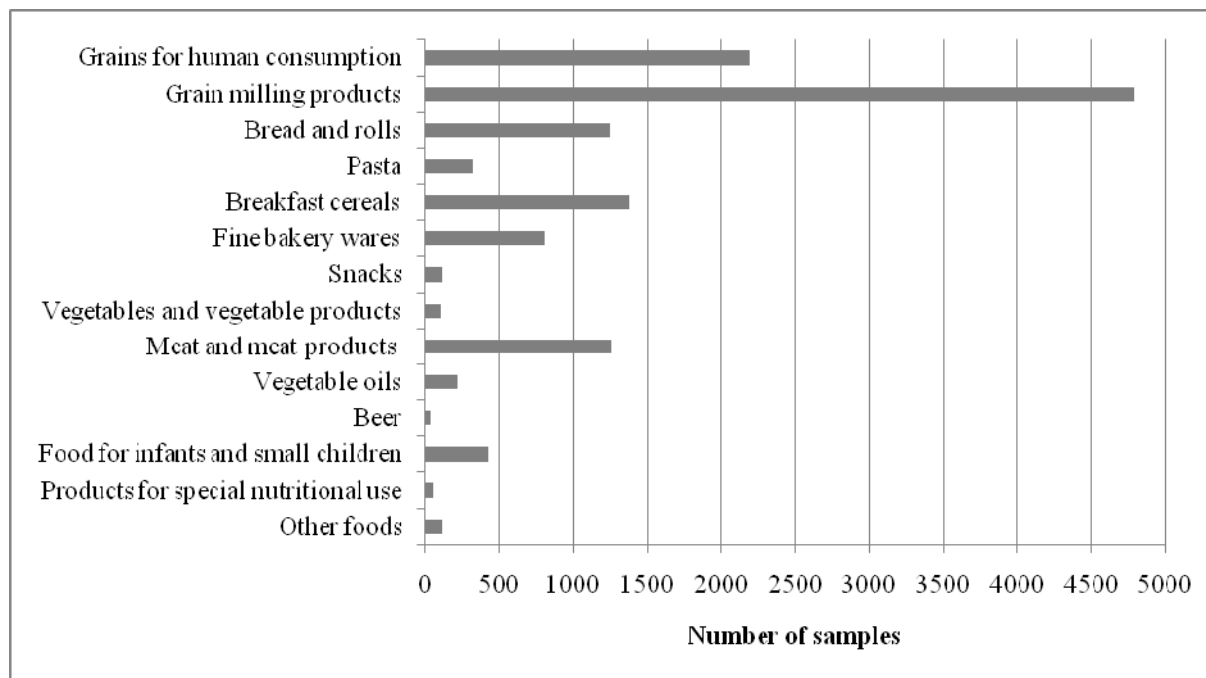


Figure 7: Distribution of samples across the aggregated food groups.

4.2.3. Analytical methods used

Data on zearalenone in food were obtained by applying HPLC-based methods (e.g. LC-MS/MS, HPLC-FLD) (45 %), ELISA (13 %) and GC-MS (0.3 %). For 42 % of the food samples, the method of analysis was not reported. However, the data provider communicated that most of the samples were analysed by LC-MS/MS and other HPLC methods.

LODs and LOQs were not reported for all observations. To enable a comparison of the LOQs applied across food groups, missing LOQs (2.4 %) were estimated by multiplying the reported LODs by three. All the measurements were converted to $\mu\text{g}/\text{kg}$ or $\mu\text{g}/\text{L}$.

The LOQs varied with the method applied, the food matrix and the laboratory (Figure 8). The median value for LOQ in grains, milling products and food groups based on cereal grains varied between 5 and 10 $\mu\text{g}/\text{kg}$. Higher LOQs have been observed for commodities with high fibre content (bran, certain breakfast cereals) which can absorb a high portion of the extraction solvent during sample extraction and thus a higher dilution factor is applied compared to matrices with low fibre content.

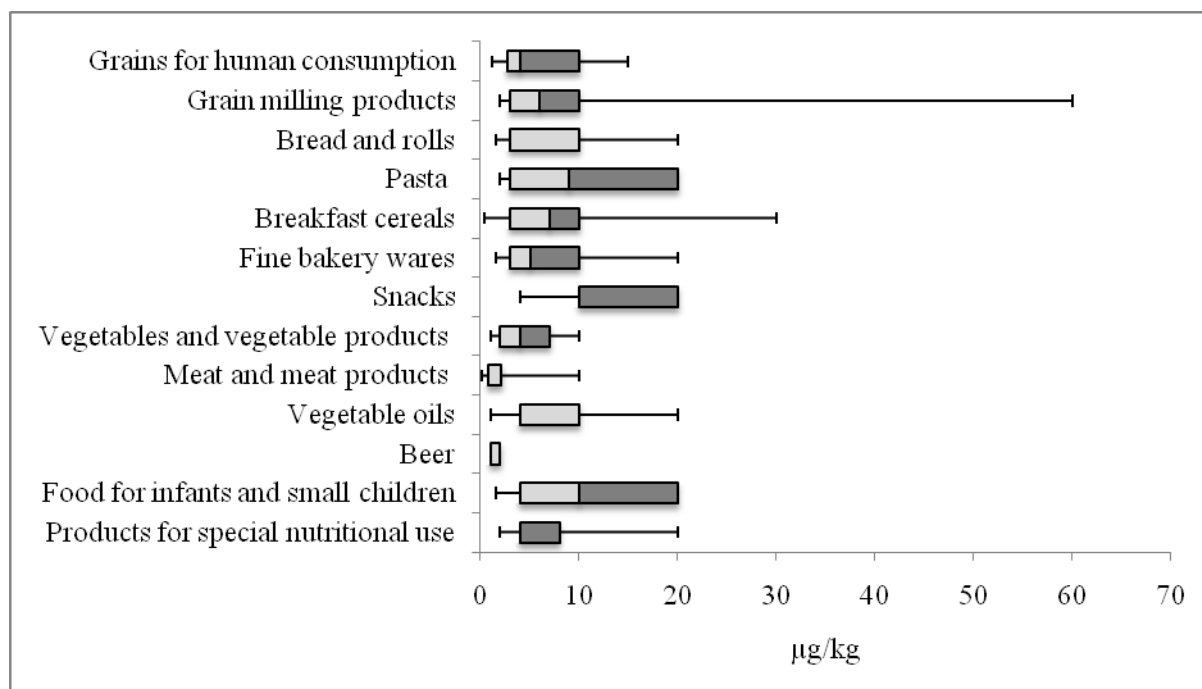


Figure 8: Distribution of the limit of quantification (LOQ) across aggregated food groups. (Box-plot: whiskers at 5th percentile and 95th percentile, box at 25th percentile and 75th percentile with line at 50th percentile).

The non-detects accounted for more than 80 % of the observations in the majority of food groups (Figure 9). The lowest proportion of non-detects was observed in ‘Vegetable oils’ (13 %). The high contamination frequency in ‘Vegetable oils’ is linked to the corn germ oil samples which dominate the group (see Section 4.2.4). Compared to other cereal-based food groups, a relatively lower percentage of non-detects (76 %) was observed in the ‘Fine bakery wares’. A possible explanation could be the high content of wheat bran in certain types of biscuits.

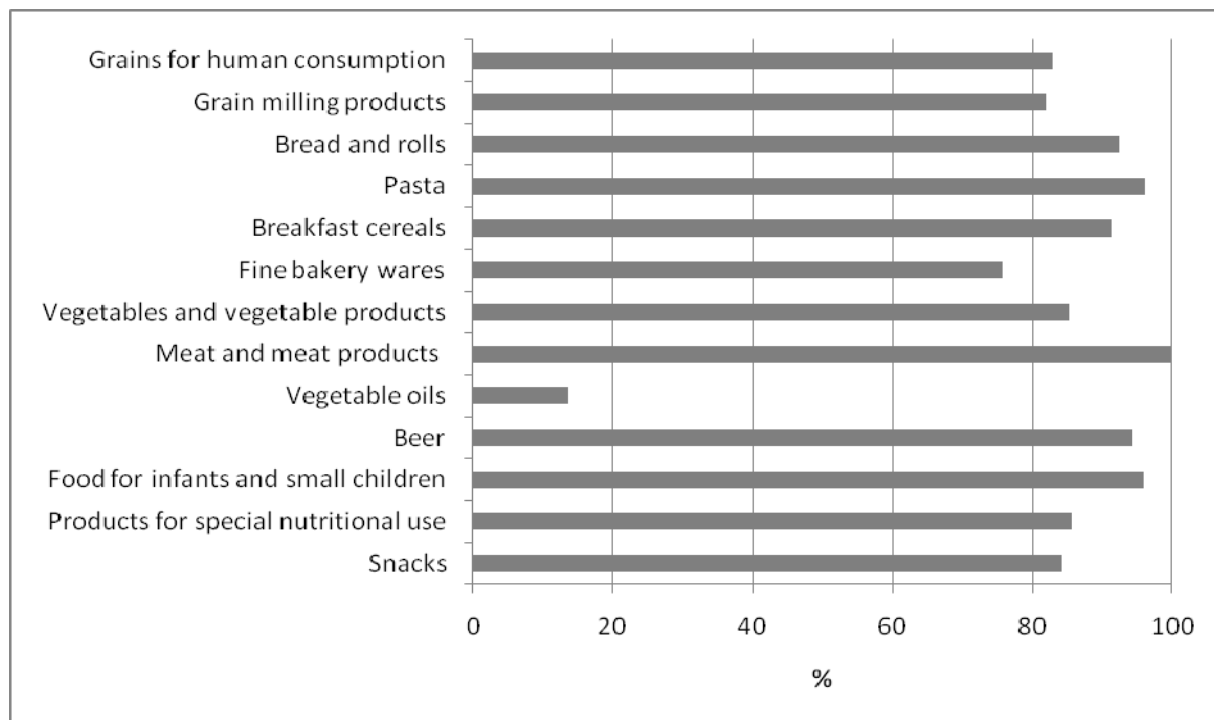


Figure 9: Percentage of analytical results below the limit of quantification (LOQ).

4.2.4. Occurrence data by food group

The proportion of analytical records reported as quantified values was only 15 % out of 13,075 observations in food. Therefore, left-censored data (non-quantifiable results) had to be carefully considered. As recommended in the ‘Principles and Methods for the Risk Assessment of Chemicals in Food’ (WHO, 2009) left-censored data were treated by the substitution method. The same method is indicated in the EFSA scientific report ‘Management of left-censored data in dietary exposure assessment of chemical substances’ (EFSA, 2010b) as an alternative in the treatment of left-censored data. The guidance suggests that the lower-bound (LB) and upper-bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD ($< \text{LOD}$) or LOQ ($< \text{LOQ}$). The UB is obtained by assigning the numerical value of LOD to values reported as $< \text{LOD}$, and LOQ to values reported as $< \text{LOQ}$ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

The analytical results were transmitted by the data providers as either corrected or not corrected for recovery. Where results were not corrected by the data provider a correction has been applied by using the reported recovery rate. Where recovery was not available, no correction has been applied.

4.2.4.1. Grains for human consumption

The food group ‘Grains for human consumption’ consists of 2,190 samples covering the most cultivated cereals. The highest contamination levels and the highest contamination frequency in the group ‘Grains for human consumption’ were observed in corn (LB mean = 13 $\mu\text{g}/\text{kg}$; UB mean = 15 $\mu\text{g}/\text{kg}$) (Table 2). Wheat, barley, rice and oats had similar mean concentrations. Lower concentrations were observed in rye and spelt.

Table 2: Occurrence of zearalenone in the food group ‘Grains for human consumption’.

Food group	N ^(a)	>LOD (%)	Concentration (µg/kg)					
			LB/UB	Mean	P50	P75	P95	Maximum
Grains for human consumption	2190	17	LB	2.7	0.0	0.0	14	140
			UB	5.7	3.0	5.0	20	140
Wheat	1013	22	LB	2.4	0.0	0.0	10	76
			UB	5.4	4.0	10	13	76
Barley	193	12	LB	2.6	0.0	0.0	15	73
			UB	5.6	3.0	4.0	26	73
Corn	137	33	LB	13	0.0	6.4	94	140
			UB	15	3.3	12	94	140
Rye	415	6.0	LB	0.7	0.0	0.0	3.7	32
			UB	3.4	2.0	5.0	6.0	32
Spelt	73	5.5	LB	0.3	0.0	0.0	2.2	14
			UB	3.5	3.0	5.0	10	14
Oats	182	15	LB	2.0	0.0	0.0	11	86
			UB	3.7	2.0	2.5	12	86
Rice	132	15	LB	3.8	0.0	0.0	30	87
			UB	9.7	2.7	20	38	87
Other grains	43	17	LB	0.9	0.0	0.0	5.0	20
			UB	3.4	2.0	4.0	14	20

N: number of samples; > LOD: indicates the percentage of results above the LOD or LOQ; LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.

(a): If N < 60 then the calculated P95 should be considered only as an indicative value (EFSA, 2011b).

4.2.4.2. Grain milling products

‘Grain milling products’ was the dominating food group (n = 4,793). Among wheat milling products the highest concentrations and contamination frequency were reported for wheat bran (LB mean = 18 µg/kg; UB mean = 33 µg/kg). Zearalenone was more frequently found in wholemeal wheat flour compared to white wheat flour but the mean concentration was higher in white wheat flour. This is explained by the high levels of zearalenone reported in a few samples (maximum = 460 µg/kg). Wheat semolina was less contaminated compared to wheat flour. This is in line with the studies which demonstrated that zearalenone is selectively distributed in the different milling fractions. Similarly to the ‘Grains for human consumption’, high concentrations and contamination frequency were observed in ‘Corn milling products’ (LB mean = 12 µg/kg; UB mean = 14 µg/kg) with higher levels in corn flour compared to corn semolina. Milling products of rye, oats and spelt were less frequently contaminated with zearalenone. There was no significant difference between the mean concentrations in the different types of rye flour (Table 3).

Table 3: Occurrence of zearalenone in the food group ‘Grain milling products’.

Food group	N ^(a)	>LOD (%)	Concentration (µg/kg)					
			LB/UB	Mean	P50	P75	P95	Maximum
Grain milling products	4793	18	LB	5.4	0.0	0.0	30	509
			UB	12	5.0	10	50	509
Wheat milling products	3088	14	LB	4.9	0.0	0.0	28	507
			UB	13	5.0	10	50	507
Wheat flour, white	1944	5.5	LB	1.8	0.0	0.0	2.0	460
			UB	9.3	5.0	10	40	460
Wheat flour, wholemeal	287	28	LB	1.5	0.0	2.4	7.7	23
			UB	4.7	3.0	5.0	11	50
Wheat bran	615	37	LB	18	0.0	17	94	507
			UB	33	23	50	97	507
Wheat semolina, Durum	123	4.1	LB	0.06	0.0	0.0	0.0	3.2
			UB	3.0	3.0	3.0	3.0	75
Wheat semolina, soft wheat	42	4.8	LB	0.21	0.0	0.0	0.0	8.2
			UB	6.6	2.0	5.0	40	50
Rye milling products	482	6.4	LB	0.6	0.0	0.0	3.7	30
			UB	4.1	3.0	5.0	10	50
Rye flour, light	162	11	LB	0.9	0.0	0.0	7.0	22
			UB	4.4	3.0	5.0	12	50
Rye flour, medium	207	4.3	LB	0.5	0.0	0.0	0.0	30
			UB	3.5	2.7	5.0	10	50
Rye flour, wholemeal	96	5.2	LB	0.4	0.0	0.0	4.3	10
			UB	4.1	3.0	5.0	10	40
Buckwheat milling products	12	8.3	LB	0.4	0.0	0.0	5.0	5.0
			UB	3.5	4.0	4.5	5.0	5.0
Corn milling products	838	44	LB	12	0.0	12	52	509
			UB	14	5.0	13	54	509
Corn flour	382	57	LB	20	4.9	22	73	509
			UB	22	7.5	24	78	509
Corn semolina	351	35	LB	4.8	0.0	6.9	22	69
			UB	6.8	4.0	10	23	69
Oat milling products	52	7.5	LB	2.9	0.0	0.0	33	50
			UB	9.0	2.0	3.0	50	50
Spelt milling products	126	7.9	LB	0.3	0.0	0.0	2.0	10
			UB	3.6	3.0	5.0	10	15
Grain milling products (undefined)	175	9.0	LB	0.8	0.0	0.0	10	16
			UB	5.7	3.0	10	16	20
Other milling products	20	10	LB	2.8	0.0	0.0	28	34
			UB	10	10	10	42	50

N: number of samples; > LOD: indicates the percentage of results above the LOD or LOQ; LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.

(a): If N < 60 then the calculated P95 should be considered only as an indicative value (EFSA, 2011b).

4.2.4.3. Bread and rolls, and pasta

Zearalenone was found in 7.5 % of the 1,247 bread samples, and in relatively low concentrations (LB mean = 0.9 µg/kg; UB mean = 5.2 µg/kg). The mean concentrations in the different types of bread were very similar independently of the type of flour used for the bread preparation (Table 4). Concentrations of zearalenone in pasta (n = 330) was similar to those observed in bread (LB mean = 0.6 µg/kg; UB mean = 5.8 µg/kg).

Table 4: Occurrence of zearalenone in the food groups ‘Bread and rolls’ and ‘Pasta’.

Food group	N ^(a)	>LOD (%)	Concentration (µg/kg)					
			LB/UB	Mean	P50	P75	P95	Maximum
Bread and rolls	1247	7.5	LB	0.9	0.0	0.0	4.6	70
			UB	5.2	4.0	6.0	12	70
Wheat bread and rolls	227	1.8	LB	0.2	0.0	0.0	0.0	20
			UB	4.7	4.0	5.0	10	50
Rye bread and rolls	72	5.6	LB	0.2	0.0	0.0	1.5	5.4
			UB	3.5	3.0	5.0	6.7	12
Mixed wheat/ rye bread and rolls	194	6.7	LB	0.9	0.0	0.0	4.9	40
			UB	4.5	4.0	5.0	12	40
Multigrain bread and rolls	56	7.1	LB	0.6	0.0	0.0	5.8	13
			UB	4.1	4.0	5.0	5.8	13
Crisp bread and rusk	181	5.0	LB	0.5	0.0	0.0	0.0	34
			UB	3.8	2.0	5.0	10.0	34
Other bread	280	12	LB	1.1	0.0	0.0	6.4	63
			UB	7.7	10	10	12	63
Pasta	330	3.9	LB	0.6	0.0	0.0	0.0	48
			UB	5.8	3.0	6.7	20	50

N: number of samples; > LOD: indicates the percentage of results above the LOD or LOQ; LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.

(a): If N < 60 then the calculated P95 should be considered only as an indicative value (EFSA, 2011b).

4.2.4.4. Breakfast cereals, fine bakery wares and snacks

In the food group ‘Breakfast cereals’ (n = 1,377), zearalenone was found most frequently in corn flakes (17.3 %) followed by wheat flakes (14.3 %) and mixed breakfast cereals (12 %). Amongst the breakfast cereals, the highest mean zearalenone concentration was recorded in wheat flakes (LB mean = 8.4 µg/kg; UB mean = 25 µg/kg) (Table 5).

A relatively high contamination frequency (24.3 %) was observed in fine bakery wares. In the sub-groups ‘Biscuits’, 33.2 % of the samples contained zearalenone which is about 3-fold higher than in ‘Pastries and cakes’. The higher contamination frequency and higher concentrations in ‘Biscuits’ compared to ‘Pastries and cakes’ could be explained by the high content of wheat bran in certain types of biscuits and by the use of zearalenone-containing vegetable oil as ingredient. The group ‘Snacks’ (n = 121) included a large variety of cereal-based snack foods. The relatively high mean concentrations (LB mean = 2.6 µg/kg; UB mean = 12 µg/kg) can be related to the high content of corn in certain snack foods and also to the addition of corn germ oil in this type of food.

Table 5: Occurrence of zearalenone in the food groups ‘Breakfast cereals’, ‘Fine bakery products’ and ‘Snacks’.

Food group	N ^(a)	>LOD (%)	Concentration (µg/kg)					
			LB/UB	Mean	P50	P75	P95	Maximum
Breakfast cereals	1377	8.7	LB	1.2	0.0	0.0	5.0	172
			UB	5.7	3.0	6.7	20	172
Corn flakes	405	17	LB	1.7	0.0	0.0	8.9	136
			UB	5.6	3.0	5.0	20	136
Cereal flakes	333	4.5	LB	2.2	0.0	0.0	10	37
			UB	10	10	10	20	37
Oat flakes	222	4.5	LB	0.5	0.0	0.0	0.1	30
			UB	3.1	1.2	4.5	10	30
Spelt flakes	77	0.0	LB	0.0	0.0	0.0	0.0	0.0
			UB	4.8	4.0	10	10	10
Wheat flakes	34	14	LB	8.4	0.0	0.0	69	69
			UB	23	10	40	69	69
Muesli	261	2.7	LB	1.2	0.0	0.0	1.7	69
			UB	5.5	3.0	5.0	15	69
Mixed breakfast cereals	50	12	LB	0.3	0.0	0.0	0.0	23
			UB	4.8	2.0	10	10	40
Grits	75	5.3	LB	2.6	0.0	0.0	1.5	172
			UB	3.9	0.9	1.0	5.0	172
Breakfast cereals (undefined)	105	12	LB	1.0	0.0	0.0	3.4	49
			UB	6.8	1.8	5.0	30	49
Other breakfast cereals	152	3.9	LB	0.3	0.0	0.0	1.0	10
			UB	6.3	4.9	10	10	50
Fine bakery wares	813	24	LB	4.2	0.0	2.2	24	98
			UB	7.7	5.0	10	25	98
Pastries and cakes	156	9.6	LB	1.4	0.0	0.0	7.1	50
			UB	4.8	4.0	5.0	12	50
Biscuits (cookies)	541	33	LB	5.8	0.0	5.3	28	98
			UB	9.0	5.0	10	29	98
Fine bakery wares (undefined)	116	3.0	LB	0.1	0.0	0.0	0.0	5.0
			UB	4.5	3.0	10	10	10
Snacks	121	16	LB	2.6	0.0	0.0	20	50
			UB	12	10	20	20	50

N: number of samples; > LOD: indicates the percentage of results above the LOD or LOQ; LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.

(a): If N < 60 then the calculated P95 should be considered only as an indicative value (EFSA, 2011b).

4.2.4.5. Vegetables and vegetable products, vegetable oils, meat and meat products and beer

The sample size of the food group ‘Vegetables and vegetable products’ was low (n = 108) and was dominated by sweet corn samples (87 %). Thus, the mean concentrations cannot be extrapolated to all vegetables and vegetable products. A very high contamination frequency (86.4 %) with zearalenone concentration up to 823 µg/kg was recorded in vegetable oils. In fact, most of the samples in this group were corn germ oil (also known as corn oil) and wheat germ oil, both highly contaminated with zearalenone. Mean concentration in corn germ oil (LB mean = 90 µg/kg; UB mean = 90 µg/kg) was about 3-fold higher compared to wheat germ oil (LB mean = 31 µg/kg; UB mean = 35 µg/kg). High zearalenone concentrations in corn germ oil and wheat germ oil have been mentioned in previous studies (Kappenstein et al., 2005).

In the group “Meat and meat products” all observations (n = 1,256) were non-detects and therefore this food group will not be considered in the exposure assessment. Under alcoholic beverages, only

35 observations on beer were available. Zearalenone was detected only in two samples and in low concentrations. The maximum UB value given in Table 6 for beer is not a quantified result but the value of the highest LOQ applied in substituting the left-censored data.

Table 6: Occurrence of zearalenone in the food groups ‘Vegetables and vegetable products’, ‘Meat and meat products’, ‘Vegetable oils’ and ‘Beer’.

Food group	N ^(a)	>LOD (%)	Concentration (µg/kg)					
			LB/UB	Mean	P50	P75	P95	Maximum
Vegetables and vegetable products	108	15	LB	2.6	0.0	0.0	13	80
			UB	5.3	3.0	4.0	26	80
Sweet corn	94	11	LB	1.4	0.0	0.0	7.1	50
			UB	4.8	4.0	5.0	12	50
Vegetables and vegetable products (undefined)	5	0.0	LB	0.0	0.0	0.0	0	0.0
			UB	2.8	1.0	1.0	10	10
Other vegetables	9	67	LB	3.8	4.5	6.9	7.1	7.1
			UB	4.6	4.5	6.9	7.1	7.1
Meat and meat products	1256	0.0	LB	-	-	-	-	-
			UB	-	-	-	-	-
Vegetable oils	221	86	LB	70	49	91	200	823
			UB	72	49	91	200	823
Corn germ oil	139	97	LB	90	59	110	243	823
			UB	90	59	110	243	823
Wheat germ oil	58	76	LB	31	23	39	110	150
			UB	35	23	39	110	150
Vegetable oils (undefined)	12	17	LB	85	93	126	163	163
			UB	87	93	126	163	163
Other vegetable oil	12	83	LB	10	0	0	74	74
			UB	20	10	30	74	74
Beer	35	5.7	LB	0.1	0.0	0.0	1.8	2.2
			UB	1.0	0.5	1.0	2.2	10

N: number of samples; > LOD: indicates the percentage of results above the LOD or LOQ; LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile; -: not calculated as all results were < LOD.

(a): If N < 0 then the calculated P95 should be considered only as an indicative value (EFSA, 2011b).

4.2.4.6. Food for infants and small children and products for special nutritional use

The group ‘Food for infants and small children’ (n = 420) was dominated by samples in the sub-group ‘Cereal-based food for infants and young children’ (67 %). The frequency of quantifiable results in all sub-groups was in the range of 0 to 5 %. Also, the concentration of zearalenone was at a low level (maximum quantified result = 19 µg/kg) (Table 7).

The group ‘Products for special nutritional use’ includes bakery products for diabetics, formulas for metabolic disorders and other specially formulated medical foods. The contamination was similar to other cereal-based food groups.

Table 7: Occurrence of zearalenone in the food groups ‘Food for infants and small children’ and ‘Products for special nutritional use’.

Food group	N ^(a)	>LOD (%)	Concentration (µg/kg)					
			LB/UB	Mean	P50	P75	P95	Maximum
Food for infants and small children	420	4.0	LB	0.3	0.0	0.0	0.0	19
			UB	7.0	6.7	10	15	20
Infant formulae, powder	19	5.0	LB	0.3	0.0	0.0	5.0	5.0
			UB	8.2	10	10	15	15
Follow-on formulae, powder	49	2.0	LB	0.2	0.0	0.0	0.0	10
			UB	11	15	15	15	15
Cereal-based food for infants and young children	280	1.8	LB	0.2	0.0	0.0	0.0	19
			UB	6.0	5.0	10	20	20
Ready-to-eat meal for infants and young children	28	0.0	LB	0.0	0.0	0.0	0.0	0.0
			UB	5.5	5.0	6.7	10	10
Food for infants and small children (undefined)	44	23	LB	1.0	0.0	0.0	10	10
			UB	4.6	1.8	10	15	15
Products for special nutritional use	49	14	LB	1.5	0.0	0.0	15	20
			UB	4.2	4.0	5.0	15	20

N: number of samples; > LOD: indicates the percentage of results above the LOD or LOQ; LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile;

(a): If N < 60 then the calculated P95 should be considered only as an indicative value (EFSA, 2011b).

4.2.4.7. Occurrence data on unprocessed grains of unknown end-use

The group ‘Unprocessed grains’ (n = 9,877) includes grains of undefined end-use. Since their end-use is not established and normally grains for human and animal consumption undergo several processing steps before being used it has been considered appropriate to evaluate them separately. High concentrations have been reported in corn grains (LB mean = 76 µg/kg; UB mean = 87 µg/kg) and in sorghum grains (LB mean = 96 µg/kg; UB mean = 116 µg/kg). The lowest zearalenone concentrations were reported in rice (LB mean = 0.8 µg/kg; UB mean = 5.5 µg/kg) (Table 8).

Comparing the mean concentrations in grains for human consumption and unprocessed grains, a constantly higher contamination was observed in unprocessed grains (Figure 10). This suggests that cleaning and selection steps applied to grains after harvesting result in lower zearalenone concentration in grains intended for human consumption.

Table 8: Occurrence of zearalenone in the group ‘Unprocessed grains’.

Food group	N ^(a)	>LOD (%)	Concentration (µg/kg)					
			LB/UB	Mean	P50	P75	P95	Maximum
Unprocessed grains	9877	41	LB	33	0.0	15	160	2969
			UB	40	7.0	27	161	2969
Wheat	5318	38	LB	22	0.0	7.0	89	2969
			UB	27	5.0	20	90	2969
Barley	1071	37	LB	10	0.0	5.0	49	775
			UB	13	5.0	10	50	775
Corn	2460	56	LB	76	16	76	319	2700
			UB	87	40	78	319	2700
Oats	596	23	LB	21	0.0	0.0	76	1590
			UB	23	1.5	5.0	98	1590
Rice	43	7.0	LB	0.8	0.0	0.0	10	15
			UB	5.5	5.0	5.0	10	15
Sorghum	55	53	LB	96	50	147	450	700
			UB	116	50	147	450	700

N: number of samples; > LOD: indicates the percentage of results above the LOD or LOQ; LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.

(a): If N < 60 then the calculated P95 should be considered only as an indicative value (EFSA, 2011b).

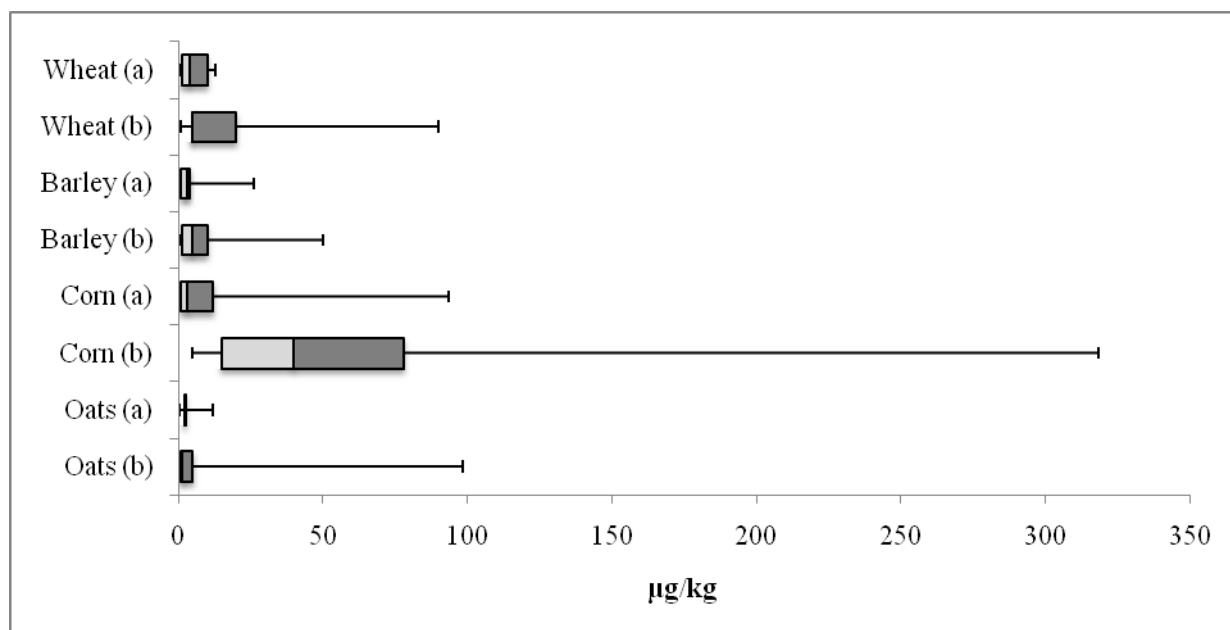


Figure 10: Comparison of zearalenone concentrations (µg/kg) in ‘Grains for human consumption’ (a) and ‘Unprocessed grains’ (b). (Box-plot: whiskers at 5th percentile and 95th percentile, box at 25th percentile and 75th percentile with line at 50th percentile).

4.2.5. Comparison of zearalenone occurrence in foods from organic and conventional farming

Of the 13,075 food samples, the production method was provided only for 948 observations (organic farming n = 862; conventional farming n = 86). For the comparison exercise, it was assumed that all samples where the method of production was not specified were from conventional farming. Food groups with more than 30 samples (‘Wheat grain’, ‘Wheat milling products’, ‘Corn milling products’, ‘Pasta’, ‘Corn flakes’ and ‘Cereal flakes’) were selected for the comparison. In the tested food groups,

the zearalenone contamination in products of organic farming was lower than in products of conventional farming (Figure 11). However, since the sampling size of the organic food was smaller compared to the conventional food and the sampling-countries and sampling years were not the same, this result should be interpreted with caution. Nevertheless, the data indicate that organic samples are not more contaminated than those conventionally cultivated.

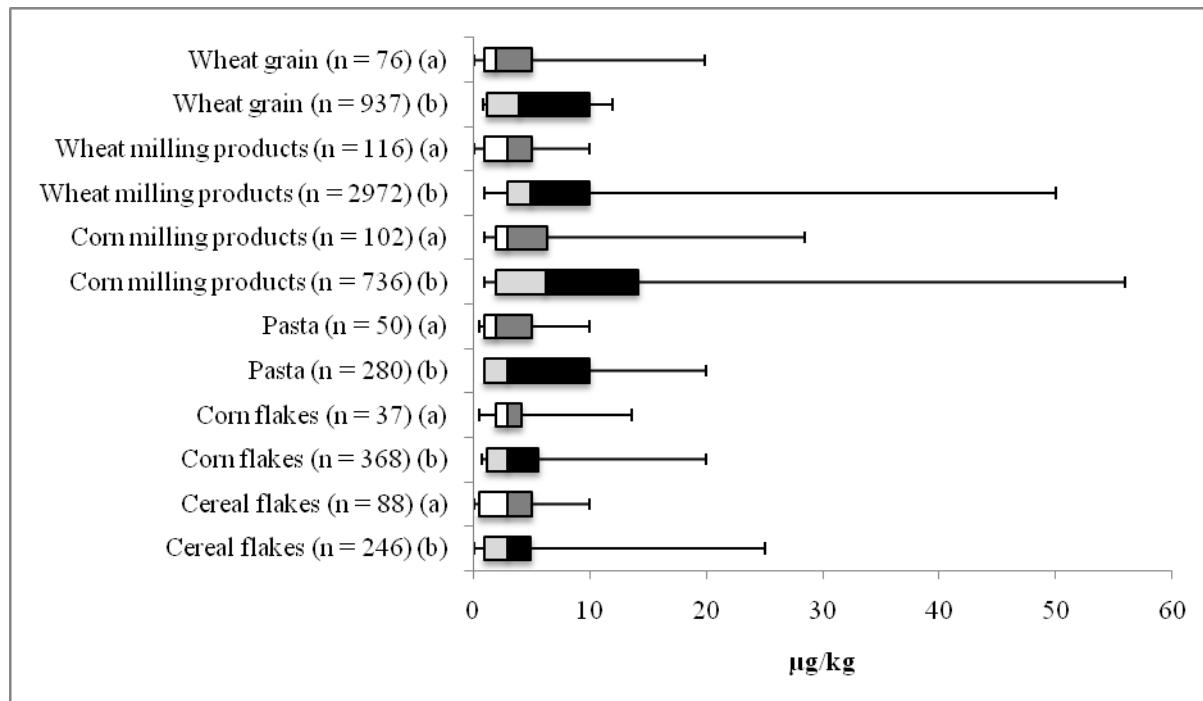


Figure 11: Comparison of the zearalenone contamination ($\mu\text{g}/\text{kg}$) in selected food groups from organic farming (a) and conventional farming (b). (Box-plot: whiskers at 5th percentile and 95th percentile, box at 25th percentile and 75th percentile with line at 50th percentile).

4.2.6. Comparison of zearalenone occurrence over the sampling years

An important factor for the development of *Fusarium* spp. and the production of *Fusarium* toxins are the climatic conditions, mainly low temperature and high humidity. As these conditions can vary between the years, it is expected that *Fusarium* toxins may occur with higher frequency and at higher concentrations in the years when the climatic conditions are favourable. It seemed therefore interesting to evaluate the contamination frequency and zearalenone concentrations in foods over the years 2005 to 2010. An important constraint in this exercise is the overlapping of at least two harvests in one sampling year. As most grains are harvested in summer they will predominantly enter into the food chain in the second half of the harvest year and in the first half of the following year. In addition, grains might be stored and used after more than one year. An exercise comparing the occurrence of zearalenone over the sampling years was performed but given the aforementioned limitations no clear variation over years could be observed (results not shown in this scientific opinion).

4.3. Milling and food processing

Concerning the milling of wheat only a few studies are available. It was shown by Palpacelli et al. (2007) that stone milling results in a 40-50 % reduction of zearalenone in wheat flour which was significantly lower compared to use of a modern roller mill. In another publication Wolff (2005) studied the effect of sorting, cleaning and milling on the zearalenone concentration. During cleaning and sorting

84 % of zearalenone could be removed. After milling the zearalenone content was low in the produced flour, however higher concentrations were found in the by-products such as wheat bran.

Generally, the milling process determines the degree of mycotoxin contamination of the resulting bran in general, and that of zearalenone in particular as demonstrated by milling experiments where the zearalenone concentration of bran made of naturally contaminated barley amounted to 38, 33, 33, 28 and 24 µg/kg when the proportion of husking losses were 17 to 22, 27, 32 and 37 %, respectively (Smith et al., 1994). The concentrations in the remaining meal decreased at the same time. Similar relationships were determined for deoxynivalenol and nivalenol. This husking loss related decrease in mycotoxin concentration of the bran is due to the increase in the proportion of lower contaminated starch endosperm in the resulting bran.

In a comprehensive German survey different by- and waste products obtained during the cleaning process of wheat and rye were analysed for an array of undesirable substances such as anthropogenic and natural contaminants (Wolff et al., 2004). Among the natural contaminants the mycotoxins zearalenone, deoxynivalenol, ochratoxin A and total ergot alkaloids were analysed. A total of 352 samples were analysed, of which 57 were cleaned cereal and 56 were bran samples. The samples were provided by 16 mills from four sample collections and represented 6,635 tonnes of cereal grains sampled. In general, the waste products from cleaning the raw cereal grains (dust, hulls and others) were characterized by 3- to 30-fold higher zearalenone concentrations than the cleaned cereal grains while bran contained up to 2-fold higher concentrations. In particular, the median zearalenone concentrations of the four sample collections varied between 1.7 and 4.9 µg/kg for the cleaned cereal grain samples, and between 2.4 and 7.2 µg/kg for the bran samples. The corresponding minimum and maximum values were 1 and 25.7 µg/kg for the grains, and 1 to 50.3 µg/kg for the bran (LOD = 1 µg/kg).

From a chemical point of view zearalenone seems to be unstable due to the macrocyclic lactone ring and the double bond. However, zearalenone is classified as a rather stable mycotoxin which e.g. is not degraded during storage (Krska et al., 2003). The degradation during food processing has been studied in different model systems where a strong difference between the various processing techniques occurred. Heating of pure zearalenone or zearalenone contaminated grain for several hours at 150°C did not lead to a significant loss of zearalenone. Starting at 200°C a moderate degradation was observed (Lauren and Smith, 2001; Yumbe-Guevara et al., 2003). Heating of zearalenone in an aqueous solution or under alkaline conditions led to degradation starting at 150°C whereas boiling in water did not influence the zearalenone content (Ryu et al., 1999).

In bread-baking experiments using wheat flour with a zearalenone concentration of 1-20 mg/kg, 34-40 % of zearalenone were degraded at approximately 200°C for 30 minutes. The production of instant noodles with the addition of 1 % potassium carbonate resulted in a 48-62 % reduction of zearalenone. In biscuits with 3 % sodium bicarbonate zearalenone was decreased by 16-27 % (Matsuura et al., 1981).

The roasting of barley kernels (3.89 mg zearalenone/kg) at 220°C resulted in a zearalenone decomposition of approximately 80 % after 18 minutes (Yumbe-Guevara et al., 2003). During the traditional preparation of tortillas using naturally contaminated corn the reduction ranged from 59 to 100 % (Abbas et al., 1988). This strong degradation can be explained by the alkaline conditions during nixtamalization (traditional preparation of corn by cooking under alkaline conditions using limewater) (Abbas et al., 1988).

A high degradation rate was also observed during extrusion cooking of maize grits or maize meal spiked with 22-38000 µg/kg of zearalenone. Depending on the extrusion parameters (temperature, moisture content, screw speed) the zearalenone concentration was reduced by 66-83 % (Ryu et al., 1999), 66-81 % (Cetin and Bullerman, 2005) and 6-54 % (Scudamore et al., 2008a). The loss of zearalenone seems not to be affected much by the presence of dextrose. The addition of salt resulted in higher zearalenone levels indicating a lower reduction rate (Scudamore et al., 2008a). Extrusion cooking of

wheat spiked with 305 µg/kg of zearalenone resulted in a reduction rate of 3-17 % depending on the parameters used (Scudamore et al., 2008b).

In most studies only the degradation of zearalenone was analysed and the chemical structures of zearalenone degradation products have not been elucidated. Only a decarboxylated zearalenone formed after opening of the lactone ring has been described (Urry et al., 1966).

In general, zearalenone is redistributed between the milling fractions. The by-products from cleaning the raw cereal grains (dust, hulls and others) were characterised by 3- to 30-fold higher zearalenone concentrations than the cleaned cereal grains while bran contained up to 2-fold higher concentrations. Generally zearalenone is not affected by cooking. Only under alkaline conditions or during extrusion cooking (heating under a high degree of pressure) a reduction of above 40 % was observed.

5. Food consumption

5.1. EFSA Comprehensive European Food Consumption Database

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been built from existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data from the most recent national dietary survey in their country at the level of consumption by the individual consumer. This included food consumption data concerning infants (2 surveys from 2 countries), toddlers (8 surveys from 8 countries), children (17 surveys from 14 countries), adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries) elderly (9 surveys from 9 countries) and very elderly (8 surveys from 8 countries) for a total of 32 different dietary surveys carried out in 22 different European countries. Surveys on children were mainly obtained through the Article 36 project “Individual food consumption data and exposure assessment studies for children” (acronym EXPOCHI) (Huybrechts et al., in press).

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, consumption data were collected by using different methodologies and thus they are not suitable for direct country-to-country comparison.

5.2. Food consumption data for different age and consumer groups

The CONTAM Panel considered that only acute dietary exposure to zearalenone did not need to be assessed. Therefore, as suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b) dietary surveys with only one day per subject were not considered for the calculation of zearalenone exposure, as they are not adequate to assess repeated dietary exposure. Similarly, subjects who participated only one day in the dietary studies where the protocol prescribed more reporting days per individual were excluded. Thus, for the present assessment, food consumption data were available from 28 different dietary surveys carried out in 17 different European countries as follows:

1. Infants: 2 countries; 2 dietary surveys
2. Toddlers: 7 countries; 9 dietary surveys
3. Other children: 13 countries; 17 dietary surveys
4. Adolescents: 10 countries; 12 dietary surveys
5. Adults: 14 countries; 15 dietary surveys
6. Elderly: 7 countries; 7 dietary surveys
7. Very elderly: 6 countries; 6 dietary surveys

Within the dietary studies, subjects were classified in different age classes as defined below:

1. Infants: < 12 months old
2. Toddlers: \geq 12 months to < 36 months old
3. Other children: \geq 36 months to < 10 years old
4. Adolescents: \geq 10 years to < 18 years old
5. Adults: \geq 18 years to < 65 years old
6. Elderly: \geq 65 years to < 74 years old
7. Very elderly: \geq 75 years old

In particular, results from consumption surveys from 13 different European countries for children gathered by means of the EFSA Article 36 project 'Individual food consumption data and exposure assessment studies for children' (acronym EXPOCHI) (Huybrechts et al., in press) were incorporated in the database. Consumption records were codified according to the FoodEx classification system, which has been developed by the DCM Unit in 2009 (EFSA, 2011a).

The dietary surveys considered for the dietary exposure assessment and number of subjects in the different age classes are presented in Table 9. Further details on how the Comprehensive Database is used are published in the Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

Table 9: Dietary surveys considered for the chronic dietary exposure assessment and number of subjects in the different age classes.

Country	Dietary survey ^(a)	Abbreviation ^(b)	Number of subjects								
			Total	Infants	Toddlers	Other	Adolescents	Adults	Elderly	Very elderly	
Belgium	Diet National 2004	BE/1	3118					584	1304	518	712
	Regional Flanders	BE/2	661				625				
Bulgaria	NUTRICHILD	BG	1721	860	428	433					
Cyprus	Childhealth	CY	303					303			
Czech Republic	SISP04	CZ	2353			389		298	1666		
Denmark	Danish_Dietary_Survey	DK	4120			490		479	2822	309	20 ^(c)
Finland	DIPP	FI/1	1430		497	933					
	FINDIET_2007	FI/2	2038						1575	463	
	STRIP	FI/3	250			250					
France	INCA2	FR	4079			482		973	2276	264	84
Germany	DONALD_2006	DE/1	303		92	211					
	DONALD_2007	DE/2	311		85	226					
	DONALD_2008	DE/3	307		84	223					
	National_Nutrition_Survey_II	DE/4	1392					1011	1041	2006	490
Greece	Regional_Crete	GR	839			839					
Hungary	National_Repr_Surv	HU	1360					1074	206	80	
Ireland	NSIFCS	IE	958					958			
Italy	INRAN_SCAI_2005_06	IT	3323	16 ^(c)	36 ^(c)	193		247	2313	290	228
Latvia	EFSA_TEST	LV	1965			189		470	1306		
	The Netherlands	DNFCS_2003	NL/1	750					750		
	VCP_kids	NL/2	1279		322	957					
	Spain	AESAN	ES/1	410						410	
AESAN_FIAB		ES/2	1067					86	981		
NUT_INK05		ES/3	1050			399		651			
enKid		ES/4	382		17 ^(c)	156		209			
Sweden	Riksmaten_1997_98	SE/1	1210						1210		
	NFA	SE/2	2491			1473		1018			
United	NDNS	UK	1724						1724		

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; (a): More information on the dietary surveys is given in the Guidance of EFSA “Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment” (EFSA, 2011b); (b): Abbreviations to be used consistently in all tables on exposure assessment; (c): The 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and therefore for these dietary surveys/age classes the 95th percentile estimates will not be presented in the exposure assessment.

5.2.1. Specific consumption patterns of breakfast cereals in European countries

Consumption data for breakfast cereals were analysed in all dietary studies specified in Section 5.1. The consumption of breakfast cereals varies between European countries and age classes. Overall, the highest median value for breakfast cereal consumption in the total population across all countries and dietary surveys was observed in the group ‘Other children’ (10 g/day) followed by ‘Adolescents’ (9.8 g/day) (Table 10). In the other age classes, the median value for breakfast cereal consumption was lower (≤ 5 g/day). However, high variation was observed between the dietary surveys within each age class (Table A1, Appendix A). In ‘Infants’, the reported consumption of breakfast cereal was negligible (0.03 g/day in one dietary study).

The 95th percentile breakfast cereal consumption in the total population followed a similar pattern to the mean consumption. The highest median values were observed in ‘Adolescents’ (50 g/day) and in ‘Other children’ (46 g/day). Maximum average consumption across the dietary surveys was recorded in ‘Other children’ (140 g/day), ‘Adolescents’ (100 g/day) and ‘Adults’ (100 g/day). Similarly to the total population, there is high variation between the dietary surveys (Table A2, Appendix A).

Table 10: Overview on breakfast cereal consumption (g/day) in the total population by age class. Minimum, median and maximum values across European countries and dietary surveys.

	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Mean consumption in the total population (g/day)						
Minimum	0.3	1.1	3.2	1.6	0.5	0.6
Median	4.0	10	9.8	5.0	2.1	1.4
Maximum	5.8	29	19	25	17	3.5
95th percentile consumption in the total population (g/day)^(a)						
Minimum	11	23	20	13	0.0 ^(b)	0.0 ^(b)
Median	21	46	50	30	11	0.0 ^(b)
Maximum	23	140	100	100	50	30

(a): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they were not included in this table.

(b): In certain dietary surveys, at least 95 % of the subjects did not report breakfast cereal consumption thus the 95th percentile equals zero.

6. Human exposure assessment

6.1. Previously reported human exposure assessments

A review of the zearalenone exposure assessments performed before the year 2000 is presented in the JECFA evaluation of zearalenone (WHO, 2000). Several exposure assessments included in this document were based on occurrence data in raw commodities thus some of those estimates have a very high degree of uncertainty.

In a total diet study carried out in France in 2000, Leblanc et al., (2005) estimated the dietary exposure to zearalenone in the French population. Zearalenone was found in concentrations above the LOD only in 5 (2 %) of the 245 analysed samples thus the left-censored data had a considerable influence on the estimates.¹⁶ The mean dietary exposure in total population was estimated at 33 ng/kg b.w. per day and the 95th percentile dietary exposure at 70 ng/kg b.w. per day. In children (3-14 years old), the mean

¹⁶ Left censored data were treated by the middle bound substitution method (non detected values = 1/2 LOD; <LOQ = 1/2 LOQ).

dietary exposure was 66 ng/kg b.w. per day and the 95th percentile dietary exposure 132 ng/kg b.w. per day. For the vegetarian population, the estimated average dietary exposure was between 50 and 200 ng/kg b.w. per day, depending on the groups studied. The 95th percentile dietary exposure was between 110 and 570 ng/kg b.w. per day.

The zearalenone dietary exposure to the European population was assessed in 2003 in the SCOOP task 3.2.10 (SCOOP, 2003). The average daily dietary exposures ranged among adults from 4 to 29 ng/kg b.w. per day. Small children had the highest average daily dietary exposures ranging from 6 to 55 ng/kg b.w. per day. Main contributors to the dietary exposure were found to be corn, wheat and the corresponding products.¹⁷

A study on occurrence of zearalenone in food and dietary exposure of the population to zearalenone was performed between 2001 and 2004 in Germany. Mean zearalenone exposure in the adult population was estimated at 6 ng/kg b.w. per day and the 95th percentile dietary exposure at 20 ng/kg b.w. The highest exposure was estimated in 4 to 6 years old children: mean dietary exposure = 16 ng/kg b.w. per day; 90th percentile dietary exposure = 57 ng/kg b.w. per day.¹⁸ No food consumption data were available for infants thus the exposure in this age class was calculated based on estimated food consumption. The estimated exposure was below 30 ng/kg b.w. per day (Curtui et al., 2006).

In 2008, the Finnish Food Safety Authority EVIRA estimated the dietary exposure to zearalenone in the Finnish population. The mean daily dietary exposure in adult Finnish women was estimated at 22 ng/kg b.w. per day and in men at 24 ng/kg b.w. per day. The 95th percentile dietary exposure,¹⁹ the dietary exposure was 44 ng/kg b.w. per day for women and 51 ng/kg b.w. per day for men (EVIRA, 2008).

Although the left-censored data were treated in different ways and different methodologies were applied for calculating exposure, the estimated dietary exposures across studies were of the same order of magnitude. Grains and grain products were the main sources of dietary intake of zearalenone.

6.2. Mean and high chronic dietary exposure to zearalenone

For calculating zearalenone exposure, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. For each country, exposure estimates were calculated per dietary survey and age class (see Section 5.2). Exposure estimates were therefore calculated for 28 different dietary surveys carried out in 17 different European countries. Not all countries provided consumption information for all age groups or in certain cases more than one consumption survey was provided by the same country.

The mean dietary exposure (average consumption in total population) and the high dietary exposure (95th percentile food consumption in total population) to zearalenone were calculated separately for each dietary survey using consumption data recorded at the individual level. Exposure estimates were calculated for both LB and UB scenarios. The LB and UB mean zearalenone concentrations of the most detailed food groups described in Section 4.2.4 ('Meat and meat products' not included) were combined with the consumption information at the individual level and each individual body weight was used to express zearalenone exposure in ng/kg b.w. per day.

¹⁷ Left-censored data were treated in the following way: results lower than the LOD were replaced by the LOD/2; results between LOD and LOQ were used; if only LOQ was available, or if numerical values between LOD and LOQ were not available, results were substituted by LOQ/6.

¹⁸ Left-censored data were treated by substitution with the half of the LOD (middle-bound scenario). The mean dietary exposure was estimated by using the median occurrence values and the median values for food consumption. For the 90th percentile dietary exposure, the median occurrence values were multiplied by the 90th percentile food consumption.

¹⁹ Average consumers: median concentration x average consumption; high consumers = median concentration x 95th percentile consumption.

In a preliminary exercise, the zearalenone exposure was calculated separately for each gender. There were only slight differences between the estimates for the two genders within the same age class and the same dietary survey. Compared to the variation observed between the dietary exposure estimates obtained across European countries and dietary surveys, the difference between the estimates for the two genders in the same dietary study was negligible. In addition, no constant higher exposure was observed in consumers of one gender across dietary studies and age classes. Therefore, the dietary exposure to zearalenone is reported from females and males combined. Minimum, median and maximum values are reported as estimated across dietary studies (Table 11). Detailed mean and 95th percentile dietary exposure estimates calculated for each of the 28 dietary surveys are presented in Tables B1 and B2 (Appendix B).

6.2.1. Infants (< 12 months old)

Despite the high proportion of non-detects, the CONTAM Panel decided to estimate the exposure to zearalenone for infants from 0 to 1 years old, by using the available consumption data from the Comprehensive Database (including EXPOCHI data). Only two dietary surveys reported consumption data for children younger than 1 year, therefore the exposure estimate should be considered as not representative of the European infant population.

Taking into account all the above mentioned limitations, the mean dietary exposure estimates ranged from 3.3 to 88 ng/kg b.w. per day (minimum LB to maximum UB). The 95th percentile exposure estimates ranged from 33 to 217 ng/kg b.w. per day (Table 11).

6.2.2. Children and adolescents (≥ 1 to < 18 years old)

The zearalenone dietary exposure in children and adolescents decreased with increasing age. This is explained by the higher intake of food per kg b.w. in younger age groups. The highest exposure was estimated in toddlers; mean exposure ranged from 9.3 to 100 ng/kg b.w. per day; the 95th percentile exposure ranged from 24 to 277 ng/kg b.w. per day (minimum LB to maximum UB) (Table 11).

6.2.3. Adults (≥ 18 to < 65 years old)

In the adult population, the mean dietary exposure to zearalenone across survey studies ranged from 2.4 to 29 ng/kg b.w. per day (minimum LB to maximum UB). The 95th percentile exposure ranged from 4.7 to 54 ng/kg b.w. per day (Table 11).

6.2.4. Elderly and very elderly (≥ 65 years old)

The mean dietary exposure to zearalenone in elderly and very elderly population was slightly lower compared to adults. The mean values across the survey studies ranged from 2 to 29 ng/kg b.w. per day (minimum LB to maximum UB). The 95th percentile dietary exposure ranged from 3.5 to 47 ng/kg b.w. per day (Table 11).

6.2.5. Summary

It can be concluded that the chronic dietary exposure to zearalenone is higher in younger consumers compared to adults. Also, there is a relatively high variation between the exposure estimates across European countries and dietary surveys within each age class. The exposure estimates found in this assessment are in line with those reported in the previous studies (see Section 6.1). A summary of the chronic dietary exposure to zearalenone in all age classes is presented in Table 11.

Table 11: Summary statistics of the chronic dietary exposure to zearalenone (ng/kg b.w. per day).

Age class	Summary statistics of exposure (ng/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants ^(a)	3.3	87	6.4	87	9.4	88
Toddlers	9.3	51	13	83	23	100
Other children	5.7	29	11	44	22	75
Adolescents	3.6	17	6.1	26	12	42
Adults	2.4	14	4.3	18	7.2	29
Elderly	2.0	13	3.4	16	6.4	26
Very elderly	2.3	12	2.9	16	7.1	29
95th percentile exposure in total population^(b)						
Infants	33 ^(c)	_(d)	_(d)	_(d)	_(d)	217 ^(c)
Toddlers	24	104	31	182	50	277
Other children	9.9	59	22	80	42	124
Adolescents	7.5	38	15	53	26	76
Adults	4.7	28	9.5	35	14	54
Elderly	3.5	25	7.5	31	12	42
Very elderly	7.0	26	7.7	35	13	47

b.w.: body weight; LB: lower-bound; UB: upper-bound.

(a): Estimates based on only two dietary surveys;

(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.

(c): Estimates are based on one dietary survey only.

(d): Not calculated.

6.3. Contributions of different food groups to zearalenone exposure

The contribution of individual food groups to total dietary exposure to zearalenone varied between the European countries and dietary surveys. This can be explained by the specific food consumption patterns in the individual European countries and even in the different regions of one country. The contribution of the individual food groups to the zearalenone exposure was calculated for both LB and UB scenarios. It is to note that in two dietary surveys foods (e.g. bread, fine bakery products) were disaggregated to ingredients (flour) and therefore these studies did not qualify for the calculation of the contribution of food groups to the exposure. A summary of the median values calculated from the average contribution of each food group across the dietary surveys and the range of the lowest and highest average contribution is shown in Table 12.

Grains and grain-based foods, in particular grains and grain milling products, bread and fine bakery wares, made the largest contribution to the zearalenone exposure in all age classes. Although the zearalenone concentration in bread was very low, the relatively high contribution to exposure is due to the high consumption in all age classes, except infants. The important contribution of fine bakery wares can be explained by the higher concentration in this food group compared to other cereal-based food groups. The higher zearalenone concentrations in this food group could be linked to the use of vegetable oils as ingredient in pastries and certain biscuits. Pasta contributed to a lesser extent to zearalenone exposure in all age classes apart from one study in infants. The contribution of breakfast cereals was

also low in all age classes with slightly higher values in adolescents and adults. In infants, the contribution of breakfast cereal was negligible (maximum 0.04 %).

Due to the high zearalenone concentrations found in vegetable oils, especially in corn germ oil and wheat germ oil, this food group made a notable contribution to the exposure. Although the amount of vegetable oil consumed is smaller compared to cereal-based foods, in some dietary studies it accounted for up to 50 % of zearalenone exposure. Beer and snacks made only a minor contribution to zearalenone exposure. Infant food accounted in UB scenarios for up to 84 % of exposure in infants and up to 60 % in toddlers.

Table 12: Contribution (%) of the different food groups to chronic dietary exposure to zearalenone in lower-bound (LB) and upper-bound (UB) scenarios. Median values across dietary surveys and range of the average contribution.

Food group	Median contribution across dietary studies (Lowest average contribution – Highest average contribution)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
Lower-bound	%													
Grains	29	(12-45)	13	(0.0-32)	13	(0.9-39)	18	(6.4-44)	18	(6.5-40)	14	(9.2-26)	11	(8.0-23)
Grain milling products	4.0	(3.8-4.3)	4.0	(0.1-8.0)	3.9	(0.0-29)	3.6	(0.2-32)	5.5	(0.3-25)	15	(0.5-26)	7.5	(0.4-36)
Bread and rolls	1.9	(0.4-3.4)	24	(3.7-40)	15	(3.4-37)	13	(5.1-33)	9.9	(6.5-30)	13	(8.8-38)	16	(9.8-37)
Pasta	12	(0.9-24)	1.4	(0.0-14)	1.3	(0.0-12)	1.6	(0.1-9.3)	1.9	(0.1-9.5)	1.2	(0.2-11)	1.7	(0.2-12)
Breakfast cereals	0.02	(0.0-0.04)	1.5	(0.1-4.5)	4.4	(0.4-11)	3.7	(0.9-12)	2.8	(0.4-17)	1.9	(0.1-2.9)	0.6	(0.1-2.4)
Fine bakery wares	21	(0.0-41)	29	(16-64)	30	(6.1-65)	33	(5.6-46)	29	(3.3-42)	19	(4.4-33)	24	(5.4-40)
Vegetables/vegetable products	0.4	(0.0-0.7)	2.1	(0.3-3.8)	1.5	(0.0-6.9)	1.6	(0.1-8.9)	2.5	(0.5-10)	3.0	(0.4-10)	1.4	(0.1-10)
Vegetable oils	10	(0.0-21)	14	(1.3-40)	7.3	(0.2-47)	11	(0.0-27)	8.6	(0.2-51)	9.5	(0.3-48)	16	(0.8-50)
Beer	0.0	(0.0-0.0)	0.0	(0.0-0.0)	0.0	(0.0-0.0)	0.1	(0.0-2.0)	4	(0.0-11)	1.2	(0.0-15)	2.6	(0.0-14)
Infant food	21	(15-27)	1.7	(0.4-7.9)	0.0	(0.0-1.3)	0.0	(0.0-0.0)	0	(0.0-0.0)	0.0	(0.0-0.0)	0.0	(0.0-0.0)
Products special nutrition	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	(0.0-0.1)	0.0	(0.0-0.1)	0.0	(0.0-0.2)
Snacks	1.0	(0.0-2.0)	3.3	(0.8-10)	5.9	(1.0-8.1)	6.4	(1.2-14)	4	(0.5-11)	0.4	(0.0-1.1)	0.2	(0.0-0.7)
Upper-bound	%													
Grains	3.9	(3.4-4.3)	5.1	(0.0-16)	9.3	(0.5-24)	10	(4.8-28)	8.9	(3.3-23)	7.1	(3.7-13)	6.4	(3.1-11)
Grain milling products	2.4	(2.1-2.7)	5.0	(0.1-9.3)	5.3	(0.1-21)	4.1	(0.3-23)	5.5	(0.2-20)	13	(0.4-19)	6.7	(0.5-20)
Bread and rolls	3.8	(0.4-7.2)	20	(13-55)	36	(21-59)	37	(28-52)	37	(29-55)	47	(36-58)	51	(36-57)
Pasta	4.6	(0.6-8.5)	1.3	(0.0-27)	2.2	(0.0-26)	3.3	(0.2-21)	3.1	(0.2-20)	2.2	(0.7-23)	3.4	(0.6-23)
Breakfast cereals	0.01	(0.0-0.02)	2.5	(0.1-5.1)	7.8	(0.5-19)	4.2	(1.4-13)	3.0	(0.6-16)	1.9	(0.2-2.2)	0.9	(0.2-1.8)
Fine bakery wares	3.8	(0.0-7.5)	7.2	(4.7-22)	18	(1.8-35)	16	(1.8-24)	13	(1.2-21)	9.5	(1.5-17)	11	(1.9-19)
Vegetables/vegetable products	0.1	(0.0-0.1)	0.8	(0.4-1.2)	0.8	(0.0-3.1)	0.6	(0.1-2.6)	1.0	(0.4-3.7)	0.7	(0.1-3.2)	0.4	(0.1-2.6)
Vegetable oils	2.2	(0.0-4.3)	4.3	(0.8-22)	4.0	(0.1-26)	5.2	(0.1-16)	4.7	(0.1-24)	7.2	(0.1-23)	10	(0.2-24)
Beer	0.0	(0.0-0.0)	0.0	(0.0-0.0)	0.0	(0.0-0.0)	0.1	(0.0-3.9)	6.5	(0.0-18)	2.0	(0.0-18)	4.1	(0.0-19)
Infant food	79	(73.8-84)	20	(6.8-60)	1.2	(0.0-7.3)	0.0	(0.0-0.2)	0.0	(0.0-0.1)	0.0	(0.0-0.0)	0.0	(0.0-0.1)
Products special nutrition	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	(0.0-0.1)
Snacks	0.5	(0.0-1.0)	2.1	(0.8-8.0)	6.7	(1.2-10)	6.6	(1.5-13)	3.4	(0.5-8.5)	0.4	(0-0.9)	0.2	(0.0-0.5)

6.4. Influence of possible increases in MLs of zearalenone in breakfast cereals on chronic total dietary exposure

EFSA has been asked to assess the potential effect on consumer health of possible increases in the currently existing maximum level of 50 µg/kg to 75, 100, 125 or 150 µg/kg for zearalenone in breakfast cereals. For this, the dietary exposure to zearalenone from other food sources, the occurrence data provided and the specific consumption patterns of breakfast cereals in the different European countries should be taken into account.

In a conservative approach, it was assumed that when setting the maximum permitted level for zearalenone in breakfast cereals to a different level from the current one, the distribution of the individual results would follow the same pattern. It was also assumed that the mean zearalenone concentration would change by the same order of magnitude. In a statistical evaluation based on random simulation of the samples distribution (see Appendix C), this conservative approach was found to be plausible. Since both the statistical evaluation and the conservative approach have several limitations it was decided to consider that the average concentration would increase by the same factor as the proposed changes to the ML (1.5, 2.0, 2.5, and 3.0).

The contribution of breakfast cereals to total dietary exposure in the different scenarios was calculated based on estimated mean concentrations (Table 13), the contribution of the breakfast cereals to the zearalenone exposure in average consumers in each dietary survey and each age class. The age class 'Infants' was not included as the consumption of breakfast cereals and their contribution to the exposure was considered negligible. The estimates obtained for each scenario were compared to the exposure calculated based on the available occurrence data (see Section 6.2). The difference between the current exposure and the exposure estimates found for the scenarios was also calculated. The ranges for the estimated exposure in each scenario and age class are presented in Table 14. The increase in exposure in relation to the assumed mean concentrations was highly variable across the dietary studies. The increase was from negligible up to 30-40 % in certain dietary surveys.

Table 13: Mean concentration used for the calculation of zearalenone dietary exposure in the different scenarios.

Scenarios	Mean LB (µg/kg)	Mean UB (µg/kg)
Current concentration (see Table 5)	1.2	5.7
Scenario 75 µg/kg	1.8	8.6
Scenario 100 µg/kg	2.4	11.4
Scenario 125 µg/kg	3.0	14.3
Scenario 150 µg/kg	3.6	17.1

LB: lower-bound; UB: upper-bound

Table 14: Influence of possible increases in maximum levels of zearalenone in breakfast cereals on chronic total dietary exposure.

Scenarios	Minimum and maximum dietary exposure estimates in ng/kg body weight per day (increase of exposure in %)											
	Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
Mean dietary exposure: Lower-bound												
Current exposure	9.3 - 23		5.7 - 22		3.6 - 12		2.4 - 7.2		2.0 - 6.4		2.3 - 7.1	
Scenario 75 µg/kg	9.3 - 24	(0.05-2.3)	5.7 - 23	(0.2-5.7)	3.6 - 13	(0.5-5.9)	2.4 - 7.8	(0.2-8.6)	2.0 - 6.6	(0.05-3.8)	2.3 - 7.2	(0.05-1.2)
Scenario 100 µg/kg	9.3 - 24	(0.10-4.6)	5.7 - 25	(0.4-12)	3.6 - 13	(0.9-12)	2.4 - 8.4	(0.4-17)	2.0 - 6.9	(0.1-7.6)	2.3 - 7.3	(0.1-2.4)
Scenario 125 µg/kg	9.3 - 25	(0.15-6.9)	5.7 - 26	(0.6-17)	3.6 - 14	(1.4-18)	2.4 - 9.1	(0.6-26)	2.0 - 7.1	(0.15-11)	2.3 - 7.4	(0.15-3.6)
Scenario 150 µg/kg	9.3 - 25	(0.20-9.2)	5.7 - 27	(0.8-23)	3.7 - 15	(1.8-24)	2.4 - 9.7	(0.8-35)	2.0 - 7.4	(0.2-15)	2.3 - 7.4	(0.2-4.8)
Mean dietary exposure: Upper-bound												
Current exposure	51 - 100		29 - 75		17 - 42		14 - 29		13 - 26		12 - 29	
Scenario 75 µg/kg	51 - 104	(0.05-4.0)	29 - 82	(0.25-9.4)	17 - 45	(0.7-6.5)	14 - 31	(0.3-7.9)	13 - 27	(0.1-3.9)	12 - 29	(0.1-0.9)
Scenario 100 µg/kg	51 - 108	(0.1-7.9)	29 - 89	(0.5-19)	17 - 45	(1.4-13)	14 - 34	(0.6-16)	13 - 28	(0.2-7.8)	12 - 30	(0.2-1.8)
Scenario 125 µg/kg	51 - 112	(0.15-12)	29 - 96	(0.75-28)	17 - 50	(2.1-19)	14 - 36	(0.9-24)	13 - 29	(0.3-12)	12 - 30	(0.3-2.7)
Scenario 150 µg/kg	51 - 116	(0.2-16)	29 - 103	(1.0-38)	17 - 53	(2.8-26)	14 - 38	(1.2-32)	13 - 30	(0.4-16)	12 - 30	(0.4-3.6)
95th percentile dietary exposure: Lower-bound^(a)												
Current exposure	24 - 50		9.9 - 42		7.5 - 26		4.7 - 14		3.5 - 12		7.0 - 13	
Scenario 75 µg/kg	24 - 51	(0.05-2.3)	9.9 - 44	(0.2-5.7)	7.5 - 28	(0.5-6.0)	4.7 - 15	(0.2-8.7)	3.5 - 12.5	(0.05-3.8)	7.0 - 13	(0.05-1.2)
Scenario 100 µg/kg	24 - 52	(0.1-4.6)	9.9 - 47	(0.4-11)	7.6 - 29	(0.9-12)	4.7 - 16	(0.4-17)	3.5 - 12.9	(0.1-7.6)	7.0 - 13	(0.1-2.4)
Scenario 125 µg/kg	24 - 54	(0.2-6.9)	10 - 49	(0.6-17)	7.6 - 31	(1.4-18)	4.7 - 18	(0.6-26)	3.5 - 13.4	(0.15-11)	7.0 - 14	(0.15-3.6)
Scenario 150 µg/kg	24 - 55	(0.2-9.2)	10 - 52	(0.8-23)	7.6 - 32	(1.8-24)	4.7 - 19	(0.8-35)	3.5 - 13.8	(0.2-15)	7.0 - 14	(0.2-4.8)
95th percentile dietary exposure: Upper-bound^(a)												
Current exposure	104 - 277		59 - 124		38 - 76		28 - 54		25 - 42		26 - 47	
Scenario 75 µg/kg	104 - 288	(0.05-3.9)	59 - 136	(0.3-9.5)	38 - 81	(0.7-6.5)	28 - 58	(0.3-7.9)	25 - 44	(0.1-3.9)	26 - 47	(0.1-0.9)
Scenario 100 µg/kg	104 - 299	(0.1-7.9)	59 - 147	(0.5-19)	39 - 86	(1.4-13)	28 - 63	(0.6-16)	25 - 45	(0.2-7.8)	26 - 48	(0.2-1.8)
Scenario 125 µg/kg	104 - 310	(0.15-12)	59 - 159	(0.8-28)	39 - 91	(2.1-19)	28 - 67	(0.9-24)	25 - 47	(0.3-12)	26 - 48	(0.3-2.7)
Scenario 150 µg/kg	104 - 321	(0.2-16)	60 - 171	(1.0-38)	39 - 96	(2.8-26)	28 - 71	(1.2-32)	25 - 49	(0.4-16)	26 - 49	(0.4-3.6)

(a): Dietary surveys/age classes with less than 60 observations were not considered for the calculation of the 95th percentile estimates as they may not be statistically robust (EFSA, 2011b).

6.5. Dietary exposure to zearalenone for specific groups

6.5.1. Vegetarians

Vegetarian diets include more cereal and cereal-based products thus it was considered that zearalenone exposure in this consumer group could be higher. The Comprehensive Database contains only limited data on food consumption of vegetarians. Dietary surveys with at least 15 adult vegetarians in each survey were selected, zearalenone dietary exposure was calculated and compared to the exposure of all subjects included in the respective dietary study. Generally, a higher mean and 95th percentile exposure was observed in vegetarians but the difference between values observed in all subjects and vegetarians only varied among the dietary studies (LB mean exposure up to 36 %; LB 95th percentile exposure: up to 228 %; UB mean exposure: up to 22 %; UB 95th percentile exposure: up to 210 %) (Table 15). Limited data on vegetarians indicate that dietary exposure to zearalenone could be up to 2-fold higher than for the general population.

Table 15: Comparative dietary exposure to zearalenone (ng/kg b.w. per day) in adult vegetarians and total adult population.

Dietary survey	N Veget.	N All	ng/kg b.w. per day			
			Mean exposure		95 th percentile exposure	
			Veget.	All	Veget.	All
Lower-bound						
FI/2	39	1575	3.0	3.0	9.3 ^(a)	6.6
FR	15	2276	6.0	4.5	13 ^(a)	10
DE/4	237	10419	4.2	3.4	12	8.7
SE/1	18	1210	4.4	3.4	18 ^(a)	7.7
UK	77	1724	6.8	5.0	15	11
Upper-bound						
FI/2	39	1575	14	15	29 ^(a)	30
FR	15	2276	21	18	47 ^(a)	35
DE/4	237	10419	17	15	40	33
SE/1	18	1210	18	15	59 ^(a)	28
UK	77	1724	27	22	51	43

FI: Finland; FR: France; DE: Germany; SE: Sweden; UK: United Kingdom; N: number of subjects in the dietary surveys; Veget.: vegetarians; All: total adult population; b.w.: body weight.

(a): The 95th percentile estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

6.5.2. Short-term dietary exposure to zearalenone by daily consumption of breakfast cereals containing zearalenone equal to the ML

Breakfast cereals are sold in packages containing several eating portions which are then consumed for several days. It is also common practice to purchase at the same time more packages from the same batch of breakfast cereals which would be consumed over a few weeks. Therefore, in a worst case scenario it was considered that breakfast cereals possibly containing zearalenone at the concentration equal to the ML are consumed daily until finishing the whole quantity purchased at once. For this scenario, the dietary exposure was calculated only for subjects who reported consumption of breakfast cereals (consumers only). Dietary surveys with limited numbers of breakfast cereal consumers (< 20) in an age class were excluded from the exposure assessment of the respective age class as they do not

provide reliable estimates. Dietary surveys excluded were: Belgium/2 (BE/2), Bulgaria (BG), Italy (IT) and Spain/4 (ES/4) in ‘toddlers’; Spain/2 (ES/2) in ‘adolescents’; France (FR), Hungary (HU) and Italy (IT) in ‘elderly’; Denmark (DK), France (FR), Hungary (HU) and Italy (IT) in ‘very elderly’. The number of subjects included in the dietary surveys considered for the exposure assessment in consumers of breakfast cereals is presented in Table 16.

The dietary exposure estimates in consumers of breakfast cereals containing zearalenone in concentrations of 50, 75, 100, 125 and 150 µg/kg are shown in Table 17. At the concentration of 50 µg/kg, the mean dietary exposure estimates across dietary surveys ranged from 13 to 136 ng/kg b.w. per day and the 95th percentile exposure ranged from 32 to 399 ng/kg b.w. per day. When considering the highest potential ML, the mean dietary exposure estimates increased up to 357 ng/kg b.w. per day and the 95th percentile exposure up to 1029 ng/kg b.w. per day. Such dietary exposure would result from daily consumption of breakfast cereals containing zearalenone at the level of ML, which would occur only for a limited period of time and is likely to be a rare event.

Table 16: Number of subjects included in the dietary surveys considered for the dietary exposure assessment in consumers of breakfast cereals.

Dietary survey	Number of subjects (consumers only)					
	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
BE/1			197	207	28 ^(a)	40 ^(a)
BE/2		300				
BG		21 ^(a)				
CY			178			
CZ		80	58 ^(a)	152		
DK		285	246	960	69	
FI/1	203	590				
FI/2				747	288	
FI/3		159				
FR		276	491	405		
DE/1	39 ^(a)	126				
DE/2	33 ^(a)	130				
DE/3	27 ^(a)	129				
DE/4			212	1516	251	62
GR		249				
HU				107		
IE				656		
IT		50 ^(a)	55 ^(a)	251		
LT		82	118	202		
NL/1				110		
NL/2	85	226				
ES/1				84		
ES/2				125		
ES/3		170	222			
ES/4		57 ^(a)	53 ^(a)			
SE/1				616		
SE/2		928	554			
UK				1049		

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom.

(a): Dietary surveys/age classes with less than 60 observations were not considered for the calculation of the 95th percentile estimates as they may not be statistically robust (EFSA, 2011b).

Table 17: Worst case scenarios for short-term dietary exposure to zearalenone through consumption of breakfast cereals at the concentration equal to the potential maximum levels (MLs). Exposure assessment on subjects who reported breakfast cereal consumption (consumers only).

Scenarios	Minimum and maximum exposure estimates in consumers only ng/kg body weight per day (increase of exposure in % compared to the 50 µg/kg scenario)											
	Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
Mean exposure: Lower-bound												
Scenario 50 µg/kg	49 - 71		27 - 117		17 - 67		13 - 58		16 - 27		21 - 29	
Scenario 75 µg/kg	68 - 96	(39-35)	37 - 173	(37-48)	24 - 98	(41-46)	18 - 85	(38-47)	24 - 39	(50-44)	30 - 42	(43-45)
Scenario 100 µg/kg	87 - 121	(78-70)	48 - 228	(78-95)	31 - 130	(82-94)	23 - 113	(77-95)	31 - 52	(94-93)	40 - 56	(90-93)
Scenario 125 µg/kg	106 - 146	(116-106)	59 - 284	(119-143)	37 - 161	(118-140)	28 - 140	(115-141)	38 - 64	(138-137)	49 - 69	(133-138)
Scenario 150 µg/kg	126 - 171	(157-141)	69 - 339	(156-190)	44 - 192	(159-187)	33 - 168	(154-190)	45 - 76	(181-181)	58 - 82	(176-183)
Mean exposure: Upper-bound												
Scenario 50 µg/kg	105 - 127		57 - 136		36 - 81		24 - 67		29 - 38		30 - 40	
Scenario 75 µg/kg	130 - 152	(24-20)	68 - 191	(19-40)	43 - 113	(19-40)	29 - 95	(21-42)	37 - 50	(28-32)	39 - 53	(30-33)
Scenario 100 µg/kg	149 - 178	(42-40)	79 - 247	(39-82)	50 - 144	(39-78)	34 - 122	(42-82)	44 - 62	(52-63)	48 - 66	(60-65)
Scenario 125 µg/kg	168 - 203	(60-60)	89 - 302	(56-122)	57 - 175	(58-116)	39 - 150	(63-124)	51 - 75	(76-97)	58 - 79	(93-98)
Scenario 150 µg/kg	187 - 229	(78-80)	100 - 357	(75-163)	63 - 206	(75-154)	44 - 177	(83-164)	58 - 87	(100-129)	67 - 92	(123-130)
95th percentile exposure: Lower-bound^(a)												
Scenario 50 µg/kg	145 - 175		70 - 342		50 - 163		32 - 122		37 - 49		44 ^(b)	
Scenario 75 µg/kg	213 - 241	(47-38)	103 - 493	(47-44)	72 - 245	(44-50)	46 - 180	(44-48)	55 - 72	(49-47)	65	(48)
Scenario 100 µg/kg	281 - 320	(94-83)	136 - 644	(94-88)	93 - 326	(86-100)	61 - 238	(91-95)	72 - 95	(95-94)	86	(95)
Scenario 125 µg/kg	349 - 399	(141-128)	169 - 795	(141-132)	115 - 407	(130-150)	76 - 297	(138-143)	90 - 117	(143-139)	108	(145)
Scenario 150 µg/kg	418 - 477	(188-173)	201 - 946	(187-177)	137 - 489	(174-200)	90 - 355	(181-191)	107 - 140	(189-186)	129	(193)
95th percentile exposure: Upper-bound^(a)												
Scenario 50 µg/kg	190 - 232		104 - 399		67 - 181		49 - 142		49 - 63		61 ^(b)	
Scenario 75 µg/kg	258 - 314	(36-35)	129 - 569	(24-43)	88 - 263	(31-45)	64 - 198	(31-39)	67 - 86	(37-37)	81	(33)
Scenario 100 µg/kg	333 - 383	(75-65)	158 - 727	(52-82)	111 - 344	(66-90)	77 - 254	(57-79)	85 - 108	(73-71)	101	(66)
Scenario 125 µg/kg	408 - 451	(115-94)	191 - 878	(84-120)	135 - 425	(101-135)	91 - 312	(86-120)	102 - 130	(108-106)	121	(98)
Scenario 150 µg/kg	476 - 520	(151-124)	223 - 1029	(114-158)	160 - 507	(139-180)	106 - 371	(116-161)	120 - 151	(145-140)	142	(133)

(a): Dietary surveys/age classes with less than 60 observations were not considered for the calculation of the 95th percentile estimates as they may not be statistically robust (EFSA, 2011b).

(b): Only one dietary study had more than 60 subjects in the age class 'Very elderly'.

7. Hazard identification and characterisation

7.1. Toxicokinetics

7.1.1. Absorption

Zearalenone appears to be rapidly and extensively absorbed after oral administration in rats, rabbits, pigs, and humans (Kuiper-Goodman et al., 1987). The total absorption in pigs after a single oral dose of 10 mg/kg b.w. was estimated to be 80-85 % (Biehl et al., 1993). The absolute bioavailability of zearalenone in rats, which is defined as the ratio of parent compound area under the time-concentration curve (AUC) following oral vs intravenous (*i.v.*) administration, was low (2.7 %) and linearly related to dose in the range of 1-8 mg/kg b.w. The low bioavailability (2.7 %) is consistent with the extensive pre-systemic metabolism of zearalenone discussed below.

7.1.2. Distribution

In male rats, the elimination half-life for unconjugated zearalenone was 0.6-2.8 hours following *i.v.* administration, 16.8 hours after oral administration, and 7.0 hours following oral administration in bile duct-cannulated rats (Shin et al., 2009). These results from comparisons of pharmacokinetic parameters using bile duct cannulation and untreated rats provided evidence for a major role of enterohepatic recirculation on the metabolism and disposition of zearalenone in rats (Shin et al., 2009). In male rats, zearalenone is extensively distributed to tissues other than the gastrointestinal (GI) tract, including kidney > liver > adipose > lung > heart, spleen, muscle, brain, and testes (Shin et al., 2009).

Zearalenone, α -zearalenol and β -zearalenol in unconjugated and glucuronide forms have been measured in pig liver and, unconjugated and sulphate conjugates of α -zearalenol and α -zearalanol were observed in pig muscle (Zöllner et al., 2002).

In pigs, the disappearance of zearalenone from plasma is closely related to the formation of its glucuronide, the reduced metabolite, α -zearalenol, and its glucuronide conjugate (Biehl et al., 1993). This finding is in general agreement with other studies where glucuronide conjugates of zearalenone and α -zearalenol were reported to be the major metabolites in the pig following *i.v.* administration (Dänicke et al., 2005a). The plasma time-concentration profile of α -zearalenol was different from that of zearalenone, because no distribution phase was observed for α -zearalenol (Dänicke et al., 2005a). An elimination half-life of 2.94 hours for α -zearalenol was close to that for zearalenone of 2.63 hours (Dänicke et al., 2005a). In pigs, there is also consistent evidence for extensive biliary secretion of the glucuronide conjugates of zearalenone and α -zearalenol resulting in enterohepatic recirculation (Biehl et al., 1993).

Placental transfer of zearalenone and α -zearalenol has been demonstrated in rats following *i.v.* administration. The zearalenone and α -zearalenol levels in the whole fetus were 5 to 38 % and 2 to 6 %, respectively, of the maternal liver levels, and the placental levels were approximately twice those of the fetus (Bernhoft et al., 2001).

The transfer of zearalenone from the pregnant sow to the piglet was evaluated by feeding a diet containing 9.57 mg deoxynivalenol and 0.358 mg zearalenone/kg from day 75 to 110 of gestation and by examining various specimens for mycotoxin metabolites (Dänicke et al., 2007a). Results were expressed as diet ratio (sum of concentrations of all metabolites in the physiological specimen divided by the dietary toxin concentration) which is also referred to as carry over factor, and as piglet ratio (sum of concentrations of all metabolites in the physiological specimen of the piglet divided by that of the sows). The zearalenone carry over factor for the liver (sows only) was 0.016. The diet ratios of zearalenone in bile reached up to 2.896 and 0.128 for sows and piglets, respectively. The piglet ratio in

bile varied up to 0.518 for zearalenone. Deoxynivalenol and its metabolite de-epoxy-deoxynivalenol were also detected in all specimens. Thus, the full-term piglet is exposed both to zearalenone and deoxynivalenol when their mothers are fed contaminated diets. Similar conclusions could be drawn for deoxynivalenol when diets with lower zearalenone and deoxynivalenol concentrations (0.048 and 4.42 mg, respectively) were fed to pregnant sows in the period of day 35 and 70 of gestation (Goyarts et al., 2007). In contrast, due to the lower dietary concentration zearalenone and its metabolites could only be detected in bile and urine of sows but were <LODs of 1, 0.5, 5, 100, 50 and 200 µg/kg for zearalenone, α-zearalenol, β-zearalenol, zearalanone, α-zearalanol and β-zearalanol, respectively, in all specimens of fetuses.

The distribution and elimination half-lives of zearalenone in goats were found to be 3.15 and 28.58 hours, respectively, after *i.v.* injection of a single bolus of 1.2 mg zearalenone/kg b.w. (Dong et al., 2010).

Distribution into bovine milk of only the conjugated forms of zearalenone, α-zearalenol, β-zearalenol has been reported (Prelusky et al., 1990). At a dose of 6 g of zearalenone given over a one day feeding period, maximum levels of conjugated forms of zearalenone (6 ng/mL), α-zearalenol (4 ng/mL), and β-zearalenol (7 ng/mL) were measured in milk with a corresponding maximum level of conjugated zearalenone in plasma of 13 ng/mL.

7.1.3. Metabolism

The *in vivo* metabolism of zearalenone has been investigated in several animal species and in humans (Kuiper-Goodman et al., 1987). Three important biotransformation pathways for zearalenone in animals have been reported as shown in Figure 12.

1. Enzymatic reduction of zearalenone catalyzed by 3α- and 3β-hydroxysteroid dehydrogenases (HSDs) produces α- and β-zearalenol, respectively, and smaller amounts of the corresponding zearalanols. The primary reduced forms of zearalenone have different oestrogenic activities relative to the parent compound in the order: α-zearalenol > zearalenone > β-zearalenol based on rat, pig, and chicken uterine/oviduct cytosolic ER binding affinity (Fitzpatrick et al., 1989) and MCF7 cell proliferation (Shier et al., 2001). Malekinejad et al. (2006) reported differences between mammalian species in hepatic transformation of zearalenone to its reduced and glucuronide metabolites. All these mammalian species converted large percentages of zearalenone and metabolites to the corresponding glucuronides (Malekinejad et al., 2006). When the specificity constants (V_{max}/K_m) for hepatic microsomal reduction to the zearalenols were compared on a normalized basis (i.e., pig formation of α-zearalenol = 1), pig (1.0) > rat (0.6) > cattle (0.5) > sheep (0.2) for α-zearalenol formation and rat (35) > cattle (2.2) > sheep (1.2) > pig (0.2) for β-zearalenol formation (Malekinejad et al., 2006). This comparison suggests that pigs, which preferentially produce α-zearalenol over the β analogue by 5-fold, are predicted to be more sensitive than these other species to the oestrogenic effects of zearalenone based on pharmacokinetics (cf. rats that preferentially form β-zearalenol by 60-fold and cows by 4.3-fold). Similar differences are observed in whole animals. A significant fraction of zearalenone was found in pig to be in the form of α-zearalenol and the respective glucuronide conjugates (Biehl et al., 1993), while cows convert zearalenone predominantly to β-zearalenol (Jodlbauer et al., 2000). Smaller amounts of further reduced metabolites (i.e., α- and β-zearalanols) are observed in some other ruminant species (Zöllner et al., 2002). Ovine metabolism of zearalenone produces at least five compounds including α- and β-zearalenols, α- and β-zearalanols, and zearalanone (Miles et al., 1996). Limited data suggest that humans produce significant amounts of both primary reduced species with α-zearalenol > β-zearalenol based on excretion in urine (Mirocha et al., 1981).

2. Zearalenone is also monohydroxylated by recombinant human cytochromes P450 (CYPs) (Bravin et al., 2009; Pfeiffer et al., 2009) and human liver microsomes (Bravin et al., 2009) *in vitro*. Hydroxylation occurs at the 6/8-position (aliphatic; see Figure 12) and 13/15-positions (aromatic). Studies with zearalenone oxidation by recombinant human CYP isoforms suggest that CYP 1A2 is the

major isoform with a lesser contribution from CYP 3A4 (Pfeiffer et al., 2009). The major oxidative metabolites appear to arise through aromatic hydroxylation and are catechols. These metabolites undergo oxidation to quinones, which can redox cycle and covalently modify biological macromolecules (Pfeiffer et al., 2009). The redox cycling of zearalenone catechols to reactive ortho- and para-quinones *in vitro* is highlighted by the reported formation of glutathione-S-conjugates and 8-oxoguanine (M. Metzler, 2011, personal communication). The catechol metabolites of zearalenone, α -zearalenol and β -zearalenol are also substrates for catechol-O-methyl transferase, which leads to identification of monomethyl catechol ethers in rat liver slices *ex vivo* (Pfeiffer et al., 2009), and rat bile *in vivo* (M. Metzler, 2011, personal communication). A study of recombinant human CYP isoforms identified CYP 2C8 and CYP 3A4/5 as the most active in aliphatic C6/8-hydroxylation of zearalenone (Bravin et al., 2009). Based on *in vitro* metabolism of zearalenone by hepatic microsomes, humans produce low amounts of C6/8 hydroxy-zearalenone relative to most other species (monkey > dog > rabbit > rat > human >> mouse) (Bravin et al., 2009). While the oestrogenic properties of zearalenone catechols are unknown, the aliphatic C6/8 hydroxy-zearalenone appears to be approximately an order of magnitude less active than the parent compound, based on transcriptional activation through human ER- α and ER- β (Bravin et al., 2009).

3. Phase II conjugation of zearalenone and its reduced metabolites with glucuronic acid and sulphate, is catalyzed by uridinediphosphate-glucuronosyltransferases (UGTs) and sulphotransferases (SULTs), respectively. Based on *in vitro* studies, zearalenone, α - and β -zearalenol, and the further reduced metabolites (α - and β -zearalanols) are readily glucuronidated both in the liver and intestine as well as in other extrahepatic organs of humans and various animal species (Pfeiffer et al., 2010). The highest activity among the human UGTs resided in UGT1A1, 1A3 and 1A8, consistent with the efficient glucuronidation by both hepatic and intestinal human microsomes. Extrapolated to the *in vivo* situation, these data imply that the zearalenone and its reduced metabolites are readily glucuronidated both in the human intestine and liver, and probably also in extrahepatic tissues. Based on *in vitro* results using human hepatic microsomal UGTs, the major glucuronide formed *in vivo* is likely to be the 14-O-glucuronide (Pfeiffer et al., 2010). The preponderance of the Phase II conjugated forms of zearalenone and its reduced metabolites in the circulation of pigs treated with zearalenone has been reported (Biehl et al., 1993; Dänicke et al., 2005b).

7.1.4. Excretion

Following *i.v.* (5 mg/kg b.w.) or oral (10 mg/kg b.w.) administration of ^3H -labeled zearalenone to pigs, the percentage of total administered radioactivity excreted into urine was 57 or 45 %, respectively, and into faeces was 7 or 22 %, respectively (Biehl et al., 1993). Rats excreted approximately 55 % of the administered dose (1 or 100 mg/kg b.w.) in the faeces with 15-20 % excreted in urine (Fitzpatrick et al., 1988). Faecal (97-98 %) and urinary zearalenone (86-88 %) was primarily in the unconjugated form. Approximately 10 % of the administered dose was excreted as α -zearalenol. The respective zearalenone, α -zearalenol and β -zearalenol concentrations found in human male urine exclusively as glucuronide conjugates after a single oral dose of 100 mg zearalenone were: 3.7 $\mu\text{g/mL}$, 3 $\mu\text{g/mL}$, and < LOD after 6 hours; 6.9, 6, and 2.7 $\mu\text{g/mL}$ after 12 hours; and 2.7, 4 and 2 $\mu\text{g/mL}$ after 24 hours (Mirocha et al., 1981). The presence of C6/8-hydroxy-zearalenone in rat liver and urine has been reported (Bravin et al., 2009). High levels of zearalenone and its reduced metabolites are excreted in the urine as glucuronides by grazing sheep (Cheeke, 1998).

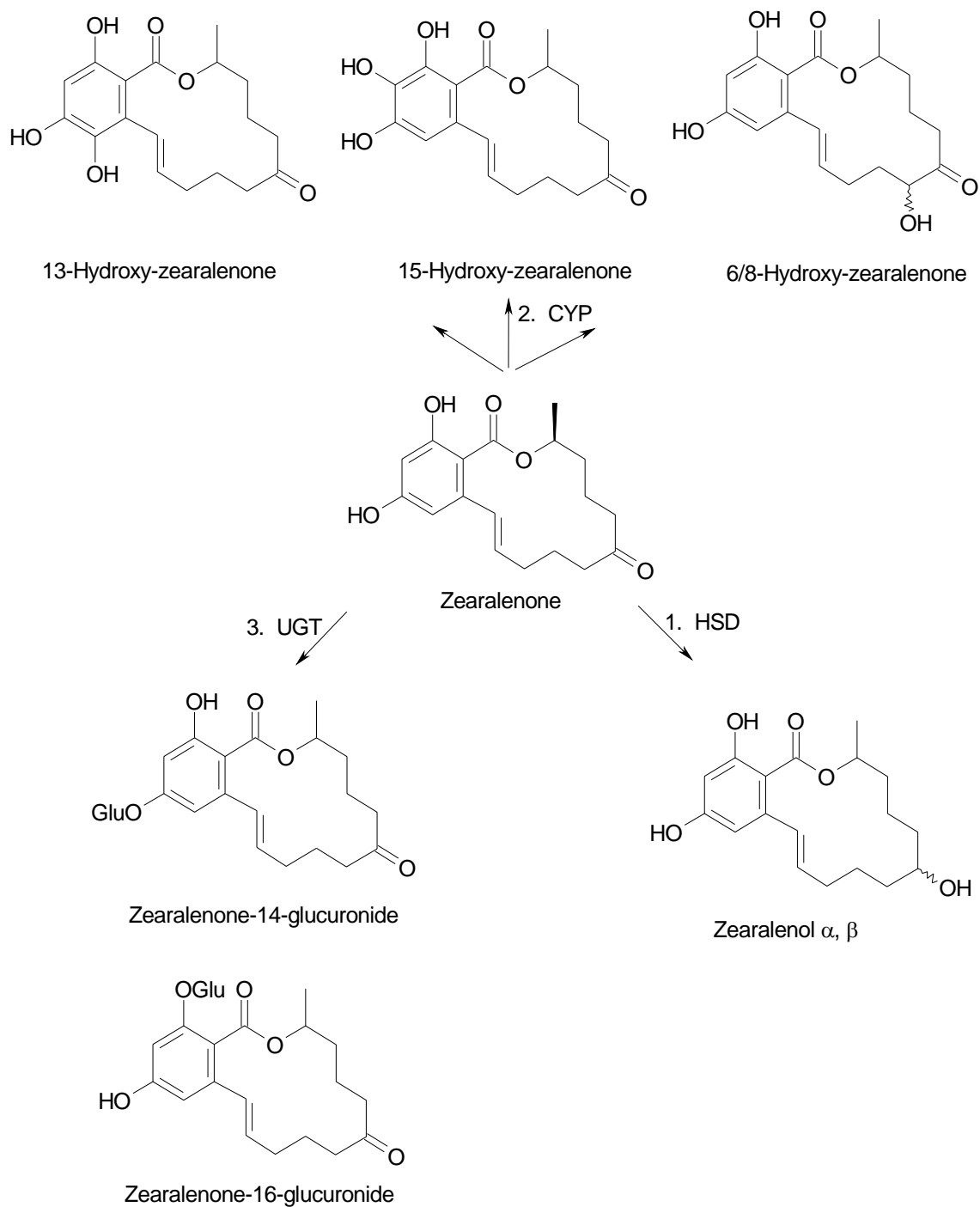


Figure 12: Major metabolic pathways for zearalenone.

7.1.5. Observations in humans

Urinary excretion data suggest that humans convert zearalenone to α - and β -zearalenols, with a small preference for the α -isomer (Mirocha et al., 1981). When comparing species differences in the *in vitro* metabolism of zearalenone to α - and β -zearalenols by hepatic microsomes (α : mouse > rat > pig > monkey > human > dog > rabbit; β : pig > rabbit >> human, mouse, rat, dog, monkey), humans are intermediate in producing the reduced metabolites with a preference for the more oestrogenic α -isomer

(Bravin et al., 2009). Human liver and intestinal microsomal preparations convert zearalenone and its reduced metabolites to the corresponding glucuronides (Pfeiffer et al., 2010). While enzyme kinetics show that human liver microsomes catalyze glucuronidation slower than other species, additional information regarding organ sizes, blood flows, extraction ratios, etc. would be needed to predict actual pharmacokinetic differences across species. The activity of several human liver and GI tract UGTs for zearalenone and its reduced metabolites would predict low oral bioavailability, as seen in other species. Recombinant human CYP isoforms convert zearalenone to catechol metabolites with a preference for 15-hydroxylation, a modification associated with greater propensity for oxidative stress associated with redox cycling relative to that in rat, pig and cow microsomes in which hydroxylation at the 13-position is preferred (M. Metzler, 2011, personal communication).

7.1.6. Conclusions

The metabolism and disposition of zearalenone are predicted to affect strongly its oestrogenic effects in mammals by the balance between competing activation and deactivation pathways. Efficient glucuronidation in the GI tract and liver significantly reduces the amounts of unconjugated (i.e., receptor-active) zearalenone that reaches the circulation through presystemic metabolism. As an example, oral administration to rats of an 8 mg/kg b.w. dose yields maximal serum levels of parent zearalenone of approximately 2 ng/mL (6 nM) (Shin et al., 2009). On the other hand, enzymatic reduction to α -zearalenol produces a metabolite with greater affinity for ER than the parent compound, whereas reduction to β -zearalenol produces a metabolite with lower affinity. While very limited toxicokinetic data from young animals are available for zearalenone, it is often assumed that fetuses and neonates could be more susceptible to the effects of such phenolic oestrogens because of metabolic and physiological immaturity (i.e., lower Phase II metabolism in liver and GI tract and renal excretory function, relative to adults). In addition, hepatic oxidation of zearalenone by CYP isoforms produces catechol metabolites that are subject to redox cycling to reactive quinones, which make possible the oxidation and/or covalent modification of biological macromolecules including glutathione, proteins and nucleic acids. While considerable inter-species differences have been demonstrated for each of these metabolic pathways, there is evidence that all are operative in humans. The known excretion of zearalenone and its metabolites in human urine suggests that oestrogenic and reactive species derived from consumption of zearalenone in foods could be monitored in different populations.

7.2. Toxicity in animals

The toxicity of zearalenone has been extensively reviewed by Nordic Council of Ministers, (1998), the JECFA (FAO/WHO, 2000) and SCF (2000). Hence, only the most relevant and recent findings are described below.

It is evident from the literature that, in addition to zearalenone and its major mammalian metabolites the zearalenols, other plant-specific metabolites may also be present (Berthiller et al., 2006; Vendl et al., 2009). They comprise glucose and sulphate conjugates of zearalenone and are known as 'masked' mycotoxins because they are not detected by the usual methods of analysis for zearalenone and its main metabolites. Such conjugates can be enzymatically cleaved to zearalenone in the GI tract of mammals such as pigs and rats (Gareis et al., 1990; Plasencia and Mirocha, 1991) and may therefore contribute to exposure to oestrogenically active substances from contaminated food and feed. It is not yet known with what frequency and at what concentrations such plant-specific metabolites may be present in contaminated food and feed. A single study on 24 wheat samples indicated that zearalenone-glucosides were present in just under half of them and that the amounts were in the range of 10-20 % that of zearalenone itself (Schneweis et al., 2002). Thus in the studies described below in which contaminated feed was used as test material, it is possible that exposure to oestrogenic substances was higher than that measured.

7.2.1. Acute toxicity

As generally reported (Kuiper-Goodman et al., 1987; FAO/WHO, 2000; SCF, 2000), zearalenone is known to exhibit low acute toxicity after oral administration in mice, rats and guinea pigs (oral LD₅₀ values of more than 2000 mg/kg b.w.).

In female rats given single intraperitoneal (*i.p.*) doses (1.5, 3 and 5 mg/kg b.w.) of zearalenone, the number of platelets was significantly reduced in all treated groups (Maaroufi et al., 1996). Haematocrit, haemoglobin, erythrocyte volume and white blood cell count were increased in animals receiving the two highest doses. Several biochemical markers like aspartate and alanine aminotransferases, alkaline phosphatase, serum creatinine and bilirubin were increased in all treated groups. These data indicated effects on liver and blood resulting from high exposure to zearalenone.

In addition, in a study by Abbès et al. (2006), mice orally treated with a single dose of zearalenone (40 mg/kg b.w.) in olive oil, were found to have lymphoid infiltration of the liver and shrunken glomeruli of the kidney.

7.2.2. Subacute and subchronic toxicity

7.2.2.1. Mice

B6C3F1 mice of each sex (groups of 10 animals) were fed diets containing zearalenone at 0, 30, 100, 300, 1000 or 3000 mg/kg of diet, equivalent to 0, 4.5, 15, 45, 150 or 450 mg/kg b.w. per day, for 13 weeks (NTP, 1982). Two of the female mice given 450 mg/kg b.w. per day died. In male mice receiving 150 and 450 mg/kg b.w. per day, the weight gain was depressed by 14 % or more. In the same animals, atrophy of the seminal vesicles and testes and cytoplasmic vacuolization of the adrenals were found. Squamous metaplasia of the prostate was observed in males given 450 mg/kg b.w. Endometrial hyperplasia of the uterus was seen in all groups of treated females, but the incidence was not dose-related. Osteoporosis was observed in animals of each sex fed doses higher than 15 mg/kg b.w. per day, and myelofibrosis of the bone marrow was seen in mice given more than 45 mg/kg b.w. per day.

Female B6C3F1 mice (26 control and 8 exposed animals) were fed diets containing 0 or 10 mg/kg of zearalenone, equivalent to 0 or 1.5 mg/kg b.w. per day, for 8 weeks (Forsell et al., 1986). No differences between treated animals and controls were seen in body-weight gain or feed intake. Gross and histopathological evaluation of the thymus, spleen, liver, kidney, uterus, small intestine, colon, heart, brain, lungs, and bone marrow showed no alterations due to zearalenone. The organ weights of treated and control animals were similar. Haematological examination revealed a statistically significant increase in the number of erythrocytes in treated animals, while other parameters appeared unchanged.

ICR mice (groups of 12 females, 15 day-old animals) were given diets containing zearalenone at 0 or 6 mg/kg, equivalent to 0 or 0.9 mg/kg b.w. per day (Underhill et al., 1995). After 5 days, the relative weight of the uterus was higher in treated mice ($p < 0.01$) than in controls.

7.2.2.2. Rats

Fischer 344/N rats of each sex (groups of 9 or 10 animals weighing 77 to 88 g, age not specified) were fed diets containing 0, 30, 100, 300, 1000 or 3000 mg/kg zearalenone, equivalent to 0, 3, 10, 30, 100 or 300 mg/kg b.w. per day, for 13 weeks (NTP, 1982). Weight gain was depressed by more than 17 % in rats of each sex receiving doses higher than 10 mg/kg b.w. per day. Atrophy of the seminal vesicles and fibromuscular hyperplasia of the prostate were observed in rats given 100 or 300 mg/kg b.w. per day of zearalenone. Ductular hyperplasia of the mammary gland was observed in animals of each sex at the highest dose. Endometrial hyperplasia of the uterus was seen in rats fed more than 10 mg/kg b.w. per day. Hyperplasia of the pituitary was seen in both males and females at the two higher doses and in one

female receiving 10 mg/kg b.w. per day. Osteoporosis was observed in males at the two highest doses and in all treated females.

7.2.2.3. Rabbits

Groups of 6 female rabbits were given zearalenone in the diet for 18 days, at concentrations of 0, 0.5 or 1 mg/kg of feed, equivalent to 0, 0.015 or 0.03 mg/kg b.w. per day (4 month-old animals), or at concentrations of 0, 1 or 4 mg/kg of feed, equivalent to 0, 0.03 or 0.12 mg/kg b.w. per day (8-month-old animals) (Abdelhamid et al., 1992). Some of the treated animals died during the study. The 4-month-old rabbits showed a treatment-related increase in body-weight gain, food and water consumption, haemoglobin percentage, packed cell volume, and serum concentrations of calcium, phosphorus and vitamin C. In contrast, the 8 month-old animals showed treatment-related decreases in these parameters. No explanation was given for these contradictory observations. Histopathological alterations due to zearalenone were observed in the liver, kidney, lungs, heart, adrenal glands, spleen, and uterus of only the 8- month-old animals. The histopathological alterations were not described quantitatively, and the number of rabbits surviving at the end of the study was not reported.

Rabbits (groups of 5 female animals, 1-year-old) received 0, 10 or 100 µg/kg b.w. per day doses of zearalenone administered orally by gavage for 14 days (Conkova et al., 2001). Selected blood serum enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and total lactate dehydrogenase (LD) were studied. In the group receiving 10 µg/kg b.w. per day of zearalenone, a significant increase in ALP activity was observed at 168 and 336 hours during the experiment. In the group receiving 100 µg/kg b.w. per day, significant increases in activities of AST, ALT, ALP, GGT, and LD were observed at 168 and 336 hours. According to the authors, these data would indicate possible liver toxicity due to chronic effects of low doses of zearalenone. However, the CONTAM Panel did not consider that the increase in ALP activity observed in rabbits receiving 10 µg/kg b.w. per day of zearalenone is a specific sign of liver toxicity. The CONTAM Panel noted also that only GGT activity was increased in rabbits exposed to 100 µg/kg b.w. per day of zearalenone when compared to corresponding sham controls receiving 1 mL of 8 % ethanol per kg b.w. Overall the CONTAM Panel considered that this subacute toxicity study was not convincing enough to be considered as pivotal for deriving a TDI for zearalenone.

7.2.2.4. Pigs

Two recent studies using pigs, investigated the adverse effects of zearalenone on growth, nutrient availability and genital organs (Jiang et al., 2010b) and on the liver and kidney functions in female piglets (Jiang et al., 2010a). The basal diet, to which 1 mg zearalenone/kg diet was added, was co-contaminated with naturally occurring zearalenone (0.3 mg/kg), aflatoxin B1 (12.9 µg/kg) and fumonisins (4.73 mg/kg), but not deoxynivalenol. The animals (35 days old) were given for 24 days, the diet containing a total of 1.3 mg/kg of zearalenone (equivalent to approximately 0.07 mg/kg b.w. per day). The efficacy of dietary montmorillonite clay in preventing zearalenone-induced adverse effects was also determined. When compared to control animals, pigs fed with zearalenone-containing diet showed reduced apparent digestibility of crude proteins and reduced metabolic rate of gross energy. Final body weight, average daily feed intake, vulva length, width and area were increased in mycotoxin-treated animals. Histopathological changes suggested that the ovaries were inflamed and hyperoestrogenized in zearalenone-treated piglets. The same group of animals showed also significant decreases in platelets, haemoglobin, globulin, triglycerides and high density lipoproteins in serum, and increases in serum enzymes activities, cholesterol, urea and creatinine. Degeneration of the liver and kidney tissues was also observed in treated animals. Dietary addition of clay showed a positive protection effect on zearalenone feeding and hepatic effects. The authors suggested that feeding zearalenone at 1.3 mg/kg diet for 24 days may result in deleterious effects in female piglets. However,

the exposure to multiple mycotoxins made this study unsuitable for evaluating the toxicity of zearalenone alone.

7.2.2.5. Poultry

As generally described (Kuiper-Goodman et al., 1987; Fink-Gremmels and Malekinedjad 2007), poultry do not appear to be significantly affected by ingestion of zearalenone. For instance, young mature chickens given high doses of zearalenone (50 mg/kg b.w. per day) for 7 days showed no signs of toxicity or impairment of reproductive performance (Chi et al., 1980).

7.2.2.6. Conclusions

These studies devoted to subacute and subchronic toxicity of zearalenone indicate that haematological changes, hepatic disturbances and oestrogenic effects are observed in rodents receiving high oral doses of zearalenone. The present data further support the view that pigs can be considered as the most sensitive animal species to the oestrogenic activity of zearalenone. Due to experimental insufficiencies or exposure to multiple mycotoxins, all these studies were unsuitable for establishing a no-observed-adverse-effect level (NOAEL) for zearalenone.

7.2.3. Chronic toxicity and carcinogenicity

7.2.3.1. Mice

B6C3F1 mice of each sex (groups of 50 70-week-old animals) were fed diets containing zearalenone at a concentration of 0, 50 or 100 mg/kg of diet (maximum tolerated dose) for 103 weeks (NTP, 1982). The daily intake of zearalenone was approximately 0, 8 and 17 mg/kg b.w. for males and 0, 9 and 18 mg/kg b.w. for females. No significant difference in survival was seen between groups. Body-weight gain was unchanged and no treatment-related non-neoplastic lesions were found in male mice. In females, oestrogen-related effects were observed in several tissues, including fibrosis in the uterus and cystic ducts in mammary glands, as well as myelofibrosis in the bone marrow. Hepatocellular adenomas were found in 8, 6 and 14 % and 0, 4 and 14 % in males and females, respectively. This effect was statistically significant only in the high-dosed females. Statistically significant trends in the incidence of pituitary adenomas were observed in both males (0, 9 and 14 %) and females (7, 5 and 31 %). Pituitary carcinomas were found in one male at the low dose and in two females at the high dose. However, the incidence of pituitary carcinomas in treated and control animals was not statistically significantly different.

7.2.3.2. Rats

Wistar rats of each sex (groups of 90 treated and 140 control animals) were fed diet containing zearalenone at doses of 0, 0.1, 1 or 3 mg/kg b.w. per day for 104 weeks (Becci et al., 1982). The rats were derived from F0 parents fed equivalent concentrations for 5 weeks before mating and throughout mating and gestation, but not during lactation. Zearalenone had no effect on reproductive parameters in the parent generation. No statistically significant differences were seen among F1 groups with respect to haematological, biochemical or urinary parameters. Significantly increased liver weights were found in males and females exposed to 3 mg/kg b.w., and the uterine weights were increased in females exposed to 1 and 3 mg/kg b.w. per day. Rats receiving the highest dose showed increased trabeculation of the femur, but no histopathological changes were seen and no treatment-related tumours were found. The CONTAM Panel noted that a NOEL of 0.1 mg/kg b.w. per day for zearalenone can be identified from this study.

Fischer 344 rats of each sex (groups of 50 5-week-old animals) were fed diets containing zearalenone at doses of 0, 1 or 2 mg/kg b.w. per day for 103 weeks (NTP, 1982). Mean body weight gains of treated rats were lower than those of control animals, and the depression in mean body weight (by 19 % in males and 11 % in females at the high dose group after 44 weeks of exposure) was dose-related. No significant difference in survival was observed between groups. Non-neoplastic lesions were observed including inflammation of the prostate gland, testicular atrophy, cysts or cystic ducts in mammary glands of males, increased incidence of hepatocellular cytoplasmic vacuolization in males, and an increased incidence of chronic progressive nephropathy in animals of each sex. Increases in the incidence of retinopathy and cataracts were observed in all treated males, and in low-dose females. No treatment-related increase in tumour incidence was found in this study.

7.2.3.3. Conclusions

These chronic toxicity and carcinogenicity studies of zearalenone confirm the oestrogenic effect in rodents exposed to long-term administration of this mycotoxin. From a long-term toxicity study in rats, a NOEL of 0.1 mg/kg b.w. per day can be derived, based on the absence of increase in weight of uterus at this dose level. These studies provide limited evidence of carcinogenic activity of zearalenone in experimental animals (hepatocellular adenomas in female mice and pituitary adenomas in both male and female mice but no effects in rats). This conclusion is in agreement with the evaluation of zearalenone by the International Agency for Research on Cancer (IARC, 1993). Zearalenone was allocated in Group 3 (not classifiable as to their carcinogenicity to humans), based on inadequate evidence in humans and limited evidence in experimental animals (IARC, 1993).

7.2.4. Genotoxicity

7.2.4.1. *In vitro* studies

As reported in previous reviews (Kuiper-Goodman et al., 1987; IARC, 1993; FAO/WHO, 2000), zearalenone did not induce SOS error-prone DNA repair in *Escherichia coli*, and mutation in *Salmonella typhimurium* or mitotic crossing over in *Saccharomyces cerevisiae*. Contrasting data were reported by Ghedira-Chekir et al. (1998) on the induction of SOS repair in *Bacillus subtilis* by this mycotoxin. Zearalenone induced sister chromatid exchanges, chromosome aberrations in Chinese hamster V79 lung cells (Thurst et al., 1983) and induced polyploidy in Chinese hamster ovary (CHO) cells in the absence of exogenous metabolic activation (Galloway et al., 1987).

More recently, Lioi et al. (2004) investigated the cytogenetic and cytotoxic effects of zearalenone in cultured bovine lymphocytes treated with 0, 0.1, 0.5, 1 and 2 μ M of the toxin. This study provides evidence of significant increases of structural chromosome aberrations and sister chromatid exchanges at all four concentrations. The increases were associated with a reduction of the mitotic index only at the two highest concentrations.

Ouanes et al. (2003) evaluated the genotoxic potential of zearalenone by using the cytokinesis block micronucleus assay in Vero monkey kidney cells. In cells treated with 5, 10 and 20 μ M of zearalenone a dose-dependent induction of micronuclei was reported. Zearalenone was also found to cause a concentration-dependent increase of unscheduled DNA synthesis and of chromosomal aberrations in Vero monkey kidney cells (Ouanes Ben-Othmen et al., 2008).

7.2.4.2. *In vivo* studies

El-Makawy et al. (2001) evaluated the genotoxic effects of zearalenone administered *i.p.* to adult males and pregnant mice with two single dose levels (5 and 10 μ g/kg b.w.). Chromosome aberrations were investigated in bone marrow and spermatocytes of the adult male mice. Chromosome analysis was

conducted on the fetuses on gestation day (GD) 13. Zearalenone was found to reduce the mitotic activity in treated males and in the fetuses at both doses. Structural abnormalities like breaks were observed on mice spermatocytes at the higher dose. Some the pregnant mice were killed on GD 17 and the fetal skeleton was examined. However, this study did not report any significant cytogenetic or teratogenic effect on albino mice treated with zearalenone.

Mice (groups of 3 females) received single or repeated *i.p.* doses (2, 4, 10, 15, 20 or 40 mg/kg b.w. per day) of zearalenone for 1, 2 or 3 days (Ouanes et al., 2005). Zearalenone induced a dose-dependent increase of chromosomal aberrations including breaks, rings, gaps and centric fusions in bone marrow cells. Interestingly, when the total dose of zearalenone (40 mg/kg b.w. per day) was fractionated into two to four repeated doses given every 24 hours, the percentage of chromosomal aberrations increased significantly as compared to the single dose. According to the authors, these findings suggest that zearalenone would act by reversible binding to receptors that could become saturated.

In the study by Ouanes et al. (2003) the genotoxic potential of zearalenone in the *in vivo* mouse bone marrow micronucleus assay was also evaluated. In mice (groups of 5 males) given single oral doses of 10, 20 and 40 mg/kg b.w., a dose-dependent increase of the frequency of micronuclei was observed. Vitamin E was found to prevent from 30 to 50 % of these toxic effects, likely acting as an anti-oxidant.

Six-week-old female Balb/c mice were treated orally with a single dose of zearalenone (40 mg/kg b.w.) and hydrated sodium calcium aluminosilicate (HSCAS) (400 mg/kg b.w., a phyllosilicate that has been shown to prevent diseases associated with aflatoxicosis in farm animals). All mice were sacrificed by cervical dislocation 48 hours after the treatment and the femur and tibia dissected out and analysed. Treatment with zearalenone increased the frequency of micronuclei and chromosomal aberrations in bonemarrow cells. Co-treatment with HSCAS resulted in a reduction of the number of micronucleated cells, a decrease in the frequency of chromosomal aberrations and an increase in the number of polychromatic erythrocytes in bone marrow (Abbès et al., 2007).

7.2.4.3. *In vivo* ³²P-postlabelling studies

Various *in vivo* postlabelling studies have been described in rodents (FAO/WHO, 2000; SCF 2000). Li et al. (1992) did not find any DNA adducts in liver, kidneys or uterus of female rats receiving a diet containing zearalenone at 0.05 mg/kg (equivalent to 5 µg/kg b.w. per day) for three weeks. DNA adducts were detected in the kidney, liver and ovary of mice treated *i.p.* (single dose of 2 mg/kg b.w. or repeated dose of 1 mg/kg b.w. every 2 days for 10 days) or orally with zearalenone (single dose of 2 mg/kg b.w.) (Pfohl-Leszkowicz et al., 1995). In another study, several DNA adducts were found by ³²P-postlabelling in female BALB/c mice treated *i.p.* with a single dose of zearalenone at 2 mg/kg b.w. in olive oil (Grosse et al., 1997).

7.2.4.4. Conclusions

Zearalenone was found to be negative in bacteria mutation studies while it was described to be clastogenic in a variety of cell culture systems such as CHO and V79 hamster cells, Vero monkey kidney cells and bovine lymphocytes. Regarding *in vivo* studies, zearalenone was found to induce micronuclei in mouse bone marrow cells following oral doses of 10-40 mg/kg b.w. per day and chromosomal aberrations following *i.p.* dosing (2-40 mg/kg b.w. per day). Overall, the CONTAM Panel concluded that zearalenone can be considered as a clastogenic compound.

DNA adducts were identified by the ³²P-postlabelling method in mice treated *i.p.* or orally with zearalenone but the interpretation of these data is hampered by the absence of structural identification of the adducts. However, the CONTAM Panel considered recent data (Pfeiffer et al., 2009) indicating that human liver cytochrome CYP1A2 catalyzed the formation of zearalenone catechols. Such catechols can also form depurinating DNA adducts that have been inferentially linked with carcinogenesis by

oestradiol (Cavalieri and Rogan, 2010). This mechanism could be relevant for genotoxicity of zearalenone but at present there are no data to evaluate its importance as a contributor to clastogenicity of zearalenone. On the other hand the amelioration of some genotoxic effects of zearalenone by antioxidants as reported above is consistent with oxidative stress related mechanisms.

7.2.5. Immunotoxicity

7.2.5.1. *In vitro* studies

As previously reviewed by JECFA (FAO/WHO, 2000), the effect of zearalenone on induced lymphocyte proliferation was measured by the incorporation of ³H-thymidine into lymphocytes. Concentrations of 2.5 and 14 µg/mL, were found to inhibit by 50 % the proliferation of rat and human peripheral lymphocytes, respectively (Atkinson and Miller, 1984; Cooray, 1984). In another study (Forsell and Pestka, 1985), zearalenone and 4 of its metabolites were also found to reduce the uptake of ³H-thymidine in mitogen-stimulated human lymphocytes. The uptake was decreased by 50 % at 3.5 µg/mL for zearalenone, 6.3 µg/mL for α-zearalenol, 36 µg/mL for β-zearalenol, 3.8 µg/mL for α-zearalanol, or 33 µg/mL for β-zearalanol. The concentration of each analogue that caused a 50 % reduction in ³H-thymidine uptake was similar for all mitogens tested (leuko-agglutinin, concanavalin A and pokeweed mitogen), suggesting that zearalenone and its metabolites can inhibit mitogen-induced proliferation by both B and T lymphocytes.

The effects of zearalenone on interleukin (IL) production were investigated by using T cells of the EL-4 murine thymoma cell line (Marin et al., 1996). These cells were stimulated with phorbol-2-myristate-13-acetate and exposed for 5 days to zearalenone or α-zearalenol at concentrations of 0.05, 0.5, 1, 5 or 10 µg/mL. Control cells were exposed to the vehicle (ethanol) only. The production of IL-2 and IL-5 was significantly increased in the presence of zearalenone or α-zearalenol at 5 and 10 µg/mL. The two compounds did not affect cell proliferation or viability, as shown in the 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay.

The immunological effects of *Fusarium* mycotoxins were tested on human peripheral blood mononuclear cells (PBMC) from different blood donors (Berek et al., 2001). Trichothecene mycotoxins, zearalenone, α-zearalenol, β-zearalenol were investigated for their effects on T and B cells in a proliferation assay, antibody-dependent cellular cytotoxicity and natural killer cell activity on human PBMC. The concentrations applied in this study were in a range of those which could be found in normal human peripheral blood system (0.0002-1.8 µg/mL). By contrast to the trichothecenes, T-2 toxin, fusarenon-X, nivalenol and deoxynivalenol, only high concentrations of zearalenone and its metabolites exerted a measurable immunosuppressing effect on human PBMC *in vitro*.

Vlata et al. (2006) investigated the *in vitro* effects of zearalenone (at concentrations of 0.1, 1, 5, 10 and 30 µg/mL) on freshly isolated human PBMC in relation to proliferation and to cell death patterns of untreated and mitogen-activated cells. The higher concentration of 30 µg/mL zearalenone was found to totally inhibit T and B lymphocytes proliferation from the stimulation with phyto-haemagglutinin and pokeweed mitogen. The inhibitory effects of zearalenone were further related to cell necrosis/apoptosis. Flow cytometry analysis showed a distinct necrotic effect on PBMC, irrespective of mitogen stimulation, whereas the induction of apoptosis was less evident. Necrosis was observed in both the lymphocyte and monocyte/granulocyte gates. Measurements of zearalenone-induced intracellular calcium ion (Ca²⁺) mobilisation showed an increase of Ca²⁺ levels and of the number of cells with high Ca²⁺ only in the monocyte/granulocyte gated cells. Using phenylmethyl sulfonyl fluoride (PMSF), a serine protease inhibitor, and ammonium chloride (NH₄Cl), a lysosomal inhibitor, both associated with cell necrosis inhibition, PMSF at 0.05 mM and NH₄Cl at 1 and 10 mM were shown to reduce the cytopathic effects induced by 30 µg/mL zearalenone, whereas apoptosis was less affected. Exposure of PBMC to 1 µg/mL zearalenone did not alter the viability of the cells. According to the authors, these results suggest that high zearalenone concentrations in the blood could exert cytotoxic effects.

Marin et al. (2010) compared the effects of zearalenone and its metabolites α -zearalenol, β -zearalenol and zearalanone on various neutrophil functions: proliferation, cytokine synthesis and oxidative stress in a porcine polymorphonuclear (PMN) model. By using the MTT test, the concentrations of toxins necessary to inhibit viability by 50 % were: 73.4 μ M for zearalenone; 59.0 μ M for α -zearalenol; 56.8 μ M for β -zearalenol and 53.1 μ M for zearalanone, with zearalenone being less toxic than its metabolites. A significant increase of superoxide-synthesis compared to the control, as shown by nitro-blue tetrazolium reduction, was observed at 1 mM concentration for only β -zearalenol and zearalanone. At 10 μ M of α -zearalenol, β -zearalenol, zearalanone, but not zearalenone, induced a significant decrease of the IL-8 synthesis in swine PMN with 49.2, 45.6 and 45.1 % respectively, compared to the control. According to the authors, these results suggest that zearalenone and its metabolites could have differential effects on important parameters of swine innate immunity like cell proliferation, IL-8 and superoxide-synthesis. In this study, metabolites of zearalenone appeared as more immunotoxic than zearalenone itself.

7.2.5.2. *In vivo* studies

As reported by JECFA (FAO/WHO, 2000), the *in vivo* immunotoxicity of zearalenone was investigated in mice receiving zearalenone by subcutaneously (*s.c.*) (Pung et al., 1984) or oral administration (Forsell et al., 1986; Pestka et al., 1987). In these studies, zearalenone did not affect either the sensitivity to *Listeria monocytogenes* infection, the serum immunoglobulins concentration or the leucocyte cell count in blood.

In order to explore the possible protective role of Tunisian radish extract against immune system disorders, Ben Salah-Abbès et al. (2008) used zearalenone as the immunotoxic agent. Balb/c mice (groups of 10 males) received a high oral dose of the toxin (40 mg/kg b.w. per day) for two weeks. Mice treated with zearalenone alone showed a significant decrease in lymphocytes of the total white blood cells, immunoglobulin profile (IgG and IgM), B cells, T-cell sub-types (CD3+, CD4+ and CD8+) and natural killer and pro-inflammatory cytokines. Mice treated with Tunisian radish extract (5, 10 or 15 mg/kg b.w. per day) for 7 days before, during or after zearalenone treatment, showed a significant effect protecting against zearalenone-induced immunological disorders. According to the authors, Tunisian radish extract would contain compounds that are able to prevent or inhibit zearalenone toxicity.

Two recent studies described the immunotoxic effects of experimental diets naturally contaminated with zearalenone in calves (Martin et al., 2010) and in primiparous sows during the perinatal period (Malovrh and Jakovac-Strajn, 2010). Unfortunately the diets used in these studies contained other mycotoxins and particularly high levels of deoxynivalenol, a well known potent immunosuppressive agent. In consequence, such exposures to multiple mycotoxins made these two studies unsuitable for assessing the specific immunotoxicity of zearalenone in these breeding animal species.

7.2.5.3. Conclusions

These data demonstrated that zearalenone exerts immunotoxic effects in *in vitro* models. These effects consist of inhibition of human and rat peripheral blood lymphocyte proliferation and increased interleukin production in cultured T-lymphocytes. In human peripheral blood mononuclear cells and porcine neutrophils, cell proliferation, and cytokine production were inhibited. In some of these studies, zearalenone appeared to be less immunotoxic than its metabolites α - and β -zearalenol. *In vivo* studies in mice did not show immunotoxicity of orally administered zearalenone, with the exception of one study in which mice were exposed to a high oral dose (40 mg/kg b.w. per day) of zearalenone for 2 weeks for assessing the protective effect of a vegetable extract.

7.2.6. Endocrine, reproductive and developmental effects

In the earlier SCF opinion (SCF, 2000), two extensive reviews on the reproductive and developmental effects of zearalenone were summarised (Kuiper-Goodman et al., 1987; FAO/WHO, 2000). Zearalenone was reported to cause alterations in the reproductive tract of females, both in laboratory animals (mice, rat, guinea-pigs, hamsters and rabbits) and in domestic animals. Various effects were described, such as persistent oestrus, decreased fertility, increased embryo-fetal resorptions, reduced litter size, alterations in the weight of adrenal, thyroid and pituitary glands, and changes in serum levels of progesterone and oestradiol. However, no teratogenic effects were reported in mice, rats, guinea pigs or rabbits. Pigs and sheep appeared to be more sensitive than rodents to reproductive disturbance after exposure to zearalenone.

7.2.6.1. Effects on males

7.2.6.1.1. Rodents

In vitro studies

In an *in vitro* study, the effect of zearalenone and its metabolite α -zearalenol on steroidogenesis in mature mouse Leydig cells was investigated (Yang et al., 2007a). Testosterone secretion and transcription level of three key steroidogenic enzymes, including 3β -hydroxysteroid dehydrogenase/isomerase (3β -HSD-1), cytochrome P450 side chain cleavage enzyme (P450scc) and steroidogenic acute regulatory protein (StAR), were measured in cells co-treated with zearalenone or α -zearalenol and human chorionic gonadotropin (hCG) at 10 ng/mL. Zearalenone and α -zearalenol, at concentrations of 10^{-4} - 10^{-8} M, significantly suppressed hCG-induced testosterone secretion. The suppressive effect was correlated with a significant decrease in the level of transcription of 3β -HSD-1, P450scc and StAR.

In vivo studies

Adult male mice were given *i.p.* injections of zearalenone or α -zearalenol at 0, 25, 50 or 75 mg/kg b.w. daily for 7 days, with 10 mice per dose group (Yang et al., 2007b). Semen quality, serum testosterone concentrations and fertility were assessed. Male mice exposed to the highest dose of zearalenone had significant reductions in body weight, as did mice exposed to the mid- and high dose of α -zearalenol. Testis and epididymal weights were unaffected, but relative seminal vesicle weights were significantly increased by all doses of zearalenone and relative preputial gland weights were significantly increased by all doses of α -zearalenol. Abnormal spermatozoa were increased and live spermatozoa were significantly decreased at all zearalenone doses. Testicular and cauda epididymal sperm counts, efficiency of sperm production and serum testosterone concentrations were significantly reduced in all mice treated with zearalenone or α -zearalenol, in a dose-dependent manner. For the investigation of fertility, the males were mated, 24 hours after the last injection, with sexually mature, untreated, superovulated females and the females were killed on GD 15 for examination of the uterine contents. Statistically significant, dose-related reductions in numbers of viable fetuses and increased numbers of animals with resorptions were observed at all doses of zearalenone and α -zearalenol. In the α -zearalenol groups given 25 or 50 mg/kg b.w. per day, there were no implantation sites, indicating that the ova were either not fertilised or fertilised ova were severely damaged.

In a study on mice, adult males were injected *i.p.* with a known toxic dose of zearalenone (4 % of the LD_{50}), with or without a simultaneous protective dose of vitamin E *i.p.* Testicular function and morphology were examined (Abou Nazel and El-Shanawany, 2007). Males were allocated into 5 groups of 8 mice per group. They comprised 3 control groups – a negative control group (no treatment), a vehicle control group given olive oil for 3 days, and a positive control group given vitamin E at 100 mg/kg b.w. per day for 3 days. Two zearalenone-treated groups were given 20 mg zearalenone/kg

b.w. per day for 3 days, or the same dose of zearalenone together with 100 mg/kg b.w. per day of vitamin E for 3 days. Serum testosterone was significantly decreased in the zearalenone-treated group compared to the control groups, while the vitamin E-protected group showed no significant difference from the control groups. Testicular tissue in animals treated with zearalenone alone showed loss of normal architecture, with seminiferous tubules showing disorganisation of spermatogenic cells and dissolution of intercellular connections. Ultrastructural examination of the same group revealed many degenerated cells with electron dense cytoplasm and dense irregular nuclei, multiple apoptotic spermatogenic cells, spermatids with various degrees of degeneration and failure of acrosomal cap formation, together with multiple abnormal spermatozoa with irregular heads. The zearalenone-treated, vitamin E-protected group showed some degeneration of testicular tissue and spermatozoa, but it was much less severe than that in the group receiving zearalenone alone. The authors noted that the effects on testis structure and testosterone secretion were similar to those seen with other oestrogens, such as diethylstilboestrol, bisphenol A and 17- β oestradiol (Nair and Shaha, 2003; Toyama and Yuasa, 2004; Yang et al., 2007a).

Ben Salah-Abbès et al. (2009) investigated the protective effect of an extract of the plant *Raphanus sativus*, which is rich in antioxidants, on zearalenone-induced male toxicity in mice. Mice were divided into 5 groups (n = 10/group): untreated controls; olive oil-treated; treated with zearalenone at 40 mg/kg b.w. per day; treated with an extract of *R. sativus* alone (15 mg/kg b.w. per day); or treated with *R. sativus* extract plus zearalenone. Treatments were given orally for 28 days. In the group treated with zearalenone alone, there were significant decreases in testis, seminal vesicle and prostate weights, and significant decreases in epididymal sperm number, sperm motility, testosterone level and antioxidant enzyme status (glutathione, catalase and superoxide dismutase) in the testis. The plant extract alone did not cause any significant toxicity compared to controls and was effective in counteracting the oxidative stress and protecting against the toxicity induced by zearalenone.

Kim et al. (2003) investigated whether apoptosis is involved in zearalenone-induced testicular toxicity in rats and identified the stage and target germ cell type affected. Ten-week-old male rats were given a single *i.p.* dose of zearalenone at 5 mg/kg b.w. and sacrificed 3, 6, 12, 24, or 48 hours later. Germ cell degeneration from apoptosis was found at stages I-VI of spermatogenesis 12 hours after dosing. This study indicates that rapid apoptosis is the principal mechanism contributing to germ cell depletion and testicular atrophy following zearalenone exposure in rats.

Yuan et al. (2010) investigated the effects of a traditional medicinal plant on zearalenone-induced apoptosis in mouse male germ cells. Groups of 10 male mice 25 days of age were given *Gynostemma pentaphyllum* at 0, 50, 100 or 200 mg/kg b.w. per day, together 0 or 10 μ g per animal per day of zearalenone. *G. pentaphyllum* had a marked effect on protecting male germ cells against apoptosis induced by zearalenone. In addition, *G. pentaphyllum* reversed other effects of zearalenone, remarkably improving the pathologic changes in testicular tissue, reducing the content of malondialdehyde (a marker of lipid oxidation), and increasing the activity of superoxide dismutase. These results indicate that *G. pentaphyllum* protects against toxicity caused by zearalenone through its properties of anti-oxidation and anti-apoptosis. A study investigated the testicular effects of zearalenone on very young male rats, 5-15 days of age (Filipiak et al., 2009). Groups of 7-13 animals were given *s.c.* injections daily of vehicle alone, 17 β -oestradiol (1.25 or 12.5 μ g), diethylstilboestrol (1.25 or 12.5 μ g), or zearalenone (4 or 40 μ g). The animals were killed at 16 days of age and the testes examined. Testis weight, seminiferous tubule diameter and length were significantly decreased by all three treatments at both doses. Both doses of 17 β -oestradiol and diethylstilboestrol also significantly reduced the numbers of germ cells (spermatogonia and spermatocytes) and the number of Sertoli cells, but zearalenone only reduced the number of spermatogonia and Sertoli cells at the higher dose. Zearalenone was the weakest of the three oestrogenic substances.

7.2.6.1.2. Pigs

In vitro studies

Tsakmakidis et al. (2006) investigated the effects of zearalenone or its metabolite, α -zearalenol, on boar sperm parameters such as motility, viability and spontaneous acrosome reaction. Semen was collected from a single boar of proven fertility and incubated for 4 hours with zearalenone or α -zearalenol at concentrations of 125, 187.5 and 250 μ M. The lowest concentration tested was equivalent to 40 μ g/mL. Sperm parameters were assessed at 1, 2, 3 and 4 hours of the incubation period. Both zearalenone and α -zearalenol significantly affected sperm characteristics in a concentration-related manner at all time points, except for the low concentration of α -zearalenol, which did not decrease the percentage of live reacted spermatozoa.

In a follow-up study (Tsakamakidis et al., 2008), 12 semen collections from 4 boars were incubated for 4 hours *in vitro* with lower concentrations of 10, 20 or 30 μ g/mL of zearalenone or α -zearalenol. Motility and nuclear chromatin integrity were assessed at 0 and 4 hours of incubation. There were no significant differences in motility and chromatin instability was significantly higher in spermatozoa of only one boar independently of the dose of zearalenone or α -zearalenol.

In another study, Tsakamakidis et al. (2007) assessed the ability of boar spermatozoa to bind to the zona pellucida of the oocyte. Boar semen was exposed to zearalenone or α -zearalenol at concentrations of 40, 60 or 80 μ g/mL for 1 hour. The semen was then washed and incubated with homologous oocyte hemizona for 4 hours. Significant decreases in the number of tightly attached spermatozoa on the hemizona were obtained at concentrations of 60 and 80 μ g/mL of both zearalenone and α -zearalenol.

Effects on boar sperm exposed to zearalenone, α -zearalenol or β -zearalenol at varying concentrations were investigated (Benzoni et al., 2008). Twelve semen collections from 3 boars of proven fertility were incubated with the mycotoxins for up to 48 hours. Viability, apoptosis and chromatin stability were assessed after 24 and 48 hours of incubation with 1×10^{-8} to 1 μ M. Motility was assessed at 5, 16 and 24 hours using higher concentrations of mycotoxins ranging from 2×10^{-7} to 20 μ M. Zearalenone and β -zearalenol reduced sperm viability at picomolar concentrations, α -zearalenol at nanomolar concentrations. Sperm chromatin stability was adversely affected by α -zearalenol at picomolar concentrations and by β -zearalenol at nanomolar concentrations; β -zearalenol reduced sperm motility at micromolar concentrations at 5 hours. These results differ from those of Tsakamakidis et al. (2007) in finding effects at much lower concentrations, but the CONTAM Panel noted that longer incubation times were used and that the short elimination half-lives for zearalenone and its metabolites *in vivo* (see Section 7.1) would not be compatible with contact times of 24-48 hours.

In vivo studies

The earlier literature records adverse effects of feeding zearalenone-contaminated corn on the male reproductive tract and sexual function of pigs. Adverse effects have been recorded at concentrations of zearalenone in feed of 20 mg/kg or more (reviewed by Diekmann and Green, 1992).

In an *in vivo* study, the absence of any effect of low levels of zearalenone in feed was confirmed (Sutkeviciene et al., 2009). Six mature boars were fed with naturally contaminated diet containing zearalenone at 1 mg/kg of feed for 2 months. No changes were observed in testis weight, epididymal weight, histopathology or the sperm quality parameters of concentration, motility, viability and abnormalities.

7.2.6.1.3. Horses

In vitro studies

A study assessed the effects of exposure of sperm *in vitro* to extracts of urine taken from male horses naturally exposed to zearalenone (Minervini et al., 2010). Zearalenone and its metabolites in urine were measured by ELISA. Thirty-eight urine extracts were tested on frozen-thawed spermatozoa to evaluate effects on sperm chromatin structure. Urine samples gave a mean level of 32.3 ng zearalenone/mL. Adverse effects of zearalenone-containing urine samples on sperm chromatin were found.

7.2.6.1.4. Conclusions on males

Adverse effects of zearalenone and its metabolites have been observed in male rodents but only at high dose levels. The protective effect of antioxidants on male reproductive disturbance in rodents suggests that the effects of zearalenone at high doses in males may be secondary to generation of reactive oxygen species.

Pigs are more sensitive, and adverse effects on testosterone concentrations, sexual behaviour, testis and secondary sex organ weight, testicular histology and spermatogenesis have been observed following exposure of boars to zearalenone or its metabolites. However, the exposures at which such effects occur in male pigs are higher than those at which effects on female pigs have been observed (see below).

7.2.6.2. Effects on females

7.2.6.2.1. Ovary, oocyte and zygote studies

7.2.6.2.1.1. Pigs

In vitro studies

The effects of α -zearalenol and β -zearalenol on *in vitro* maturation and degeneration rates of porcine cumulus-oocyte complexes (COCs) were investigated (Alm et al., 2002). Concentrations tested ranged from 3.75-90 μ M and 60-88 COCs were examined at each concentration. Both substances affected maturation and degeneration rates in a dose-dependent manner, but to different extents. Significant delays in maturation of COCs were obtained at concentrations of α -zearalenol > 7.5 μ M and at concentrations of β -zearalenol > 30.0 μ M. Significant increases in degeneration were seen at concentrations > 30 μ M for both α -zearalenol and β -zearalenol. The influence of α -zearalenol at concentrations of 3.75-30 μ M on the developmental competence of *in vivo*-derived zygotes during 5 days of *in vitro* culture was also investigated. The number of zygotes examined at each concentration ranged from 29-32. Significant reductions in transition from zygote to blastocyst and reduced numbers of cell nuclei in blastocysts were observed at concentrations of α -zearalenol > 15 μ M. This study demonstrated an adverse effect of direct exposure to zearalenone metabolites on meiosis progression in oocytes and on embryonic development.

Tiemann et al. (2003a) investigated whether α -zearalenol and β -zearalenol *in vitro* affected the enzymes P450_{scc} and 3 β -HSD involved in progesterone synthesis in cultured porcine granulosa cells. No differences in basal progesterone levels or numbers of viable cells were observed between untreated granulosa cells and those treated with α -zearalenol or β -zearalenol at 15 and 30 μ M. Follicle stimulating hormone (FSH) (0.01 μ g/mL) or forskolin (10 μ M) enhanced the low basal progesterone secretion in the absence of the mycotoxin metabolites. The addition of α -zearalenol or β -zearalenol at 7.5, 15 and 30 μ M to cultures stimulated with FSH or forskolin reduced progesterone synthesis and the levels of P450_{scc} and 3 β -HSD transcripts in a dose-dependent manner, reaching statistical significance at the highest

concentration of 30 μM . The enzymatic activity of 3 β -HSD and the abundance of P450_{scc} protein were also reduced by these mycotoxins.

Jakimiuk et al., (2010a) studied the impact of zearalenone *in vitro* on the production and secretion of steroid sex hormones by granulosa cells in monoculture and granulosa cells with theca interna cells in co-culture. Follicular cells were obtained from ovarian follicles of pre-pubertal gilts. They were exposed to zearalenone at concentrations of 0, 0.4, 4, 40, or 400 ng/mL (0, 1.2, 12, 120 or 1200 μM) for 48 hours. The concentrations of progesterone, testosterone, and 17 β -oestradiol in the medium were determined by radioimmunoassay. In accordance with the results of Tiemann et al. (2003a), who used the main zearalenone metabolites, there was no effect of zearalenone on progesterone secretion in granulosa cells in monoculture. Nor was there any effect on 17 β -oestradiol secretion in granulosa cells in monoculture. Testosterone secretion was slightly but significantly reduced by zearalenone at 0.4, 4 and 400 ng/mL, but not at 40 ng/mL. However, in co-cultures, progesterone secretion was significantly increased at all tested concentrations of zearalenone, and secretion of 17 β -oestradiol and testosterone were increased at 400 ng/mL. Thus, the stimulating and inhibitory effect of zearalenone on basal steroidogenesis in porcine ovarian follicular cells was dependent on the type of cultures and concentrations of zearalenone.

Ranzenigo et al. (2008) investigated the effect of the metabolite α -zearalenol *in vitro* on cell proliferation, steroidogenesis and gene expression in cultured pig granulosa cells. α -Zearalenol had biphasic effects on oestradiol production induced by insulin-like growth factor (IGF-1) and FSH in combination, increasing production at smaller concentrations of 30 ng/mL (0.0937 μM) and inhibiting it at larger concentrations of 300 (0.937 μM) and 3000 ng/mL (9.37 μM). At 3000 ng/mL, α -zearalenol also increased FSH plus IGF-I-induced progesterone production but not at the two lower concentrations. Progesterone production induced by FSH alone was increased by the lower concentrations of 30 and 300 ng/mL α -zearalenol, but was decreased by the higher concentration of 3000 ng/mL. At 3000 ng/mL α -zearalenol inhibited FSH plus IGF-I-induced CYP19A1 and CYP11A1 mRNA abundance. α -Zearalenol had no effect on granulosa cell numbers. This study also illustrates the stimulatory and inhibitory effects of a zearalenone metabolite on steroidogenesis in porcine cells in culture, dependent on the concentration of the test substance and the culture conditions.

Chen et al. (2009) studied the effects of maturing pig oocytes *in vitro* in the presence of zearalenone or its metabolites, α - and β -zearalenol at concentrations of 0, 0.312, 3.12 or 31.2 $\mu\text{mol/L}$. They caused a concentration-related inhibition of oocyte, maturation abnormal formation of aberrant spindle and abnormal nuclei. The developmental competence of oocytes matured in the presence of zearalenone (3.12 $\mu\text{mol/L}$) was also investigated after *in vitro* fertilization. Exposure during maturation significantly reduced the percentages of oocytes that cleaved and formed a blastocyst and increased the percentage of aneuploid blastomeres.

In vivo studies

Of the studies commented on by the SCF, there were several experimental studies with pigs. The pivotal one was a study with sexually mature non-pregnant gilts, given 2 kg of feed per day containing 0, 1, 5 or 10 mg purified zearalenone/kg of feed between day 5 and day 20 of oestrus, equivalent to 0, 40, 200 or 400 $\mu\text{g/kg}$ b.w. per day (Edwards et al., 1987). The study was replicated in winter and summer and the number of gilts per dose group from the two seasons combined was 24-25. Similar results were obtained from the two seasons. In the combined results, the inter-oestrous interval increased significantly from 21.0 ± 0.3 days in the control group to 29.2 ± 2.9 and 32.7 ± 3.3 days in gilts fed 5 or 10 mg zearalenone/kg of feed. The inter-oestrous interval was not affected in gilts given 1 mg zearalenone/kg diet. Increased plasma progesterone and prolonged maintenance of corpora lutea were observed in gilts with prolonged oestrous cycles. The corpora lutea regressed when zearalenone was withdrawn from the diet. A NOEL of 40 $\mu\text{g/kg}$ b.w. per day can be taken from this study.

In a limited study (Bauer et al., 1987) using a low number of prepubertal female pigs, and pure zearalenone added to the diet, oestrogenic effects were reported at lower doses than in the previous study by Edwards et al. (1987). Two pigs were fed 0.25 mg zearalenone/kg diet (equivalent to 10 µg/kg b.w. per day) for 11 days, followed by 5 days of feed without zearalenone. Two additional pigs were fed 0.05 mg zearalenone/kg diet (equivalent to 2 µg/kg b.w. per day) for 21 days. Only 1 pig was used as a control. The higher dose resulted in redness and swelling of the vulva, swelling of the mammarys, and numerous vesicular follicles and some cystic follicles on the ovaries. With the low dose, no external changes were seen at the end of the experimental period, but autopsy showed that the number of vesicular follicles on the ovaries was higher in treated animals than in the control animal. The SCF considered that these reported effects would require confirmation using larger numbers of animals to establish a clear NOEL.

Obremski et al. (2003a) studied ovarian cell proliferation in sexually immature gilts given zearalenone orally at doses of 0.2 and 0.4 mg/kg b.w. per day for 7 days. At the end of the treatment period, numerous histopathological changes in ovarian follicle structure were observed, including ovarian follicle atresia and apoptosis-like changes in granule cells. Intensified cell proliferation was also observed in the uterus and oviduct.

In a study on apoptosis in the ovaries (Wasowicz et al., 2005), gilts before first oestrus were given zearalenone in gelatine capsules at doses of 0, 20 or 40 µg/kg b.w. per day for 63 days. They were then sacrificed and the ovaries were studied for apoptosis and for the presence of apoptosis-promoting protein Bax. Apoptosis was detected only in medium-sized antral ovarian follicles in control and low-dose animals. There were no differences in the distribution and intensity of staining for Bax. The results indicate that zearalenone did not induce apoptosis in pig ovaries, and that inhibition of proliferation observed in earlier studies is associated with other mechanisms.

Zwierzchowski et al. (2005) investigated how an oral dose of zearalenone of 200 µg/kg b.w. per day given for 8 days influences the level of zearalenone and α -zearalenol in blood plasma and the occurrence of histopathological changes in the cells of the ovarian follicles in sexually immature gilts (4 treated, 4 controls). On day 8 the animals were sacrificed and their ovaries were taken for histopathological examination. In the treated animals, the highest blood serum concentration of zearalenone plus α -zearalenol was noted on day 5; external symptoms of oestrus without standing reflex were observed on day 4. A few ovarian follicles were found, but they were located in the cortical layer and were filled with a liquid substance rich in protein and without the granulosa layer. There was disintegration with apoptotic-like changes of cells in the granulosa layer of single mature follicles. The CONTAM Panel noted that this effect level is the same as that in the earlier pivotal study by Edwards et al. (1987). A NOEL was not established.

Following up on their earlier *in vitro* study (Tiemann et al., 2003a), Alm et al. (2006) investigated *in vivo*, the influence of *Fusarium*-toxin contaminated feed on cumulus cell morphology and maturation of porcine oocytes. Wheat that was naturally contaminated with deoxynivalenol and zearalenone was included in feed for gilts in increasing dietary concentrations. In mg toxin/kg feed the amounts were: Group 1 (control), 0.21 and 0.004, Group 2, 3.07 and 0.088, Group 3, 6.1 and 0.235, Group 4, 9.57 and 0.358, for deoxynivalenol and zearalenone, respectively. There were 9 gilts per group. Feed was refused by some gilts in Groups 2, 3 and 4 during the first 21 days of the 35 days of treatment. The mean daily exposure to deoxynivalenol and zearalenone during this period amounted to 3.6 and 0.1, 52.4 and 1.5, 104.2 and 4.0, and 169.1 and 6.3 µg/kg b.w. for animals of Groups 1-4, respectively. Deoxynivalenol and zearalenone exposures increased to 3.8 and 0.1, 54.9 and 1.6, 104.6 and 4.0, to 146.5 and 5.5 µg/kg b.w. for Groups 1-4, respectively, when the average feed-intake over the whole experimental period was used. At the end of the treatment period, the gilts were anaesthetised and oocytes and granulosa cells recovered from the ovaries were analyzed for the expression of enzymes involved in progesterone synthesis (P450scc and 3 β -HSD mRNA and additionally for P450scc protein). At post-mortem, no signs of hyperoestrogenism or uterotrophic effects were observed due to feed treatments. In contrast to their earlier observations *in vitro*, progesterone synthesis enzyme parameters were unchanged. Cumulus cell

morphology did not differ significantly between treatment groups. The proportion of oocytes with degenerated meiotic chromatin was significantly higher in Group 4 than in the other groups. The proportion of oocytes reaching metaphase II in culture was significantly lower in Groups 3 and 4 than in Group 1, and tended to be lower in Group 2 than in Group 1.

7.2.6.2.1.2. Cattle

In vitro studies

Zearalenone, α -zearalenol and zearalanone were tested to evaluate effects on the *in vitro* maturation rate of bovine oocytes and on the formation of 17β -oestradiol in supernatants of mural granulosa cell cultures (Minervini et al., 2001). Concentrations tested ranged from 0.3-30 $\mu\text{g/mL}$ and 7-25 oocytes or 2-4 supernatants were examined at each concentration. Maturation of oocytes to metaphase II stage was inhibited in the presence of 30 $\mu\text{g/mL}$ zearalenone, α -zearalenol or zearalanone, with a significant increase in chromatin abnormalities occurring in the presence of zearalenone and α -zearalenol. Higher concentrations of 17β -oestradiol were found in the presence of α -zearalenol compared with zearalenone and zearalanone. These data demonstrated an adverse effect of zearalenone and its metabolites on meiotic progression of bovine oocytes, possibly attributable to independent effects on steroidogenesis not related to the binding affinity of these compounds to ER sites, and supports previous observations (Olsen, 1989) that α -zearalenol acts as a stronger oestrogenic inducer than zearalenone.

Bovine oocytes were cultured in a medium containing zearalenone concentrations of 0, 1, 10, 100, and 1000 $\mu\text{g/L}$, fertilised, and cultured further (Takagi et al., 2008). There were 108-131 oocytes per concentration. The oocyte maturation rate was significantly decreased at the highest concentration of 1000 $\mu\text{g/L}$ and 50 % of oocytes in the 1000 $\mu\text{g/L}$ group were arrested in metaphase I, without affecting the fertilisation rate. Blastocyst-formation rates did not significantly differ among the groups.

7.2.6.2.2. Reproductive tract studies

7.2.6.2.2.1. Rodents

In vivo studies

Nikaido et al. (2003) gave rats zearalenone by *s.c.* injection of 0, 0.1 or 10 mg/kg b.w. per day ($n = 30$ per group) for 4 consecutive days, from 15-19 days of age. Six rats per group were killed at 28 days of age and the remaining animals later. At 28 days of age there were no histological effects on the uterus, vagina, ovaries or mammary glands. Vaginal opening was significantly accelerated by one day at the high dose of zearalenone, and oestrous cycles were significantly disturbed, with the majority of animals in both treated groups showing either persistent oestrus or prolonged dioestrus. The absence of effects in this study on mammary gland tissue, which was investigated both qualitatively and quantitatively, is in contrast to a later study in which effects on the mammary gland were observed at 30 days of age when zearalenone exposure occurred prenatally as opposed to postnatally (see Belli et al., 2010 in Section 7.2.6.2.3.1.).

Female rats were fed with zearalenone (0.03-10 mg/kg b.w. per day) for 3 days starting on postnatal day 21 using a validated OECD protocol for the immature rat uterotrophic assay (Heneweer et al., 2007). Zearalenone significantly increased uterine weight at doses of 1 and 10 mg/kg b.w. per day, and significantly increased uterine epithelial cell height at 10 mg/kg b.w. per day. Zearalenone, at doses as low as 0.1 mg/kg b.w. per day, produced oedema and vacuolization in some animals. Zearalenone doses greater than 1 mg/kg b.w. per day of zearalenone produced extensive degeneration and necrosis of the uterine epithelial layer. The main uterine gene-regulated pathways that were significantly up- or down-regulated by zearalenone treatment included inflammation, water regulation, remodelling of

extracellular matrix, alternative complement activation, cell proliferation, and oestrogen-mediated calcium signalling. Zearalenone also recruited coregulator inhibitor of kappaB and induced expression of the matrix metalloproteinase 7 gene (6.9-fold upregulation), which plays an important role in the maintenance of the integrity of the epithelial layer of the uterus during proliferation and growth.

Several measures of oestrogenic responses to α -zearalanol and zearalenone were compared to the reference oestrogens, oestradiol or diethylstilboestrol, using female CD-1 mice injected *s.c.* on postnatal day 17 at various doses ranging from 0.01 to 1,000,000 $\mu\text{g}/\text{kg}$ b.w. per day (Jefferson et al., 2002). A uterotrophic bioassay showed that α -zearalanol and zearalenone caused significant increases in uterine wet weight at doses $> 10\,000$ $\mu\text{g}/\text{kg}$ b.w. per day. Uterine morphological parameters (e.g., uterine epithelial cell height and gland number) also showed significant increases at doses of α -zearalanol and zearalenone $> 10,000$ $\mu\text{g}/\text{kg}$ b.w. per day, and induction of the oestrogen-responsive protein, lactoferrin, occurred at doses of α -zearalanol and zearalenone $> 1,000$ $\mu\text{g}/\text{kg}$ b.w. per day. In addition, the *in vivo* results were compared with an *in vitro* assay using the BG1Luc4E2 cell line to measure transcriptional activation of ER- α (i.e., effective concentration (EC_{50}) values for α -zearalanol (122 pM) and zearalenone (1,700 pM) in comparison with oestradiol (8 pM) and diethylstilboestrol (18 pM)).

7.2.6.2.2.2. Pigs

In vitro studies

A study by Tiemann et al. (2003b) investigated the *in vitro* effects α -zearalenol and β -zearalenol at concentrations of 7.5, 15 and 30 μM on porcine uterine cell cycle distribution in combination with the proliferating cell nuclear antigen marker by flow cytometry. The viability of uterine cells was not impaired at 30 μM α -zearalenol, whereas β -zearalenol at this concentration significantly decreased cell number. Some cells showed ultrastructural features of cell death indicated by swollen mitochondria, disrupted cell membranes, and many vacuoles. After 24 and 48 hours of exposure to α -zearalenol up to 30 μM , the cell cycle distribution was still comparable to the control groups. An anti-proliferative effect of β -zearalenol up to 30 μM was detected by a significant reduction in the S-phase together with arrest of cells in the G0/G1-phase. A significant decrease in the expression of the proliferation marker proliferating cell nuclear antigen (PCNA) amounts indicated that β -zearalenol disengaged cells from active cycling. The study confirmed that α -zearalenol binds to the porcine uterine cytoplasmic ER.

In vivo studies

As previously reported by JECFA (FAO/WHO, 2000), pathological alterations were observed in the reproductive tract of female pigs given *Fusarium culmorum* extracts containing 80 mg/kg of zearalenone (equivalent to 3.2 mg/kg b.w. per day) and 5 mg/kg of deoxynivalenol (Palyusik et al., 1990). Vulvar swelling and reddening were observed in gilts within 7 days of exposure to diet containing purified zearalenone at a concentration of 2 mg/kg (equivalent to 0.08 mg/kg b.w. per day) (Rainey et al., 1990). In ovariectomized piglets weighing ~ 41 kg the feeding of diets containing 0.18 and 0.36 mg of crystalline zearalenone/kg diet the weights of the uteri were increased dose-independently (Coenen and Boyens, 2001). This study is reported in abstract only.

Feeding of diets containing increasing zearalenone concentrations of 0.01, 0.06, 0.15, 0.22, 0.42 mg/kg from naturally contaminated maize to female piglets for 5 weeks corresponding to a mean exposure of 0.5, 3.0, 7.4, 10.4 and 17.6 μg zearalenone/kg b.w. per day (mean body weight range ~ 12 -33 kg) resulted in an apparent dose related increase in the number of piglets with swollen and reddened vulva and cervix at all exposure levels, which was only statistically significant at the highest dose ($n = 20$ per group) (Döll et al., 2003b). Also, the weights of the uteri were significantly increased only at the highest dose when evaluated both on an absolute weight basis (13.9, 18.0, 13.3, 16.4 and 24.0 g, respectively) and relative to body weight (0.41, 0.53, 0.40, 0.51 and 0.81 g/kg b.w., respectively). As naturally contaminated maize was used in this study the piglets were additionally exposed to deoxynivalenol at

0.2, 0.8, 1.0, 1.9 and 3.9 mg deoxynivalenol/kg diet (9.8, 39.6, 49.1, 90.2 and 163.5 µg deoxynivalenol/kg b.w. per day). It should be noted that the effects on the uteri were detected although body weight declined at the same time (significant at the highest dose), probably due to the feed intake depressing effects of deoxynivalenol (34, 34, 34, 32 and 30 kg of final b.w., respectively). This study indicates a NOEL 10.4 µg/kg b.w. per day for zearalenone.

No effects were found on luteinising hormone (LH) while FSH decreased independently of dose. Histopathologically, no marked effects on the general appearance of the uterus sections were seen and no conspicuous alterations in morphometric parameters were found (n = 10 per group) (Döll et al., 2003a).

Feeding zearalenone containing capsules to pigs weighing ~ 49 kg corresponding to 0.18 and 0.36 mg zearalenone/kg b.w. per day for 7 days the observed clinical hyperoestrogenism (oedema and reddening of vulva) was more pronounced at the higher dosage whereas no correlations between plasma zearalenone and α -zearalenol concentrations and signs of hyperoestrogenism could be established (Obremski et al., 2003b).

The effects of *in vivo* perinatal zearalenone exposure on the expression of oestrogen-sensitive, uterine genes associated with endometrial development in neonatal female pigs were studied (Chen et al., 2009). The genes were relaxin/insulin-like factor 3 receptors RXFP1 and RXFP2, the morphoregulatory genes Wnt4, Wnt7a and Hoxa10, and ER- α . Pregnant sows were fed zearalenone at a concentration of 1500 µg zearalenone/kg of feed per day or vehicle from 14 days before farrowing until postnatal day (PND) 20-21. It is unclear whether the diet was naturally contaminated or if purified zearalenone was added. At birth, the piglets were cross-fostered to generate four zearalenone exposure groups (5-6/group), i.e. unexposed controls, prenatal exposure only, postnatal exposure only, or pre- and postnatal (continuous) exposure. Neonatal uterine tissues were collected at PND 20-21. Uterine Wnt7a, Hoxa10, ER- α and RXFP2 mRNA levels were significantly decreased in neonates exposed continuously to zearalenone. Uterine RXFP1 mRNA was decreased in postnatally and continuously exposed groups. Neonatal uterine Wnt4 mRNA levels were unchanged.

Jiang et al. (2010b) investigated the effect of zearalenone added to the diet in pre-pubertal female pigs. The basal diet, to which 1 mg zearalenone/kg diet was added, was co-contaminated with naturally occurring zearalenone, aflatoxins and fumonisins, but not deoxynivalenol. The total zearalenone concentration in the diet was 1.3 mg/kg. The diet was fed from 42 days of age for 24 days and the animals were then killed. There were 5 animals per group. The dimensions of the vulva were measured at 4-day intervals during the study and the weight of the uterus plus ovary at termination. Data on body weight and feed intake from the same study but reported separately (Jiang et al., 2010a) indicate that exposure to zearalenone ranged from 0.150 mg/kg b.w. per day at the start of the study to 0.06 mg/kg b.w. per day at the end of the study. The length, width and area of the vulva were significantly increased in those given added zearalenone in the diet, as was the weight of the uterus plus ovary. Other observations on general toxicity from this study have been described in Section 7.2.2.4.

The performance, heart, liver, kidney and spleen weights, and morphology of the vulva were evaluated in pre-pubertal gilts fed control diet without zearalenone, or diet containing zearalenone at a concentration of 2 mg/kg for 28 days (Andretta et al., 2008). There were 6 animals per group. The amount of zearalenone consumed ranged from 0.17 mg/kg b.w. per day on day 1 to 0.08 mg/kg b.w. per day on day 28. There were no differences between the two groups in daily feed intake, average daily weight gain, feed conversion ratio, or organ weights. However, zearalenone significantly increased the length and weight of the reproductive tract. The final volume of the vulva was on average 820 % larger in gilts fed diets containing zearalenone compared with those fed control diet. Similar results were obtained in a subsequent replica experiment (Andretta et al., 2010).

In a study on prepubertal gilts (Oliver et al., 2010), the effects of zearalenone on growth performance and reproductive tract development were investigated. After weaning at 21 days of age, animals were

randomly assigned after one further week to consume a commercial basal diet containing zearalenone at 0 or 1.5 mg/kg of feed for 4 weeks (controls 9 gilts, treated 10 gilts). They were then killed, urine collected, and tissue collected and frozen. Zearalenone, α -zearalenol, and β -zearalenol were detected at levels of less than 4 $\mu\text{g}/\text{kg}$ in urine of controls, but were significantly increased to 292 ± 76 , 113 ± 20 , and 15 ± 3 $\mu\text{g}/\text{kg}$, respectively, in pigs consuming zearalenone. There were no effects on feed intake or growth. In gilts fed zearalenone, reproductive tract size was significantly increased 1.5-fold and uterine endometrial gland development was increased by 50 %. Uterine ER- α mRNA and protein were unchanged, but in those fed zearalenone, there was a 2- and 3-fold higher abundance of ER- β mRNA and protein, respectively, compared with controls. This study is reported in abstract only. It is described here because the study subjects were prepubertal gilts, pure zearalenone was given rather than naturally contaminated feed, and the concentration in feed was only slightly higher than the level in feed of 1 mg/kg that was without any effect in the earlier pivotal study using mature gilts by Edwards et al. (1987). The exposure on a body weight basis was not stated but young gilts would be lighter than mature gilts.

Twelve 2-month-old, sexually immature gilts, weighing on average 40 kg at the start of the study, were given zearalenone orally in gelatine capsules at doses of 0, 20 or 40 $\mu\text{g}/\text{kg}$ b.w. per day for 48 days and the ovaries and uterus were examined morphometrically at the end of the period of treatment (Jakimiuk et al., 2010b). There were 4 gilts per group. The authors stated that the standard feed was analysed for aflatoxin, ochratoxin, zearalenone, α -zearalenol and deoxynivalenol, but no information was given on the LODs or the results of the analyses. No significant changes were noted, except for an increase in the number of medium-sized ovarian follicles in those given the lower dose of zearalenone. However, the size of the follicles would also depend on the stage of the cycle at which they were examined. There were no changes in the numbers of small or large ovarian follicles and the authors concluded that these doses of zearaleneone were without effect.

The same group have investigated whether oral administration of zearalenone resulted in changes in uterine histology in sexually immature gilts (Gajecka et al., 2011). The description of the study was the same as that above with 4 gilts per group (and possibly the same animals were used). The authors again stated that the standard feed was analysed for other mycotoxins but no further information was given. Both doses of zearalenone of 20 and 40 $\mu\text{g}/\text{kg}$ b.w. per day given for 48 days induced hyperoestrogenism and atypical endometrial hyperplasia. The accompanying uterine hyperaemia caused uterine reddening and swelling. The authors suggested that this study raises the possibility of detrimental health effects when the level of endogenous oestrogen is low and the body is supplied with an additional dose of exogenous oestrogen. This study indicates an effect of zearalenone in immature gilts at exposures as low as 20 $\mu\text{g}/\text{kg}$ b.w. per day. Although it seems contrary to what they reported previously (Jakimiuk et al., 2010b), it is possible that histopathological examination is more sensitive than morphometry (weight and length only).

7.2.6.2.2.3. Horses

In vivo studies

In a study on horses, mares were given daily oral doses of 7 mg of zearalenone starting 10 days after ovulation and continuing until the next ovulation (Juhasz et al., 2001). There was no effect on inter-ovulatory intervals, or the luteal or follicular phases of the oestrous cycle. Similarly, there were no effects on plasma progesterone, follicular number, growth and size, or uterine oedema.

Six mature, fertile mares were fed oats twice daily that were free from mycotoxins or were naturally contaminated with zearalenone and deoxynivalenol for 5 consecutive oestrous cycles (Aurich et al., 2006). There was no separate control group, animals serving as their own controls. In cycles 1, 3 and 5 the mares received mycotoxin-free oats and in cycles 2 and 4 mycotoxin-contaminated oats. The concentrations of mycotoxins were 1 and 12 mg/kg for zearalenone and deoxynivalenol, respectively. In

cycle 2 or 4 they were fed 3 kg of contaminated oats per day and in cycle 4 or 2 they were fed 1.5 kg of contaminated oats and 1.5 kg of uncontaminated oats per day. They were monitored every other day by rectal palpation and ultrasound examination of the ovaries and uterus. Samples were taken at 2-day intervals and analysed for concentrations of progesterone, oestradiol and LH with assays validated for the horse. Endometrial biopsies were collected on day 10 (ovulation = day 0) in all cycles. There were no effects on the release of reproductive hormones, cycle length or uterine histology. Although the cycle length was shorter than reported for untreated mares, the authors considered this was not an effect of the mycotoxins, but most likely caused by collection of endometrial biopsies on day 10 of the cycle. However, the mycotoxins tended to increase the number of growing follicles during the second half of the cycle. In addition, in the cycles when there was exposure to mycotoxins, there was a high incidence of haemorrhagic corpora lutea and follicular haematomas, which did not occur during control cycles. The authors concluded that, in contrast to pigs and ruminants, horses seem to be less sensitive to the effects of mycotoxins on reproductive functions.

7.2.6.2.3. Effects on the pregnant animal, embryo, fetus and offspring

7.2.6.2.3.1. Rodents

In vivo studies

The effects of zearalenone on pregnancy and embryo-fetal development of rats were investigated (Collins et al., 2006). Pregnant female Sprague-Dawley rats were given zearalenone once daily by oral gavage at doses of 0, 1, 2, 4, or 8 mg/kg b.w. per day on GD 6-19, and killed on GD 20. There were 27 females per dose group. Reproductive and developmental parameters were measured and blood was taken for gonadotrophin (LH, FSH, prolactin) and sex steroid hormone analyses. Significant, dose-related decreases were seen in maternal feed consumption and body weight gain in all treated groups. At 4 and 8 mg/kg per day, maternal liver-body weight ratios were significantly increased and organ-brain weight ratios for weights of liver, heart, spleen, kidneys, and ovaries were significantly decreased. At 8 mg/kg b.w. per day, the pregnancy rate was significantly reduced, the number of viable fetuses was significantly reduced, and the proportion of embryo-fetal deaths was significantly increased. Fetal body weight was significantly decreased in both sexes in all treated groups. Skeletal ossification was significantly retarded at 4 and 8 mg/kg b.w. per day, in a dose-related manner. Fetal anogenital distance was not affected but fetal anogenital index (anogenital distance normalized for body weight) was increased in all treated groups, indicating an androgenic effect of zearalenone. LH and FSH were not significantly affected. Prolactin was significantly increased at 8 mg/kg b.w. per day. Progesterone was decreased at 2, 4, and 8 mg/kg b.w. per day and the decreases were significant at 2 and 4 mg/kg b.w. per day. There was a dose-related decrease in oestradiol at 2, 4, and 8 mg/kg, which was statistically significant at 8 mg/kg b.w. per day. This study showed that zearalenone was maternally toxic and fetotoxic but not teratogenic. The NOEL for reproductive and fetotoxic effects was less than 1 mg/kg b.w. per day. The CONTAM Panel noted the unexpected observation of an androgenic effect.

In a study in mice (Nikaido et al., 2004), pregnant animals were given s.c. injections of zearalenone at either 0.5 or 10 mg/kg b.w. per day for 4 consecutive days, beginning on GD 15. The female offspring were examined at 4, 8, 12 and 16 weeks of age. In the group given the high dose, mammary growth was reduced and there was a lack of corpora lutea and vaginal cornification.

A study in female Wistar rats investigated the effect of administration of zearalenone during the fetal period and the first days of life on mammary gland development (Belli et al., 2010). Zearalenone was given to groups of 9 pregnant animals from GD 9 until delivery by s.c. injection, at doses of 0, 0.2 µg, 20 µg, 1 mg and 5 mg/kg b.w. per day, and then the same doses were administered to the pups by s.c. injection into the neck for the first 5 PNDs. In the group treated with 5 mg/kg b.w. per day of zearalenone, two dams died during pregnancy and one during delivery; another was euthanized for dystocia. The necropsy observations showed traces of fetal resorption and abnormalities in the size of

the fetuses (no details given), which the authors considered may have been responsible for the deaths by dystocia. The remaining mothers presented no clinical or behavioural changes. Mammary tissue was examined for development and maturation by morphologic analyses and immunochemistry at PND 30, a period of active terminal bud development, and/or at PND 180 (adulthood). At PND 30, the mean length of terminal buds was significantly enhanced in all of the zearalenone-exposed females, in a dose-related manner. Mammary tissue was more differentiated in the zearalenone-exposed groups in a dose-related manner compared with controls, reaching statistical significance in the 1 mg/kg b.w. per day treated group. There was no effect of zearalenone on epithelial cell proliferation at PND 30. At PND 180, mammary tissue was more differentiated in all of the zearalenone-exposed groups and some females exposed to 5 mg/kg b.w. per day showed hyperplasia of the ductal epithelium. No such lesions were seen in the lower dose groups. These effects differ from those observed in the mouse at a higher *s.c.* dose of 10 mg/kg b.w. per day (Nikaido et al., 2004). The CONTAM Panel noted that although the above-described rat study also showed effects at low doses given by *s.c.* injection, the bioavailability in rats of zearalenone administered by that route is considerably higher than bioavailability from oral dosing (Mallis et al., 2003).

7.2.6.2.3.2. Pigs

In vivo studies

A study investigated the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on reproductive performance, serum chemistry, feed intake and weight gain of gilts, and tested the ability of a polymeric glucomannan mycotoxin adsorbent (GMA) to reduce or eliminate the effects of the contaminated feeds (Díaz-Llano and Smith, 2006). Only deoxynivalenol (5.5 mg/kg of feed), 15-acetyl deoxynivalenol (0.5 mg/kg of feed) and zearalenone (0.3 mg/kg of feed) were detected in the feed. Gilts were fed one of 3 diets (n = 12 gilts/diet) from GD 91 ± 3 until farrowing. Diets were (1) control, (2) contaminated grains, and (3) contaminated grains plus 0.2 % GMA. Control diet contained deoxynivalenol at 0.2 mg/kg of feed; zearalenone was not detected (LOD 0.2 mg/kg of feed). Diets contaminated with mycotoxins did not affect average daily feed intake, but average daily weight gain and gain:feed ratio were significantly reduced in groups 2 and 3. Serum chemistry was unaffected. The frequency of stillborn piglets was significantly greater in gilts fed contaminated grains (15.5 %), compared with that of gilts fed contaminated grains plus GMA (4.6 %), or controls (6.3 %). Since only average starting weight on the day of artificial insemination, average daily feed intake and average daily weight gain were reported, only a rough estimate of the exposure to zearalenone during the feeding of contaminated grains can be made. Estimated exposure in group 2 would have ranged from approximately 2.7 down to 2.5 µg/kg b.w. per day on GDs 91-114. The exposure to deoxynivalenol was higher and, as with other studies on co-contaminated feed, it is not possible to reach a conclusion about the contribution of the individual contaminants to the stillbirths.

Pregnant sows were fed either a control diet (n = 8, 0.21 mg deoxynivalenol and 0.004 mg zearalenone/kg diet) or a diet containing 40 % of a *Fusarium* toxin contaminated wheat (n = 7, 9.57 mg deoxynivalenol and 0.358 mg zearalenone/kg diet) from day 75 to 110 of gestation (Dänicke et al., 2007a). There was observed a clear deoxynivalenol-related decrease in feed intake resulting in practically no weight gain during the exposure period (+ 43 g/day), while control sows gained 636 g/day at the same time. However, no abortion occurred during the experimental period. Piglets were delivered by Caesarean section at the end and 12.5 and 12.7 live piglets were delivered in the control and the exposed group, respectively. Piglets of the exposed group appeared macroscopically normal. Necrotic alterations of tails and ears were not observed in any of the piglets. The vulvae and mammary ridge were neither reddened nor swollen. The piglet weight of control piglets amounted to 1.34 kg and was reduced by 16 % in exposed piglets. The weights of the spleens of the *in utero* exposed piglets were significantly reduced by 25 %, whereas liver, kidneys and heart weights remained unaffected. As the insulin-like growth factor (IGF) system regulates fetal and placental growth and can potentially be influenced by dietary factors some major IGF genes in the livers of sows and fetuses were examined.

Feeding of the contaminated diet exerted no effects on these genes. This suggests that deoxynivalenol and zearalenone-contaminated diets can induce growth depression in sows and piglets during pregnancy without affecting hepatic transcription of major IGF genes (Tiemann et al., 2008a).

Haemoglobin concentration and haematocrit were significantly decreased in exposed piglets while no changes were observed in their mothers (Dänicke et al., 2007a). However, in those sows enhanced iron particle deposition was detected in the spleen and the liver cells. As this was not accompanied by anaemia this effect can be viewed as a mild haemosiderosis without clinical relevance. Moreover, beside the hepatic haemosiderosis other hepatocellular effects such as ultrastructural organelle changes were observed in exposed sows (Tiemann et al., 2008c). Based on the appearance of more fatty vacuoles, a subsequent impairment of hepatocyte function was hypothesized. Moreover, a significant decrease in cellular immune response to the mitogen concanavalin A in splenocytes was observed in these sows; an effect which was ascribed rather to deoxynivalenol than to zearalenone. Similarly, haemosiderosis was observed in sows fed diets containing 0.048 mg zearalenone and 4.42 mg deoxynivalenol per kg in the period of day 35 and 70 of gestation (Tiemann et al., 2008b). In this experiment indirect effects of deoxynivalenol on feed (and hence energy and nutrient) intake were avoided by applying a restricted feeding regimen to ensure that control sows and exposed sows consumed similar feed amounts.

7.2.6.2.3.3. Conclusions on females

Taking into account both the earlier literature and more recent literature (see above and also reviews by Kuiper-Goodman et al., 1987; FAO/WHO, 2000; Zinedine et al., 2007; Kanora and Maes, 2009; Golinski et al., 2010), the effects of zearalenone in female laboratory species such as mice, rats, guinea pigs and rabbits, illustrate its oestrogenic activity. They include decreases in fertility, reproductive tract alterations, increases in embryonic resorptions, reductions in litter size, changes in weight of adrenal, thyroid and pituitary glands, and changes in serum levels of progesterone and 17 β -oestradiol. However, zearalenone is not teratogenic in rodents. The overall NOEL for these effects is 0.1 mg/kg b.w. per day orally (Kuiper-Goodman et al., 1987; Fink-Gremmels and Malekinejad, 2007).

Pigs appear to be the most susceptible species to zearalenone and readily exhibit signs of hyperoestrogenism, such as swelling and thickening of the vagina and vulva within a few days of onset of exposure. There is uterine hypertrophy and disturbance of oestrous cycles, ovulation, conception and implantation. Zearalenone exposure during pregnancy causes embryonic death, reduced fetal weight, reduction in litter size and impaired neonatal viability. Some of these effects have been reported in pigs given pure zearalenone, while others have been reported in pigs fed cereals that have been naturally co-contaminated with zearalenone and other mycotoxins such as deoxynivalenol. *In vitro* studies indicate that zearalenone also affects granulosa cell steroidogenesis, oocytes and fertilised ova.

At low oral exposures, gilts are more susceptible than sows and some evidence suggests that prepubertal gilts may be more susceptible than sexually mature gilts. In mature gilts, prolongation of the luteal phase of the oestrous cycle, accompanied by high serum progesterone levels, appears to be the most sensitive adverse effect, with a lowest effect level of 200 μ g/kg b.w. per day and a NOEL of 40 μ g/kg b.w. per day (Edwards et al., 1987). In immature gilts, ovarian follicle atresia, apoptosis-like changes in granulosa cells and intensified cell proliferation in the uterus and oviduct have been reported at 200 μ g/kg b.w. per day (Obremski et al., 2003b; Zwierzchowski et al., 2005). NOELs were not established in those studies but a NOEL of 40 μ g/kg b.w. per day for these effects was reported by the same group in later studies (Wasowicz et al., 2005; Jakimiuk et al., 2010b). However, histological observation of uterine cell proliferation and hyperaemia in immature gilts has also been reported by the same group at 20 μ g/kg b.w. per day (Gajecka et al., 2011). Swelling and lengthening of the vulva has been reported in immature gilts at oral exposures of zearalenone of 17.6 μ g/kg b.w. per day (Döll et al., 2003b), 80-170 μ g/kg b.w. per day (Andretta et al., 2008; 2010) and at doses of 60-150 μ g/kg b.w. per day (Jiang et al., 2010b).

Thus, some of the more recent oral studies in immature gilts, based on observations of various effects in the uterus and the ovary have reported effect- and no-effect levels similar to those noted in the study by Edwards et al. (1987). There are also indications from other studies on immature gilts of oestrogenic effects on the uterus and vulva at zearalenone exposures ranging from 17.6 to 170 µg/kg b.w. per day. The study of Döll et al. (2003b) indicated a NOEL of 10.4 µg/kg b.w. per day for these effects. In this study, which used feed co-contaminated with zearalenone and deoxynivalenol, there was no effect on body weight at the LOEL or NOEL, indicating that there was unlikely to be any influence on the outcome measures from the co-exposure to deoxynivalenol.

7.3. Mode of action

7.3.1. Oestrogenic activity

In vivo and *in vitro* studies have shown that zearalenone, and its metabolites bind to both ER- α and ER- β , with a higher affinity for ER- α , inducing oestrogenic-like effects by activating gene transcription via oestrogen-responsive elements. The stereoisomers differ considerably in their oestrogenic activity, since the activity of α -zearalenol and α -zearalanol is greater than zearalenone and β -zearalenol. Beside stereoisomer differences, there are also species differences in binding affinity. Relative binding affinity of α -zearalenol to ERs in different species has been shown to be pig > rat > chicken (as reviewed by Metzler et al., 2010).

The proliferation assay with MCF-7 human mammary carcinoma cells has shown that zearalenone induces cell cycle progression and that the metabolite α -zearalenol is the most potent in inducing cell proliferation. In contrast, β -zearalenol blocks cell cycle in granulosa cells. α -Zearalenol is more potent than zearalenone also in the uterotrophic assay. Oocyte maturation is also affected by zearalenone at a concentration (0.3 µM) that is equimolar to the physiological concentrations of oestradiol in follicle fluid. Zearalenone also acts as an oestrogen agonist in the brain (reviewed in Fink-Gremmels and Malekinejad, 2007).

7.3.2. Effects on transcription factors

Recent data indicate that zearalenone can activate the pregnane X receptor (PXR), a human xenobiotic receptor member of ligand activated nuclear transcription factors (Ding et al., 2006). The expression of genes involved in the metabolism of endobiotics and xenobiotics, such as the cytochrome P450 enzymes CYP3A4, is regulated by PXR. In addition, PXR has a role in the transcriptional regulation of glutathione-S-transferases, sulfotransferases and UGTs, organic anion transporters and ATP-binding cassette (ABC) efflux transporters (Kast et al., 2002; Maher et al., 2005). In primary cultures of human hepatocytes, zearalenone at concentrations as low as 0.1 µM, activates, in addition to PXR, constitutive androstane receptor (CAR) and aryl hydrocarbon receptor (AhR) mRNA levels. In addition some of the phase I target genes are also activated, mainly CYP3A4, CYP2B6 and CYP1A1 and to a lesser extent CYP3A5 and CYP2C9 (Ayed-Boussema et al., 2010).

7.3.3. Oxidative stress and cell death

Exposure of Vero cells (from green monkey kidney) and Caco-2 cells (human colon cancer epithelial cell line) to 1-60 µM zearalenone for 72 hours induces cell cycle perturbation, inhibition of protein and DNA syntheses and increased malondialdehyde (MDA) formation in a concentration-dependent manner (Abid-Essefi et al., 2004). Caco-2 cells exposed to 0-100 µM of zearalenone, or to its two major metabolites α -zearalenol or β -zearalenol for 24-48 hours undergo apoptotic cell death as revealed by DNA fragmentation, caspase-3 activation and poly (adenosine diphosphate (ADP)-ribose) polymerase (PARP) cleavage. Oxidative stress is also induced as shown by MDA generation (Abid-Essefi et al., 2009), but also in the Chinese Hamster Ovary cells CHO-K1 (Ferrer et al., 2009). Exposure of the

CaCo-2 cells to zearalenone for 24 hours has also been shown to result in lysosomal destabilization (Kouadio et al., 2005). Human liver carcinoma cell line HepG2 exposed to zearalenone (1-100 μM) show an inhibition of cell proliferation and total protein synthesis as well as an induction of Hsp 70 expression observed at early time points (Hassen et al., 2005; Gazzah et al., 2010). At later time points, cells undergo apoptosis with increased Bax, decreased Bcl-2, altered mitochondrial membrane potential ($\Delta\psi\text{m}$), release of cytochrome *c* and activation of caspase-9 and -3 (Gazzah et al., 2010). Pre-exposure of HepG2 cells to sub-lethal heat shock, which is known to increase tolerance to cellular insults, reduces zearalenone (25-100 μM) cytotoxic effects (Hassen et al., 2005). Also the antioxidant 4-(methylthio)-3-butenyl isothiocyanate (MTBITC) (32 μM) could counteract the toxic effects of zearalenone (40 μM) in Balb/c mice keratinocyte cell line C5-O cells by reducing lipid peroxidation and subsequently apoptotic cells death (Ben Salah-Abbès et al., 2010). Altogether, these data indicate that oxidative stress plays a major role in zearalenone cytotoxicity. However, zearalenone exerts anti-apoptotic effects in MCF-7 cells at 32-100 nM (Ahamed et al., 2001; Yu et al., 2005).

7.4. Combined effects with other mycotoxins

In addition to zearalenone *Fusarium* species produce trichothecenes, such as deoxynivalenol, and fumonisins. Consequently there is a need to consider whether the combined effects of exposure to the different mycotoxins, could be additive or interactive.

The effects of combinations of *Fusarium* toxins have been studied *in vitro*. Tajima et al. (2002) studied the effects of zearalenone, deoxynivalenol, nivalenol, T-2 toxin and fumonisin B1 on DNA synthesis in mouse fibroblast L929 cells. Individually, zearalenone, deoxynivalenol, nivalenol and T-2 toxin caused a concentration-related inhibition of DNA synthesis whereas fumonisin B1 increased it at the highest concentrations tested. The effects of combinations of mycotoxins were mostly additive or less than additive. Kouadio et al. (2007) studied the effects of zearalenone, deoxynivalenol and fumonisin B1 on human intestinal Caco-2 cells, with a number of endpoints of viability, lipid peroxidation and effects on DNA. The authors reported the combination of zearalenone and deoxynivalenol resulted in greater than additive lipid peroxidation, whereas fumonisin B1 antagonised the effects of zearalenone on cell viability. Fumonisin B1 also antagonised the effects of β -oestradiol on cell viability, indicating an anti-oestrogenic effect. These studies were conducted at cytotoxic concentrations of the mycotoxins, and without full investigations of the concentration-response relationships.

In an early study, the potential effects of zearalenone on the toxicity of deoxynivalenol were investigated in female B6C3F1 mice (Forsell et al., 1986). Administration of deoxynivalenol in the diet for 8 weeks resulted in a dose-related decrease in body weight gain and changes in white blood cell counts and serum immunoglobulins, with a NOAEL at a dietary concentration of 0.5 mg/kg. Co-administration of zearalenone at 10 mg/kg diet with deoxynivalenol at 5 mg/kg diet did not modify the effects observed with deoxynivalenol alone.

In pigs, many of the cited studies have used naturally contaminated feedstuffs as a source for zearalenone. In many cases such contaminated feedstuffs are often co-contaminated with deoxynivalenol. The interactive effects of zearalenone and deoxynivalenol on porcine reproductive tracts were recently addressed in a review (Tiemann and Dänicke, 2007). From a mode of action viewpoint, deoxynivalenol affects protein synthesis and influences humoral and cellular immune responses in pigs depending on dose, exposure and timing of the functional immune assay. The modifying effects of deoxynivalenol might provide a mechanistic rationale for its effects on fertility and reproduction. High deoxynivalenol concentrations impair oocyte and embryonic development both *in vivo* and *in vitro*. *In vitro* exposure of uterine cells to deoxynivalenol inhibits their proliferation rate and modulates the process of translation at a different molecular level when compared with the *in vivo* application. As the effects of oestrogen or zearalenone effects might be mediated via protein synthesis too, it seems reasonable to assume interactive effects of zearalenone or its metabolites and deoxynivalenol at the molecular level. For example, β -zearalenol and deoxynivalenol exert pronounced effects on mitogen-activated protein kinases (MAPKs), which are involved in different signalling

pathways important for transcription and thus translation, resulting in the inhibition of cell proliferation after β -zearalenol and deoxynivalenol exposure (Wollenhaupt et al., 2004, 2006).

Beside the effects at the molecular level, it needs to be considered further that deoxynivalenol is known to reduce feed intake, which determines not only the amount of toxins entering the organism but also the metabolically available nutrients and energy which might also markedly modulate processes involved in fertility.

The key effects of zearalenone result from its oestrogenicity. The studies using naturally contaminated feed do not allow direct comparison of combined effects with the effects of the individual mycotoxin. However, they indicate that at doses not resulting in reduced feed intake, body weight gain and protein synthesis, the observed effects are likely to be due to zearalenone, with no impact of deoxynivalenol, whereas at higher doses interaction between zearalenone and deoxynivalenol can occur.

In view of the available studies on combined effects of zearalenone and other mycotoxins, and also the fact that zearalenone exerts its effects via oestrogenicity whereas other mycotoxins have different modes of action, the CONTAM Panel concluded that combined effects are not expected to occur in animals at doses below those at which the individual toxins have effects. Similarly, combined effects of zearalenone and other mycotoxins are not expected to arise in humans at dietary exposures below the respective health-based guidance values of the individual toxins.

However, it is possible that zearalenone could have additive or interactive effects with other oestrogens in the diet, such as phytoestrogens in soya, or with xenoestrogens present in food or the environment. The impact of combined exposure to dietary oestrogens are not predictable since it would depend on the relative potency in binding to ERs and any competitive antagonism between oestrogenic substances present in the body. Thus there is potential for additivity, or for antagonism by weakly oestrogenic substances (Riley, 1998).

7.5. Observations in humans

There is little substantive information on the effects of zearalenone in humans. However, observations of high concentrations of zearalenone in foodstuffs and the occurrence of oestrogen-related pathologies in humans, such as precocious puberty and breast cancer has resulted in speculation that zearalenone may contribute to such effects.

Between 1978 and 1981, a 3-fold increase in premature thelarche (premature breast development in girls under 8 years of age) compared with previous years was reported in Puerto Rico (Pérez-Comas, 1982). There was no increase in other forms of early sexual development in boys or girls. From 552 cases recorded in a 1982 survey of endocrinologists in Puerto Rico, 120 cases were compared with 120 controls matched for age (Freni-Titulaer et al., 1986). Family medical histories and potential exposures to oestrogenic substances via food, occupation of household members, use of creams, drugs, ointments and pesticides were evaluated by home interview. Seventy percent of subjects were under 2 years of age at diagnosis. In subjects with onset of premature thelarche before 2 years of age, there were significant positive associations with maternal history of ovarian cysts and consumption of soy-based infant formula, various meat products and fresh chicken. There was a statistically significant negative association with consumption of corn products, suggesting that zearalenone was unlikely to be a causal factor. A subsequent analysis for oestrogenic activity in a limited number of food samples and for zearalenone, its metabolites and α -zearalanol in serum samples collected from cases and controls did not provide clear evidence for a role in the epidemic (Hannon et al., 1987).

In 1997, an increased incidence since 1989 of early thelarche was reported in patients in south-eastern Hungary. Oestrogenic mycotoxins were detected in 5 of 36 early thelarche patients with serum zearalenone levels of 18.9-103 $\mu\text{g/mL}$. Food samples collected from the patients were also analysed but no quantitative results were given (Szuets et al., 1997).

A high incidence of precocious puberty has been reported in Viareggio, a small region of North-West Tuscany, Italy (Massart and Saggese, 2010). The incidence of precocious puberty was 22-29 times higher than that in neighbouring areas or currently published. Of 63 subjects studied, high serum zearalenone levels of 933.7 ± 200.3 pg/mL and α -zearalenol levels of 106.5 ± 1.9 pg/mL were detected in 6 precocious puberty patients at diagnosis. The authors commented that although this finding might be incidental, it may be related to the precocious puberty occurrence in zearalenone-exposed girls, but that zearalenone could not explain the epidemic, suggesting that other environmental factors may be involved.

Fusarium-contaminated grain has been linked to 'endemic breast enlargement disease' in China (Yonghang et al., 1995 cited by Fugh-Berman, 2003). It was later determined that all samples of buckwheat grown in the area were infected with mold; 34 % were infected with *Fusarium* and zearalenone was extracted from the affected buckwheat (Zhang et al., 1995 cited by Fugh-Berman, 2003).

The hypothesis that zearalenone in foodstuffs may contribute to the increasing occurrence of breast cancer has been discussed but not widely investigated. In *in vitro* studies, zearalenone stimulates the proliferation of human breast cancer MCF-7 cells containing human ER by activation of cell cycle progression, and this forms the basis of a widely used *in vitro* assay for the detection of oestrogenic activity of chemicals (reviewed by Fink-Gremmels and Malekinejad, 2007).

The concentrations of zearalenone and its metabolites, α -zearalenol and β -zearalenol, present in the plasma of patients with breast (n = 28) and cervical carcinoma (n = 54) were compared with levels in patients presenting with other diagnoses (n = 26) and healthy volunteers (n = 24). There were no significant differences between the groups (Pillay et al., 2002).

Zearalenone was measured in normal and abnormal endometrial tissue from 49 women. Zearalenone concentrations were 47.8 ± 6.48 , 167 ± 17.69 ng/mL, and < LOD in 27 endometrial adenocarcinoma, 11 endometrial hyperplasia, and 11 normal proliferative endometrium samples, respectively. In 8 cases of hyperplastic and 5 cases of neoplastic endometrial tissue zearalenone was not detected (Tomaszewski et al., 1998).

In summary, human observations have not definitively linked exposure to zearaleneone in the diet with adverse outcomes due to oestrogenicity.

7.6. Dose response modelling

Since, zearalenone has shown limited evidence of carcinogenicity, and is clastogenic, the CONTAM Panel modelled the dose response relationship in order to identify a point of departure. Liver and pituitary adenomas were observed in a mouse carcinogenicity assay. As an example, the incidence of pituitary adenomas in male B6C3F1 mice (NTP, 1982) was selected for dose-response modelling (see Section 7.2.3.1.). There was an increase in incidence of the pituitary adenomas at the low dose in male mice and tumours in pituitary glands of mice are more likely to be treatment related than liver tumours. Zearalenone was administered in the diet resulting in doses of approximately 0, 8 and 17 mg/kg b.w. per day. The incidence of pituitary adenomas was 0/40, 4/45 and 6/44 (in Section 7.2.3.1. expressed as 0, 9 and 14 %, respectively).

The benchmark response (BMR) was chosen as 10 % extra risk, the default response level for quantal response data as recommended by EFSA (2009) in its Scientific Opinion on the use of the benchmark dose (BMD) approach. The outcomes of that dose-response analysis are shown in Appendix D. All quantal dose response models in the US EPA's Benchmark dose software²⁰ (BMDS) 2.1.2 were used. Acceptability of a model was assessed using the log-likelihood value associated with the fitted model

²⁰ <http://www.epa.gov/ncea/bmds/about.html>

(when tested versus the full model). In accordance with the Scientific Opinion of the EFSA (EFSA, 2009) a goodness-of-fit was judged as sufficient if the fit showed a p-value not smaller than 0.1 (i.e. $p \geq 0.1$), using the likelihood ratio test.

The models providing acceptable fits resulted in BMD_{10} values ranging from 10.95-14.67 mg/kg b.w. per day, and 95 % lower confidence limit of the benchmark dose ($BMDL_{10}$) values ranging from 6.39 to 11.32 mg/kg b.w. per day. In line with the guidance of EFSA (2009), the CONTAM Panel selected the lowest $BMDL_{10}$ of 6.39 mg/kg b.w. per day, obtained using the restricted Log-Logistic model, as the appropriate point of departure to be used in calculating the margins between the $BMDL_{10}$ and the TDI.

7.7. Derivation of TDI

Zearalenone does not cause gene mutations in bacterial test systems but is clastogenic and aneugenic *in vitro* and has been confirmed as an *in vivo* clastogen in the mouse. The CONTAM Panel noted that a plausible mechanism for the clastogenic effects has been proposed, namely formation of catechols that can be oxidised to quinones that undergo redox-cycling.

In well-conducted carcinogenicity bioassays, no increase in tumors was observed in two rat studies, while in mice, significant increases in pituitary and liver adenomas, but not carcinomas, were observed in one study. This was considered by IARC as limited evidence of carcinogenicity in animals, and they allocated it, along with other related mycotoxins, to group 3 (not classifiable as to carcinogenicity in humans).

The CONTAM Panel took into account that there is wide variability between species in sensitivity to the oestrogenic effects of zearalenone and that such effects are observed in pigs at doses around 3 orders of magnitude lower than doses reported to cause clastogenicity and increases in adenomas in mice. The CONTAM Panel therefore decided to establish a TDI for zearalenone based on its oestrogenic effects.

Review of the recent literature on laboratory and domestic animals confirms that the female pig is the most sensitive species and sex to the oestrogenic effects of zearalenone. It is possible that the greater formation of α -zearalenol in the pig, relative to other animal species studied, is in part responsible for this sensitivity. The available *in vivo* information from pigs includes both studies using pure zearalenone mixed into the feed and studies using naturally contaminated feed in which zearalenone and other mycotoxins were present.

The difficulties of interpreting studies using feed that is co-contaminated with zearalenone and other mycotoxins have been considered and such studies were treated as follows for the risk assessment:

- studies showing no effects were taken into account;
- studies showing effects for which it was not possible to attribute causality to zearalenone alone were not taken into account;
- studies showing effects that were clearly attributable to an oestrogenic mode of action of zearalenone and its metabolites were taken into account.

With this in mind, the CONTAM Panel made a weight-of-evidence assessment, considering studies on both immature and mature female pigs. The study by Edwards et al. (1987) that was previously used to establish the t-TDI, showing lengthening of the oestrous cycle in mature gilts, with an effect level of 200 $\mu\text{g}/\text{kg}$ b.w. per day and a NOEL of 40 $\mu\text{g}/\text{kg}$ b.w. per day, remains important for the risk assessment. Some of the more recent studies in immature gilts, based on observations of various effects in the uterus and the ovary (Obremski et al., 2003a; Wasowicz et al., 2005; Zwierzchowski et al., 2005; Jakimiuk et al., 2010b), have reported effect- and no-effect levels similar to those noted above in the study by Edwards et al. (1987). There are also indications from other studies on immature gilts of oestrogenic effects on the uterus and vulva at zearalenone exposures ranging from 17.6 to 170 $\mu\text{g}/\text{kg}$ b.w. per day (Döll et al., 2003b; Andretta et al., 2008, 2010; Oliver et al., 2010; Gajecka et al., 2011).

The study of Döll et al. (2003b) indicated a NOEL of 10.4 µg/kg b.w. per day for these effects. In this study, which used feed co-contaminated with zearalenone and deoxynivalenol, there was no effect on body weight at the LOEL or NOEL, indicating that there was unlikely to be any influence on the outcome measures from the co-exposure to deoxynivalenol. The CONTAM Panel considered that these are NOELs rather than NOAELs because it is not possible to conclude that the effects described above in pigs at the LOEL are adverse in terms of later fertility and reproductive performance. However, although the estrogenicity *per se* may not be adverse it is undesirable and indicative of adverse effects and therefore was considered as the appropriate basis for establishing a TDI.

The study of Döll et al. (2003b), supported by the data from other studies in female pigs, was considered the critical study for establishment of a TDI. In the selection of an appropriate uncertainty factor to apply to the NOEL of 10 µg/kg b.w. per day, the comparative sensitivity of the female pig and human to oestrogens and to zearalenone and its metabolites was considered.

The CONTAM Panel noted that in both pigs and humans the main estrogenic metabolite of zearalenone is α -zearalenol, which is more potent than zearalenone in estrogenic activity assays. Comparison of the range of endogenous 17 β -oestradiol concentration in plasma during the pig oestrous cycle (0-70 pg/mL) and the human menstrual cycle (30-350 pg/mL) indicates that the two ranges are within an order of magnitude (Baird and Guevara, 1969; Knobil and Neill, 1994; Knox et al., 2003). The background levels of 17 β -oestradiol during the early follicular phase are similar between the two species and the amount of 17 β -oestradiol required to trigger the preovulatory surge of gonadotrophins is less than an order of magnitude higher in women compared with pigs.

From the analysis above, it is likely that the human female would not be more sensitive to oestrogens in general, or zearalenone and its metabolites in particular, than the female pig. It is therefore not necessary to include an uncertainty factor of 2.5 for toxicodynamic differences between pigs and humans. Using the NOEL of 10 µg/kg b.w. per day and an uncertainty factor of 40 (4 for interspecies differences in toxicokinetics and 10 for interhuman variability), a TDI of 0.25 µg/kg b.w. can be derived. As a number of relevant studies, including in the pig, have become available since the previous t-TDI was set by the SCF in 2000, the CONTAM Panel concluded that a full TDI of 0.25 µg/kg b.w. can now be established. The results of these new studies also account for the difference between the TDI established by the CONTAM Panel and the PMTDI established by the JECFA.

8. Risk characterisation

The margin between the BMDL₁₀ of 6.39 mg/kg b.w. per day for 10 % extra risk of pituitary adenomas in male mice, and the newly established TDI of 0.25 µg/kg b.w. was in the region of 25,000, which in line with the guidance of EFSA (2009), would be of low concern for a genotoxic carcinogen. Taking also into account that the genotoxicity of zearalenone may be related to oxidative stress mediated mechanisms, and that it is at most a weak carcinogen, the CONTAM Panel concluded that the critical effects of zearalenone relate to its oestrogenicity.

It is possible that elevated circulating levels of oestrogens could lead to adverse effects either from long-term exposure or from short-term exposure during a sensitive stage of development. The CONTAM Panel therefore focussed its risk characterisation on estimates of chronic dietary exposure to zearalenone and, a worst case scenario in which dietary exposure could be elevated for a relatively short period of time.

Estimates of mean and high level chronic total dietary exposure to zearalenone were based on the available data on mean occurrence of zearalenone in foods, and mean and high level consumption of those foods in different European countries. For adults, the estimated exposures are within the ranges of 2.4 to 29 ng/kg b.w. per day and 4.7 to 54 ng/kg b.w. per day for mean and 95th percentile consumers, respectively (range represents the minimum LB to maximum UB from the different countries). The highest estimated chronic dietary exposures are for the toddler age group (≥ 12 months to < 36 months),

being in the ranges of 9.3 to 100 ng/kg b.w. per day and 23 to 277 ng/kg b.w. per day for mean and 95th percentile consumers, respectively. Due to the high percentage of samples with levels of zearalenone below the LOD, it is unlikely that the actual exposures are close to the UB, and therefore dietary exposure would be below the TDI of 0.25 µg/kg b.w. and not a health concern.

In line with the terms of reference provided by the EC, the CONTAM Panel assessed the impact of increasing the ML for zearalenone from 50 µg/kg up to 150 µg/kg in breakfast cereals. Such an increase has the potential to increase chronic total dietary exposure to zearalenone by up to 35 % in adults, and by up to 16 % in toddlers. The maximum 95th percentile estimate of chronic dietary exposure in toddlers would be 55-321 ng/kg b.w. per day (LB to UB). Again, due to the high percentage of samples with levels of zearalenone below the LOD, it is unlikely that the actual exposures would be close to the UB. In addition, the CONTAM Panel assumed that the distribution of occurrence data would be unchanged if the ML increases, i.e. if the ML were to be doubled, then the mean concentration would also double, which is considered to be a conservative assumption. Thus it is likely that dietary exposure would be below the TDI of 0.25 µg/kg b.w. Therefore the CONTAM Panel concluded that the chronic dietary exposure that would result from increasing the ML from 50 µg/kg up to 150 µg/kg is not a health concern.

In a worst case scenario it is possible that an individual could consume the same batch of breakfast cereal containing zearalenone at the ML every day for 2 to 4 weeks. The highest estimated exposure calculated for consumers of breakfast cereals were for children aged ≥ 3 to < 10 years old. At the current ML of 50 µg/kg zearalenone in breakfast cereal, this scenario would result in short-term dietary exposure in this age group within the ranges of 27 to 136 ng/kg b.w. per day and 70 to 399 ng/kg b.w. per day for mean and 95th percentile consumers, respectively (minimum LB to maximum UB from the different countries). This highest estimated exposure is 60 % above the TDI. Increasing the ML from 50 µg/kg up to 150 µg/kg has the potential to increase the short-term exposure of children aged ≥ 3 to < 10 years old to up to approximately 1000 ng/kg b.w. per day.

High fibre breakfast cereals, which are the most likely to be contaminated with zearalenone due to the high content of wheat bran, are more likely to be consumed by adults than by children. For the adults the highest estimates of short-term exposure are below the TDI for ML scenarios up to a concentration of 100 µg/kg zearalenone in breakfast cereals.

There are limited data on dietary habits of vegetarians with data available for only 5 European countries, with very few subjects in 4 of them. Estimates of dietary exposure for these vegetarian populations indicate that dietary exposure to zearalenone could be up to 2-fold higher than for the general population.

9. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to zearalenone has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006b). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006b) the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters).

9.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference. The CONTAM Panel reviewed the t-TDI established by the SCF in 2000 and revised it in the light of results of toxicological studies published since that time. The CONTAM Panel also assessed the new occurrence data that were

collected by EFSA, carried out an exposure assessment and assessed the impact of potential changes in the ML for zearalenone in breakfast cereals taking into account exposure from other food sources. The uncertainty in the assessment objectives is considered to be negligible.

9.2. Exposure scenario and exposure model

The occurrence data provided were mainly on grains and grain-based foods, there were insufficient data for soy-based products, which could lead to underestimation of exposure. The use of the UB approach for high percentage of occurrence data < LOQs is conservative.

There is uncertainty in the impact on dietary exposure to zearalenone of potential changes to the MLs for breakfast cereal. The CONTAM Panel assumed that the distribution of occurrence data would be unchanged if the ML increases, i.e. if the ML were to be doubled, then the mean concentration would also double. This is considered to be a conservative approach. The worst case scenario of assuming an individual consumes breakfast cereal containing zearalenone at the ML repeatedly for 2-4 weeks is also conservative.

The estimates of the dietary exposure for children assume they may eat any type of breakfast cereal. However, high fibre breakfast cereals, which are the most likely to be contaminated with zearalenone due to the high content of wheat bran, are more likely to be consumed by adults than by children.

9.3. Model input (parameters)

There is uncertainty regarding the mode of action and human relevance of the increased incidence of adenomas in a carcinogenicity study of zearalenone in mice. The calculation of the margin between the BMDL₁₀ and the newly established TDI suggests that this would be a low concern, even if a genotoxic mechanism was involved. The CONTAM Panel established a TDI based on the NOEL for oestrogenic effects of zearalenone in pigs, which are not clearly adverse in terms of later fertility and reproductive performance. An uncertainty factor for toxicodynamic differences between pigs and humans was not considered necessary because of the evidence that the human female would not be more sensitive to zearalenone and its metabolites than the female pig. In addition, pigs preferentially produce the more active metabolite α -zearalenol over the β analogue by 5-fold, indicating that an uncertainty factor lower than the default value of 4 for toxicokinetic differences. Overall the approach to establishing the TDI is considered to be conservative.

It is possible that zearalenone could have combined effects with other oestrogenic substances in the diet, such as phytoestrogens.

9.4. Summary of uncertainties

In Table 18, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

Table 18: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure of zearalenone.

Sources of uncertainty	Direction
Occurrence data not representative of all foods in which zearalenone could be present	-(^a)
Impact of increasing the ML on the distribution of zearalenone occurrence data	+
Use of LB bound occurrence data in the dietary exposure estimations	-
Use of UB bound occurrence data in the dietary exposure estimations	+
Consumption data for vegetarians from few European countries	+/-
Use of oestrogenic activity without clear adverse consequences as the basis for deriving a TDI	+
Assumption that humans could be as sensitive as pigs to the oestrogenic effects of zearalenone	+
Combined effects with other oestrogenic substances in food	+/-

ML: maximum level; LB: lower bound; UB upper bound; TDI: tolerable daily intake.

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk.

The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of exposure to zearalenone, and of possible increases in the ML for breakfast cereals, and concluded that the risk assessment is likely to be conservative, i.e. more likely to over- than to under-estimate the risk.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Zearalenone is a phenolic resorcylic acid lactone mycotoxin produced by several *Fusarium* species, particularly *F. graminearum* (formerly called *F. roseum*).
- It is commonly found in maize but can be found also in other crops such as wheat, barley, sorghum and rye throughout various countries of the world. Generally, the *Fusarium* species grow and invade crops in moist cool field conditions. *F. graminearum* also produces trichothecenes, such as deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, 4-acetylnivalenol and fusarenon-X. Toxin production may also occur under poor storage conditions.

Methods of analysis

- Analysis of zearalenone in food and feed as well as biological samples is mostly done with high performance liquid chromatography coupled to fluorescence detection or triple quadrupole mass spectrometers. Quantification can be achieved via matrix calibration or by using stable isotope labelled standards.
- The application of clean-up steps is required for samples for special dietary purposes (baby food, infants and young children) as the European Union (EU) maximum levels (MLs) are much lower for these products, and also for biological samples. Immunoaffinity columns are mainly used for clean-up.

- A standardised method is available for the determination of zearalenone in maize-based baby food, barley flour, maize flour, polenta, wheat flour and cereal-based foods for infants and young children using high performance liquid chromatography with immunoaffinity clean-up and fluorescence detection.

Occurrence and effects of processing

- A total of 13,075 analytical results obtained on food samples and 9,877 results on unprocessed grains sampled by 19 European countries in 2005-2010 were used in the evaluation. Zearalenone was reported at quantifiable levels in 15 % of the samples.
- The highest concentrations of zearalenone were reported for wheat bran, corn and products thereof (e.g. corn flour, cornflakes). Notably high levels have been found in corn germ oil and wheat germ oil.
- There were indications that soy can be contaminated with zearalenone but there were insufficient data to draw conclusions.
- Limited data indicate that organic samples are not more contaminated than those conventionally cultivated.
- Levels of zearalenone in the group 'Unprocessed grains' were considerably higher than in the group 'Grains for human consumption'. This suggests that cleaning and selection steps applied to grains after harvesting result in lower zearalenone concentrations in grains intended for human consumption.
- In general, zearalenone is redistributed between the milling fractions. The by-products from cleaning the raw cereal grains (dust, hulls and others) were characterised by 3- to 30-fold higher zearalenone concentrations than the cleaned cereal grains while bran contained up to 2-fold higher concentrations.
- Generally zearalenone is not affected by cooking. Only under alkaline conditions or during extrusion cooking (heating under a high degree of pressure) a reduction of above 40 % was observed.

Exposure

- The estimated chronic total dietary exposures to zearalenone of adults across 19 European countries, using lower bound and upper bound concentrations, range from 2.4 to 29 ng/kg body weight (b.w.) per day for average consumers, and 4.7 to 54 ng/kg b.w. for 95th percentile consumers.
- The highest chronic exposure was estimated in toddlers (age > 12 months to < 36 months) ranging from 9.3 to 100 ng/kg b.w. per day for average consumers, and 23 to 277 ng/kg b.w. for 95th percentile consumers.
- Grains and grain-based foods, in particular grains and grain milling products, bread and fine bakery wares, made the largest contribution to the zearalenone dietary exposure in all age classes. Vegetable oils, especially corn germ oil and wheat germ oil, make an important contribution to the zearalenone exposure.

- From the average values across the European countries, breakfast cereals provide a contribution of 0.4-17 % to total dietary exposure of zearalenone in adults. Increasing the ML from 50 µg/kg up to 150 µg/kg has the potential to increase chronic total dietary exposure to zearalenone by up to 35 %.
- From the average values across the European countries, breakfast cereals provide a contribution of 0.1-5.1 % to total dietary exposure of zearalenone in toddlers (age ≥ 12 months to < 36 months). Increasing the ML from 50 µg/kg up to 150 µg/kg has the potential to increase chronic total dietary exposure to zearalenone by up to 16 %.
- Short-term mean dietary exposure in consumers of breakfast cereals could increase to up to 357 ng/kg b.w. per day and the 95th percentile exposure up to 1029 ng/kg b.w. per day if the ML for zearalenone were to be increased from 50 µg/kg up to 150 µg/kg.
- Limited data indicate that dietary exposure of vegetarians to zearalenone could be up to 2-fold higher than for the general population.

Hazard identification and characterisation

- Zearalenone is rapidly and extensively absorbed from the gastrointestinal tract in mammals.
- Efficient glucuronidation of zearalenone in the small intestine and liver significantly reduces the amounts of unconjugated (i.e. receptor-active) parent compound that reaches the circulation.
- It is possible that fetuses and neonates could be more susceptible than adults to the oestrogenic effects of zearalenone, based on higher internal exposures due to metabolic and physiological immaturity.
- Enzymatic reduction of zearalenone produces α-zearalenol, a metabolite with greater affinity for oestrogen receptors than the parent compound, and β-zearalenol, a metabolite with lower affinity.
- Cytochrome P450-mediated oxidation of zearalenone produces catechol metabolites that are subject to redox cycling to reactive quinones.
- Toxicity studies of zearalenone and its main reductive metabolites demonstrate that the oestrogenic activity is the critical mode of action.
- Of the laboratory and domestic animals studied, pigs are the most sensitive species for oestrogenic effects, with females being more sensitive than males. It is possible that the greater formation of α-zearalenol in the pig, relative to other animal species studied, is in part responsible for this sensitivity.
- In well-conducted carcinogenicity bioassays, no increase in tumours was observed in two rat studies, while in mice, significant increases in pituitary and liver adenomas, but not carcinomas, were observed in one study, providing limited evidence of carcinogenicity.
- Zearalenone does not cause gene mutations in bacterial test systems but is clastogenic and aneugenic *in vitro* and has been confirmed as an *in vivo* clastogen in the mouse.
- A plausible mechanism for the clastogenic effects has been proposed, namely formation of catechols that can be oxidised to quinones that undergo redox-cycling.

- There is wide variability between species in sensitivity to the oestrogenic effects of zearalenone and such effects are observed in pigs at doses around three orders of magnitude lower than doses reported to cause clastogenicity and increases in adenomas in mice. The Panel on Contaminants in the Food Chain (CONTAM Panel) therefore decided to establish a tolerable daily intake (TDI) for zearalenone based on its oestrogenic effects.
- Adverse effects of zearalenone and its metabolites on testosterone synthesis, sexual behaviour, sex organ weights, testicular histology and spermatogenesis have been observed. Effects occur in male rodents only at high dose levels. Effects in male pigs occur at lower doses, but not as low as the doses causing effects in female pigs.
- In females, adverse effects of zearalenone on the reproductive tract, fertility and embryo survival have been found in laboratory animals such as mice, rats, guinea pigs and rabbits but zearalenone is not teratogenic. Such effects generally occur at doses in the range 1-10 mg/kg b.w. per day and above. The overall no-observed-adverse-effect-level (NOAEL) for these effects is 0.1 mg/kg b.w. per day.
- Among domestic animals, such as horses, cattle, sheep and pigs, the female pig is the most sensitive, with immature pigs possibly more sensitive than mature pigs. Effects of zearalenone include disturbance of the oestrous cycle, ovulation, conception and implantation, embryonic death, reduced fetal weight, reduced litter size and impaired neonatal survival.
- In female pigs, the tissues that are most sensitive to the oestrogenic effect of zearalenone and its metabolites are the ovary, uterus and vulva. Lowest-observed-effect-levels (LOELs) for these tissues in mature and immature gilts range from 17 to 200 µg/kg b.w. per day, with an overall no-observed-effect-level (NOEL) of 10 µg/kg b.w. per day.
- Toxicodynamic information indicates that it is likely that the human female would not be more sensitive to zearalenone and its metabolites than the female pig. For derivation of a TDI, it is therefore not necessary to include an uncertainty factor of 2.5 for toxicodynamic differences between pigs and humans.
- Using the NOEL of 10 µg/kg b.w. per day and an uncertainty factor of 40 (4 for interspecies differences in toxicokinetics and 10 for interhuman variability), a TDI of 0.25 µg/kg b.w. can be derived. As a number of relevant studies, including in the pig, have become available since the previous temporary TDI (t-TDI) was established by the SCF in 2000, the CONTAM Panel concluded that a full TDI of 0.25 µg/kg b.w. can now be established.

Risk characterisation

- Estimates of chronic dietary exposure to zearalenone based on the available occurrence data are below or in the region of the TDI for all age groups and not a health concern.
- A potential increase in the ML for zearalenone in breakfast cereals from 50 µg/kg to 75, 100, 125 or 150 µg/kg is unlikely to result in a chronic dietary exposure exceeding the TDI and is also not a health concern.
- In a worst case scenario it is possible that an individual could consume the same batch of breakfast cereal containing zearalenone at the ML every day for 2 to 4 weeks. The highest estimated short-term exposure is for children aged ≥ 3 to < 10 years old and, at the current ML, is 60 % above the TDI.
- Increasing the ML from 50 µg/kg up to 150 µg/kg has the potential to increase the short-term exposure of children aged ≥ 3 to < 10 years old to up to approximately 1 µg/kg b.w. per day.

- High fibre breakfast cereals, which are the most likely to be contaminated with zearalenone due to the high content of wheat bran, are more likely consumed by adults than by children. For adults the highest estimates of short-term exposure are below the TDI for ML scenarios up to a concentration of 100 µg/kg zearalenone in breakfast cereals.
- The mycotoxins which usually co-occur with zearalenone do not have oestrogenic effects. Combined effects of zearalenone and other mycotoxins are not expected to arise in humans at dietary exposures below the respective health-based guidance values of the individual toxins.
- The possible impact of combined exposure to zearalenone with other oestrogenic substances in food (such as phytoestrogens in soya) or the environment could be additive or antagonistic.

RECOMMENDATIONS

- More occurrence data on zearalenone in soy and soy-based foods should be collected.
- More food consumption data for vegetarians should be obtained.
- Any proposal to raise the MLs for zearalenone would need to take into account the likely consequence that exposure to other *Fusarium* toxins might also be increased.
- If the MLs for zearalenone in wheat bran and breakfast cereals are increased, monitoring should be conducted to establish whether the distribution of concentrations of zearalenone in these products changes.

DOCUMENTATION PROVIDED TO EFSA

1. Cressey P and Thomson B, 2006. Risk profile: Mycotoxins in the New Zealand food supply. Prepared as part of a New Zealand Food Safety Authority contract for scientific services by Peter Cressey and Barbara Thomson, April 2006, 177 pp.

REFERENCES

- Abbas HK, Mirocha CJ, Rosiles R and Carvajal M, 1988. Decomposition of zearalenone and deoxynivalenol in the process of making tortillas from corn. *Cereal Chemistry*, 65, 15-19.
- Abbès S, Ouanes Z, ben Salah-Abbès J, Houas Z, Oueslati R, Bacha H and Othman O, 2006. The protective effect of hydrated sodium calcium aluminosilicate against haematological, biochemical and pathological changes induced by Zearalenone in mice. *Toxicol*, 47, 567-574.
- Abbès S, Ouanes Z, Salah-Abbès J, Abdel-Wahhab MA, Oueslati R and Bacha H, 2007. Preventive role of aluminosilicate clay against induction of micronuclei and chromosome aberrations in bone-marrow cells of Balb/c mice treated with Zearalenone. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 631, 85-92.
- Abdelhamid AM, Kelada IP, Ali MM and el-Ayouty SA, 1992. Influence of zearalenone on some metabolic, physiological and pathological aspects of female rabbits at two different ages. *Archiv für Tierernährung*, 42, 63-70.
- Abid-Essefi S, Bouaziz C, Golli-Bennour EE, Ouanes Z and Bacha H, 2009. Comparative study of toxic effects of zearalenone and its two major metabolites alpha-zearalenol and beta-zearalenol on cultured human Caco-2 cells. *Journal of Biochemical and Molecular Toxicology*, 23, 233-243.

- Abid-Essefi S, Ouanes Z, Hassen W, Baudrimont I, Creppy E and Bacha H, 2004. Cytotoxicity, inhibition of DNA and protein syntheses and oxidative damage in cultured cells exposed to zearalenone. *Toxicology In Vitro*, 18, 467-474.
- Abou Nazel MW and El-Shanawany S, 2007. Effect of the mycotoxin Zearalenone on the testis of mice and the protective role of vitamin E: histological and toxicological study. *Bulletin of the Alexandria Faculty of Medicine*, 43, 451-452.
- AFSSA (Agence Française de Sécurité Sanitaire des Aliments), 2006. Risk assessment for mycotoxins in human and animal food chains. Summary report. December 2006. 14 pp.
- AFSSA (Agence Française de Sécurité Sanitaire des Aliments), 2009. Évaluation des risques liés à la présence de mycotoxines dans les chaînes alimentaires humaine et animale. Rapport final. Mars 2009. 308 pp.
- Ahamed S, Foster JS, Bukovsky A and Wimalasena J, 2001. Signal transduction through the Ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell-cycle progression in MCF-7 cells. *Molecular Carcinogenesis*, 30, 88-98.
- Alm H, Brüssow KP, Torner H, Vanselow J, Tomek W, Dänicke S and Tiemann U, 2006. Influence of *Fusarium*-toxin contaminated feed on initial quality and meiotic competence of gilt oocytes. *Reproductive Toxicology*, 22, 44-50.
- Alm H, Greising T, Brüssow KP, Torner H and Tiemann U, 2002. The influence of the mycotoxins deoxynivalenol and zearalenol on *in vitro* maturation of pig oocytes and *in vitro* culture of pig zygotes. *Toxicology In Vitro*, 16, 643-648.
- Andretta I, Lovatto PA, Hauschild L, Dilkin P, Garcia GG, Lanferdini E, Cavazini NC and Mallmann CA, 2008. Feeding of pre-pubertal gilts with diets containing zearalenone. *Arquivo Brasileiro De Medicina Veterinaria E Zootecnia*, 60, 1227-1233.
- Andretta I, Lovatto PA, Lanferdini E, Lehnen CR, Rossi CAR, Hauschild L, Fraga BN, Garcia GG and Mallmann CA, 2010. Feeding of pre-pubertal gilts with diets containing aflatoxins or zearalenone. *Archivos de Zootecnia*, 59, 123-130.
- Arranz I, Mischke C, Stroka J, Sizoo E, van Egmond H and Neugebauer M, 2007. Liquid chromatographic method for the quantification of zearalenone in baby food and animal feed: interlaboratory study. *Journal of AOAC International*, 90, 1598-1609.
- Atkinson HA and Miller K, 1984. Inhibitory effect of deoxynivalenol, 3-acetyldeoxynivalenol and zearalenone on induction of rat and human lymphocyte proliferation. *Toxicology Letters*, 23, 215-221.
- Aurich JE, Hoppen HO, Trampler R, Zentek J, Boehm J, Razzazi-Fazeli E and Aurich C, 2006. Effects of mycotoxins on reproductive function in mares. *Animal Reproduction Science*, 94, 238-241.
- Ayed-Boussema I, Pascussi JM, Maurel P, Bacha H and Hassen W, 2010. Zearalenone activates pregnane X receptor, constitutive androstane receptor and aryl hydrocarbon receptor and corresponding phase I target genes mRNA in primary cultures of human hepatocytes. *Environmental Toxicology and Pharmacology*, 31, 79-87.
- Baird DT and Guevara A, 1969. Concentration of unconjugated estrone and estradiol in peripheral plasma in nonpregnant women throughout the menstrual cycle, castrate and postmenopausal women and in men. *The Journal of Clinical Endocrinology and Metabolism*, 29, 149-156.
- Bankole SA, Schollenberger M and Drochner W, 2010. Survey of ergosterol, zearalenone and trichothecene contamination in maize from Nigeria. *Journal of Food Composition and Analysis*, 23, 837-842.
- Bauer J, Heinritzi K, Gareis M and Gedek B, 1987. Changes in the genital tract of female swine after feeding with practice-relevant amounts of zearalenone. *Tierärztliche Praxis*, 15, 33-36.

- Becci PJ, Voss KA, Hess FG, Gallo MA, Parent RA, Stevens KR and Taylor JM, 1982. Long-term carcinogenicity and toxicity study of zearalenone in the rat. *Journal of Applied Toxicology*, 2, 247-254.
- Belli P, Bellaton C, Durand J, Balleydier S, Milhau N, Mure M, Mornex JF, Benahmed M and Le Jan C, 2010. Fetal and neonatal exposure to the mycotoxin zearalenone induces phenotypic alterations in adult rat mammary gland. *Food and Chemical Toxicology*, 48, 2818-2826.
- Ben Salah-Abbès J, Abbès S, Abdel-Wahhab MA and Oueslati R, 2009. *Raphanus sativus* extract protects against Zearalenone-induced reproductive toxicity, oxidative stress and mutagenic alterations in male Balb/c mice. *Toxicology*, 53, 525-533.
- Ben Salah-Abbès J, Abbès S, Abdel-Wahhab MA and Oueslati R, 2010. *In-vitro* free radical scavenging, antiproliferative and anti-zearalenone cytotoxic effects of 4-(methylthio)-3-butenyl isothiocyanate from Tunisian *Raphanus sativus*. *The Journal of Pharmacy and Pharmacology*, 62, 231-239.
- Ben Salah-Abbès J, Abbès S, Houas Z, Abdel-Wahhab MA and Oueslati R, 2008. Zearalenone induces immunotoxicity in mice: possible protective effects of radish extract (*Raphanus sativus*). *The Journal of Pharmacy and Pharmacology*, 60, 761-770.
- Benzoni E, Minervini F, Giannoccaro A, Fornelli F, Vigo D and Visconti A, 2008. Influence of *in vitro* exposure to mycotoxin zearalenone and its derivatives on swine sperm quality. *Reproductive Toxicology*, 25, 461-467.
- Berek L, Petri IB, Mesterhazy A, Teren J and Molnar J, 2001. Effects of mycotoxins on human immune functions *in vitro*. *Toxicology In Vitro*, 15, 25-30.
- Bernhoft A, Behrens GH, Ingebrigtsen K, Langseth W, Berndt S, Haugen TB and Grotmol T, 2001. Placental transfer of the estrogenic mycotoxin zearalenone in rats. *Reproductive Toxicology*, 15, 545-550.
- Bernhoft A, Clasen PE, Kristoffersen AB and Torp M, 2010. Less *Fusarium* infestation and mycotoxin contamination in organic than in conventional cereals. *Food Additives & Contaminants - Part A*, 27, 842-852.
- Berthiller F, Schuhmacher R, Buttinger G and Krska R, 2005. Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography. A*, 1062, 209-216.
- Berthiller F, Werner U, Sulyok M, Krska R, Hauser MT and Schuhmacher R, 2006. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) determination of phase II metabolites of the mycotoxin zearalenone in the model plant *Arabidopsis thaliana*. *Food Additives & Contaminants. Part A*, 23, 1194-1200.
- Biehl ML, Prelusky DB, Koritz GD, Hartin KE, Buck WB and Trenholm HL, 1993. Biliary excretion and enterohepatic cycling of zearalenone in immature pigs. *Toxicology and Applied Pharmacology*, 121, 152-159.
- Biselli S and Hummert C, 2005. Development of a multicomponent method for *Fusarium* toxins using LC-MS/MS and its application during a survey for the content of T-2 toxin and deoxynivalenol in various feed and food samples. *Food Additives & Contaminants*, 22, 752-760.
- Blokland MH, Sterk SS, Stephany RW, Launay FM, Kennedy DG and van Ginkel LA, 2006. Determination of resorcylic acid lactones in biological samples by GC-MS. Discrimination between illegal use and contamination with *Fusarium* toxins. *Analytical and Bioanalytical Chemistry*, 384, 1221-1227.
- Bravin F, Duca RC, Balaguer P and Delaforge M, 2009. *In vitro* cytochrome p450 formation of a mono-hydroxylated metabolite of zearalenone exhibiting estrogenic activities: possible occurrence of this metabolite *in vivo*. *International Journal of Molecular Sciences*, 10, 1824-1837.

- Cavaliere C, D'Ascenzo G, Foglia P, Pastorini E, Samperi R and Lagana A, 2005. Determination of type B trichothecenes and macrocyclic lactone mycotoxins in field contaminated maize. *Food Chemistry*, 92, 559-568.
- Cavaliere EL and Rogan EG, 2010. Depurinating estrogen-DNA adducts in the etiology and prevention of breast and other human cancers. *Future Oncology*, 6, 75-91.
- CEN (The European Committee for Standardization), 2010. - Determination of zearalenone in maize based baby food, barley flour, maize flour, polenta, wheat flour and cereal based foods for infants and young children - HPLC method with immunoaffinity column cleanup and fluorescence detection. EN 15850:2010, Foodstuffs, CEN/TC 275 - Food analysis - Horizontal methods. Available from <http://www.cen.eu/cen/pages/default.aspx>.
- Cetin Y and Bullerman LB, 2005. Evaluation of reduced toxicity of zearalenone by extrusion processing as measured by the MTT cell proliferation assay. *Journal of Agricultural and Food Chemistry*, 53, 6558-6563.
- Cheeke PR, 1998. Mycotoxins in cereal grains and supplements. In: *Natural Toxicants in feeds, forages and Poisonous plants*. Ed Cheeke PR. Interstate Publishers Inc., Danville, IL, 7-136.
- Chełkowski J, 1998. *Fusarium* and mycotoxins. In: *Mycotoxins in Agriculture and Food Safety*. Eds Sinha KS, Bhatnagar D. Marcel Dekker, New York, 45-64.
- Chen JC, Wiley AA, Kauffold J, Wahner M, Bartol FF and Bagnell CA, 2009. Perinatal Zearalenone Exposure Affects RXFP1, RXFP2, and Morphoregulatory Gene Expression in the Neonatal Porcine Uterus. *Relaxin and Related Peptides: Fifth International Conference*, 1160, 188-189.
- Chi MS, Mirocha CJ, Weaver GA and Kurtz HJ, 1980. Effect of zearalenone on female white leghorn chickens. *Applied and Environmental Microbiology*, 39, 1026-1030.
- Choi SW, Chang HJ, Lee N, Kim JH and Chun HS, 2009. Detection of mycoestrogen zearalenone by a molecularly imprinted polypyrrole-based surface plasmon resonance (SPR) sensor. *Journal of Agricultural and Food Chemistry*, 57, 1113-1118.
- Coenen M and Boyens B, 2001. Capacity of zeolithe to depress the oestrogenic effects of zearalenone. *Proceedings of the Society of Nutrition and Physiology*, 10,
- Collins TF, Sprando RL, Black TN, Olejnik N, Eppley RM, Alam HZ, Rorie J and Ruggles DI, 2006. Effects of zearalenone on *in utero* development in rats. *Food and Chemical Toxicology*, 44, 1455-1465.
- Conkova E, Laciakova A, Pastorova B, Seidel H and Kovac G, 2001. The effect of zearalenone on some enzymatic parameters in rabbits. *Toxicology Letters*, 121, 145-149.
- Cooray R, 1984. Effects of some mycotoxins on mitogen-induced blastogenesis and SCE frequency in human lymphocytes. *Food and Chemical Toxicology*, 22, 529-534.
- Cramer B, Bretz M and Humpf HU, 2007. Stable isotope dilution analysis of the *Fusarium* mycotoxin zearalenone. *Journal of Agricultural and Food Chemistry*, 55, 8353-8358.
- Cressey P and Thomson B, 2006. Risk profile: Mycotoxins in the New Zealand food supply. Prepared as part of a New Zealand Food Safety Authority contract for scientific services by Peter Cressey and Barbara Thomson, April 2006. 177 pp.
- Cunha SC and Fernandes JO, 2010. Development and validation of a method based on a QuEChERS procedure and heart-cutting GC-MS for determination of five mycotoxins in cereal products. *Journal of Separation Science*, 33, 600-609.
- Curtui V, Brockmeyer A, Dietrich R, Kappenstein O, Klaffke H, Lepschy J, Märtlbauer E, Schneider E, Seidler C, Thielert G, Usleber E, Weber R and Wolff J, 2006. Analytik und Vorkommen wichtiger Fusarientoxine (Deoxynivalenol, Zearalenon) sowie Aufnahme dieser Toxine durch den deutschen

- Verbraucher. Schriftenreihe des Bundesministeriums für Verbraucherschutz, Ernährung und Landwirtschaft: Reihe A, Angewandte Wissenschaft Münster, Landwirtschaftsverl. 161 pp.
- Dänicke S, Brüßow KP, Goyarts T, Valenta H, Ueberschär KH and Tiemann U, 2007a. On the transfer of the *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) from the sow to the full-term piglet during the last third of gestation. *Food and Chemical Toxicology*, 45, 1565-1574.
- Dänicke S, Brüßow KP, Valenta H, Ueberschär KH, Tiemann U and Schollenberger M, 2005b. On the effects of graded levels of *Fusarium* toxin contaminated wheat in diets for gilts on feed intake, growth performance and metabolism of deoxynivalenol and zearalenone. *Molecular Nutrition & Food Research*, 49, 932-943.
- Dänicke S, Gädeken D, Ueberschär KH, Meyer U and Scholz H, 2002a. Effects of *Fusarium* toxin contaminated wheat and of a detoxifying agent on performance of growing bulls, on nutrient digestibility in wethers and on the carry over of zearalenone. *Archiv für Tierernährung*, 56, 245-261.
- Dänicke S, Swiech E, Buraczewska L and Ueberschär KH, 2005a. Kinetics and metabolism of zearalenone in young female pigs. *Journal of Animal Physiology and Animal Nutrition*, 89, 268-276.
- Dänicke S, Ueberschär KH, Halle I, Matthes S, Valenta H and Flachowsky G, 2002b. Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or *Fusarium* toxin-contaminated maize on performance of hens and on carryover of zearalenone. *Poultry Science*, 81, 1671-1680.
- Dänicke S, Ueberschär KH, Valenta H, Matthes S, Matthaus K and Halle I, 2004. Effects of graded levels of *Fusarium*-toxin-contaminated wheat in Pekin duck diets on performance, health and metabolism of deoxynivalenol and zearalenone. *British Poultry Science*, 45, 264-272.
- Dänicke S, Valenta H, Ueberschär KH and Matthes S, 2007b. On the interactions between *Fusarium* toxin-contaminated wheat and non-starch-polysaccharide hydrolysing enzymes in turkey diets on performance, health and carry-over of deoxynivalenol and zearalenone. *British Poultry Science*, 48, 39-48.
- Díaz-Llano G and Smith TK, 2006. Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins with and without a polymeric glucomannan mycotoxin adsorbent on reproductive performance and serum chemistry of pregnant gilts. *Journal of Animal Science*, 84, 2361-2366.
- Diekman MA and Green ML, 1992. Mycotoxins and reproduction in domestic livestock. *Journal of Animal Science*, 70, 1615-1627.
- Ding X, Lichti K and Staudinger JL, 2006. The mycoestrogen zearalenone induces CYP3A through activation of the pregnane X receptor. *Toxicological Sciences*, 91, 448-455.
- Döll S, Dänicke S and Schnurrbusch U, 2003a. The effect of increasing concentrations of *Fusarium* toxins in the diets for piglets on histological parameters of the uterus. *Mycotoxin Research*, 19, 73-76.
- Döll S, Dänicke S, Ueberschär KH, Valenta H, Schnurrbusch U, Ganter M, Klobasa F and Flachowsky G, 2003b. Effects of graded levels of *Fusarium* toxin contaminated maize in diets for female weaned piglets. *Archiv für Tierernährung*, 57, 311-334.
- Dong M, He XJ, Tulayakul P, Li J-Y, Dong K-S, Manabe N, Nakayama H and Kumagai S, 2010. The toxic effects and fate of intravenously administered zearalenone in goats. *Toxicon*, 55, 523-530.
- Driehuis F, Spanjer MC, Scholten JM and te Giffel MC, 2008. Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. *Journal of Dairy Science*, 91, 4261-4271.
- Driffield M, Hird SJ and MacDonald SJ, 2003. The occurrence of a range of mycotoxins in animal offal food products by HPLC-MS/MS. *Aspects of Applied Biology*, 205-210.

- Edwards S, Cantley TC, Rottinghaus GE, Osweiler GD and Day BN, 1987. The effects of zearalenone on reproduction in swine. I. The relationship between ingested zearalenone dose and anestrus in non-pregnant, sexually mature gilts. *Theriogenology*, 28, 43-49.
- EFSA (European Food Safety Authority), 2004a. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Deoxynivalenol (DON) as undesirable substance in animal feed. *The EFSA Journal*, 73, 1-42.
- EFSA (European Food Safety Authority), 2004b. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Zearalenone as undesirable substance in animal feed. *The EFSA Journal*, 89, 1-35.
- EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to fumonisins as undesirable substances in animal feed. *The EFSA Journal*, 235, 1-32.
- EFSA (European Food Safety Authority), 2006a. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to ochratoxin A in food. *The EFSA Journal*, 365, 1-56.
- EFSA (European Food Safety Authority), 2006b. Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment *The EFSA Journal*, 438, 1-54.
- EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. *The EFSA Journal*, 1150, 1-72.
- EFSA (European Food Safety Authority), 2010a. Standard sample description for food and feed, *The EFSA Journal*. 1457, 54 pp.
- EFSA (European Food Safety Authority), 2010b. Scientific Report: Management of left-censored data in dietary exposure assessment of chemical substances. *The EFSA Journal*, 1557, 96 pp.
- EFSA (European Food Safety Authority), 2011a. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. *The EFSA Journal*, 1970, 27 pp.
- EFSA (European Food Safety Authority), 2011b. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. *The EFSA Journal*, 2011, 34 pp.
- El-Makawy A, Hassanane MS and Abd Alla ES, 2001. Genotoxic evaluation for the estrogenic mycotoxin zearalenone. *Reproduction, Nutrition, Development*, 41, 79-89.
- EVIRA (Finnish Food Safety Authority), 2008. *Fusarium* toxins: adult intake from cereals and cereal-based products in Finland. November 2008. *Evira Research Reports 5/2008*. 40 pp.
- FAO/WHO (Food and Agriculture Organization - World Health Organization), 1988. Joint FAO/WHO Expert Committee on Food Additives, 32nd Meeting. Toxicological evaluation of certain veterinary drug residues in food. *WHO Food Additives Series 23*.
- FAO/WHO (Food and Agriculture Organization - World Health Organization), 2000. Zearalenone. Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In: *Safety Evaluation of Certain Food Additives and Contaminants*, *WHO Food Additives Series 44*. International Programme on Chemical Safety, World Health Organization, Geneva.
- Ferrer E, Juan-Garcia A, Font G and Ruiz MJ, 2009. Reactive oxygen species induced by beauvericin, patulin and zearalenone in CHO-K1 cells. *Toxicology In Vitro*, 23, 1504-1509.
- Filipiak E, Walczak-Jedrzejowska R, Oszukowska E, Guminska A, Marchlewska K, Kula K and Slowikowska-Hilczer J, 2009. Xenoestrogens diethylstilbestrol and zearalenone negatively influence pubertal rat's testis. *Folia Histochemica et Cytobiologica*, 47, S113-120.

- Fink-Gremmels J and Malekinejad H, 2007. Clinical effects and biochemical mechanisms associated with exposure to the mycoestrogen zearalenone. *Animal Feed Science and Technology*, 137, 326-341.
- Fitzpatrick DW, Arbuckle LD and Hassen AM, 1988. Zearalenone metabolism and excretion in the rat: effect of different doses. *Journal of Environmental Science and Health B*, 23, 343-354.
- Fitzpatrick DW, Picken CA, Murphy LC and Buhr MM, 1989. Measurement of the relative binding affinity of zearalenone, alpha-zearalenol and beta-zearalenol for uterine and oviduct estrogen receptors in swine, rats and chickens: an indicator of estrogenic potencies. *Comparative Biochemistry and Physiology. C*, 94, 691-694.
- Forsell JH and Pestka JJ, 1985. Relation of 8-ketotrichothecene and zearalenone analog structure to inhibition of mitogen-induced human lymphocyte blastogenesis. *Applied and Environmental Microbiology*, 50, 1304-1307.
- Forsell JH, Witt MF, Tai JH, Jensen R and Pestka JJ, 1986. Effects of 8-week exposure of the B6C3F1 mouse to dietary deoxynivalenol (vomitoxin) and zearalenone. *Food and Chemical Toxicology*, 24, 213-219.
- Freni-Titulaer LW, Cordero JF, Haddock L, Lebron G, Martinez R and Mills JL, 1986. Premature thelarche in Puerto Rico. A search for environmental factors. *American Journal of Diseases of Children*, 140, 1263-1267.
- Fugh-Berman A, 2003. "Bust enhancing" herbal products. *Obstetrics and Gynecology*, 101, 1345-1349.
- Gajecka M, Rybarczyk L, Jakimiuk E, Zielonka L, Obremski K, Zwierzchowski W and Gajecki M, 2011. The effect of experimental long-term exposure to low-dose zearalenone on uterine histology in sexually immature gilts. *Experimental and Toxicologic Pathology*, in press.
- Gareis M, Bauer J, Thiem J, Plank G, Grabley S and Gedek B, 1990. Cleavage of zearalenone glycoside, a 'masked' mycotoxin during digestion in swine. *Journal of Veterinary Medicine B*, 37, 236-240.
- Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S and et al., 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environmental and Molecular Mutagenesis*, Suppl 10, 1-175.
- Gazzah AC, Bennour EE, Bouaziz C, Abid S, Ladjimi M and Bacha H, 2010. Sequential events of apoptosis induced by zearalenone in cultured hepatocarcinoma cells. *Mycotoxin Research*, 26, 187-197.
- Ghali R, Hmaissia-Khlifa K, Ghorbel H, Maaroufi K and Hedili A, 2008. Incidence of aflatoxins, ochratoxin A and zearalenone in tunisian foods. *Food Control*, 19, 921-924.
- Ghedira--Chekir L, Maaroufi K, Zakhama A, Ellouz F, Dhouib S, Creppy EE and Bacha H, 1998. Induction of a SOS repair system in lysogenic bacteria by zearalenone and its prevention by vitamin E. *Chemico-Biological Interactions*, 113, 15-25.
- Giraud F, Pasquali M, El Jarroudi M, Vrancken C, Brochot C, Cocco E, Hoffmann L, Delfosse P and Bohn T, 2010. *Fusarium* head blight and associated mycotoxin occurrence on winter wheat in Luxembourg in 2007/2008. *Food Additives & Contaminants. Part A*, 27, 825-835.
- Goertz A, Zuehlke S, Spittler M, Steiner U, Dehne HW, Waalwijk C, de Vries I and Oerke EC, 2010. *Fusarium* species and mycotoxin profiles on commercial maize hybrids in Germany. *European Journal of Plant Pathology*, 128, 101-111.
- Golinski P, Waskiewicz A and Gromadska K 2010. Zearalenone and its derivatives: known toxins in new aspects. Chapter 8. In: *Mycotoxins in Food, Feed and Bioweapons*. Eds Rai M and Varma A, Springer-Verlag, Berlin, Germany.

- Goyarts T, Dänicke S, Valenta H and Ueberschär KH, 2007. Carry-over of *Fusarium* toxins (deoxynivalenol and zearalenone) from naturally contaminated wheat to pigs. *Food Additives & Contaminants*, 24, 369-380.
- Grosse Y, Chekir-Ghedira L, Huc A, Obrecht-Pflumio S, Dirheimer G, Bacha H and Pfohl-Leszkowicz A, 1997. Retinol, ascorbic acid and alpha-tocopherol prevent DNA adduct formation in mice treated with the mycotoxins ochratoxin A and zearalenone. *Cancer Letters*, 114, 225-229.
- Hannon WH, Hill RH, Jr., Bernert JT, Jr., Haddock L, Lebron G and Cordero JF, 1987. Premature thelarche in Puerto Rico: a search for environmental estrogenic contamination. *Archives of Environmental Contamination and Toxicology*, 16, 255-262.
- Hassen W, El Golli E, Baudrimont I, Mobio AT, Ladjimi MM, Creppy EE and Bacha H, 2005. Cytotoxicity and Hsp 70 induction in Hep G2 cells in response to zearalenone and cytoprotection by sub-lethal heat shock. *Toxicology*, 207, 293-301.
- Heneweer M, Houtman R, Poortman J, Groot M, Maliepaard C and Peijnenburg A, 2007. Estrogenic effects in the immature rat uterus after dietary exposure to ethinylestradiol and zearalenone using a systems biology approach. *Toxicological Sciences*, 99, 303-314.
- Herebian D, Zuhlke S, Lamshoft M and Spiteller M, 2009. Multi-mycotoxin analysis in complex biological matrices using LC-ESI/MS: experimental study using triple stage quadrupole and LTQ-Orbitrap. *Journal of Separation Science*, 32, 939-948.
- Hervas M, Lopez MA and Escarpa A, 2009. Electrochemical immunoassay using magnetic beads for the determination of zearalenone in baby food: an anticipated analytical tool for food safety. *Analytica Chimica Acta*, 653, 167-172.
- Hoogenboom LA, Bokhorst JG, Northolt MD, van de Vijver LP, Broex NJ, Mevius DJ, Meijs JA and Van der Roest J, 2008. Contaminants and microorganisms in Dutch organic food products: a comparison with conventional products. *Food Additives & Contaminants. Part A*, 25, 1195-1207.
- Huffman J, Gerber R and Du L, 2010. Recent advancements in the biosynthetic mechanisms for polyketide-derived mycotoxins. *Biopolymers*, 93, 764-776.
- Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, De Neve M, Arcella D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T, Oltarzewski M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-Majem L, Volatier JL, Trolle E, Tornaritis M, Busk L, Kafatos A, Fabiansson S, De Henauw S and Van Klaveren J, in press. Dietary Exposure Assessments for Children in Europe (the EXPOCHI project): rationale, methods and design. *Archives of Public Health*, in press.
- IARC (International Agency for Research on Cancer), 1993. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 56, Some Naturally Occurring Substances: Heterocyclic Aromatic Amines and Mycotoxins, Lyon. 39-444.
- Jakimiuk E, Gajecka M, Jana B, Obremski K and Gajecki M, 2010a. Effect of zearalenone on steroid secretion by porcine follicular cells in mono- and coculture. *Bulletin of the Veterinary Institute in Pulawy*, 54, 419-423.
- Jakimiuk E, Rybarczyk L, Zwierzchowski W, Obremski K, Gajecka M, Zielonka L and Gajecki M, 2010b. Effect of experimental long-term exposure to low-dose zearalenone mycotoxicosis on selected morphometric parameters of the reproductive tract in sexually-immature gilts. *Bulletin of the Veterinary Institute in Pulawy*, 54, 25-28.
- Jefferson WN, Padilla-Banks E, Clark G and Newbold RR, 2002. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. *Journal of Chromatography. B*, 777, 179-189.

- Jiang SZ, Yang ZB, Yang WR, Gao J, Liu FX, Chen CC and Chi F, 2010a. Physiopathological effects of zearalenone in post-weaning female piglets with or without montmorillonite clay adsorbent. *Livestock Science*, 131, 130-136.
- Jiang SZ, Yang ZB, Yang WR, Yao BQ, Zhao H, Liu FX, Chen CC and Chi F, 2010b. Effects of Feeding Purified Zearalenone Contaminated Diets with or without Clay Enterosorbent on Growth, Nutrient Availability, and Genital Organs in Post-weaning Female Pigs. *Asian-Australasian Journal of Animal Sciences*, 23, 74-81.
- Jimenez M, Huerta T and Mateo R, 1997. Mycotoxin production by *Fusarium* species isolated from bananas. *Applied and Environmental Microbiology*, 63, 364-369.
- Jin PG, Han Z, Cai ZX, Wu YJ and Ren YP, 2010. Simultaneous determination of 10 mycotoxins in grain by ultra-high-performance liquid chromatography-tandem mass spectrometry using (1)(3)C-deoxynivalenol as internal standard. *Food Additives & Contaminants. Part A*, 27, 1701-1713.
- Jodlbauer J, Zöllner P and Lindner W, 2000. Determination of zearanol, taleranol, zearalenone, alpha- and beta-zearalenol in urine and tissue by high-performance liquid chromatography-tandem mass spectrometry. *Chromatographia*, 51, 681-687.
- Josephs RD, Schuhmacher R and Krska R, 2001. International interlaboratory study for the determination of the *Fusarium* mycotoxins zearalenone and deoxynivalenol in agricultural commodities. *Food Additives & Contaminants*, 18, 417-430.
- Juhasz J, Nagy P, Kulcsar M, Szigeti G, Reiczigel J and Huszenicza G, 2001. Effect of low-dose zearalenone exposure on luteal function, follicular activity and uterine oedema in cycling mares. *Acta Veterinaria Hungarica*, 49, 211-222.
- Kaklamanos G, Theodoridis G and Dabalís T, 2009. Determination of anabolic steroids in muscle tissue by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography. Part A*, 1216, 8072-8079.
- Kanora A and Maes D, 2009. The role of mycotoxins in pig reproduction: a review. *Veterinari Medicina*, 54, 565-576.
- Kappenstein O, St. Klaffke H, Mehlitz I, Tiebach R, Weber R, Lepschy J and Wittkowski R, 2005. Determination of zearalenone in edible oils with SEC and LC-ESI-MS/MS. *Mycotoxin Research*, 21, 3-6.
- Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM and Edwards PA, 2002. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *The Journal of Biological Chemistry*, 277, 2908-2915.
- Kim IH, Son HY, Cho SW, Ha CS and Kang BH, 2003. Zearalenone induces male germ cell apoptosis in rats. *Toxicology Letters*, 138, 185-192.
- Kleinova M, Zöllner P, Kahlbacher H, Hochsteiner W and Lindner W, 2002. Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zearanol in urine, liver, and muscle of heifers. *Journal of Agricultural and Food Chemistry*, 50, 4769-4776.
- Klötzel M, Lauber U and Humpf HU, 2006. A new solid phase extraction clean-up method for the determination of 12 type A and B trichothecenes in cereals and cereal-based food by LC-MS/MS. *Molecular Nutrition & Food Research*, 50, 261-269.
- Knobil E and Neill JD, 1994. *The physiology of reproduction*, 2nd edition. Raven Press; New York. 3302 pp.
- Knox RV, Vatzias G, Naber CH and Zimmerman DR, 2003. Plasma gonadotropins and ovarian hormones during the estrous cycle in high compared to low ovulation rate gilts. *Journal of Animal Science*, 81, 249-260.

- Kouadio JH, Dano SD, Moukha S, Mobio TA and Creppy EE, 2007. Effects of combinations of *Fusarium* mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. *Toxicol*, 49, 306-317.
- Kouadio JH, Mobio TA, Baudrimont I, Moukha S, Dano SD and Creppy EE, 2005. Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. *Toxicology*, 213, 56-65.
- Krska R, Baumgartner S and Josephs R, 2001. The state-of-the-art in the analysis of type-A and -B trichothecene mycotoxins in cereals. *Fresenius' Journal of Analytical Chemistry*, 371, 285-299.
- Krska R, Pettersson H, Josephs R D, Lemmens M, MacDonald S and Welzig E, 2003. Zearalenone in maize: stability testing and matrix characterisation of a certified reference material. *Food Additives & Contaminants*, 20, 1141-52.
- Kuiper-Goodman T, Scott PM and Watanabe H, 1987. Risk assessment of the mycotoxin zearalenone. *Regulatory Toxicology and Pharmacology*, 7, 253-306.
- Kuo CH, Taub D, Hoffsommer RD and L. WN, 1967. The resolution of α -zearalenone: determination of the absolute configuration of the natural enantiomorph. *Chemical Communications*, 15, 761-762.
- Launay FM, Young PB, Sterk SS, Blokland MH and Kennedy DG, 2004. Confirmatory assay for zearanol, taleranol and the *Fusarium* spp. toxins in bovine urine using liquid chromatography-tandem mass spectrometry. *Food Additives & Contaminants*, 21, 52-62.
- Lauren DR and Smith WA, 2001. Stability of the *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone in ground maize under typical cooking environments. *Food Additives & Contaminants*, 18, 1011-1016.
- Leblanc JC, Tard A, Volatier JL and Verger P, 2005. Estimated dietary exposure to principal food mycotoxins from the first French Total Diet Study. *Food Additives & Contaminants*, 22, 652-672.
- Li D, Chen S and Randerath K, 1992. Natural dietary ingredients (oats and alfalfa) induce covalent DNA modifications (I-compounds) in rat liver and kidney. *Nutrition and Cancer*, 17, 205-216.
- Lioi MB, Santoro A, Barbieri R, Salzano S and Ursini MV, 2004. Ochratoxin A and zearalenone: a comparative study on genotoxic effects and cell death induced in bovine lymphocytes. *Mutation Research*, 557, 19-27.
- Lucci P, Derrien D, Alix F, Perollier C and Bayouhd S, 2010. Molecularly imprinted polymer solid-phase extraction for detection of zearalenone in cereal sample extracts. *Analytica Chimica Acta*, 672, 15-19.
- Maaroufi K, Chekir L, Creppy EE, Ellouz F and Bacha H, 1996. Zearalenone induces modifications of haematological and biochemical parameters in rats. *Toxicol*, 34, 535-540.
- Maher JM, Cheng X, Slitt AL, Dieter MZ and Klaassen CD, 2005. Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. *Drug Metabolism and Disposition*, 33, 956-962.
- Malekinejad H, Maas-Bakker R and Fink-Gremmels J, 2006. Species differences in the hepatic biotransformation of zearalenone. *Veterinary Journal*, 172, 96-102.
- Mallis LM, Sarkahian AB, Harris HA, Zhang MY and McConnell OJ, 2003. Determination of rat oral bioavailability of soy-derived phytoestrogens using an automated on-column extraction procedure and electrospray tandem mass spectrometry. *Journal of Chromatography. Part B*, 796, 71-86.
- Malovrh T and Jakovac-Strajn B, 2010. Feed contaminated with *Fusarium* toxins alter lymphocyte proliferation and apoptosis in primiparous sows during the perinatal period. *Food and Chemical Toxicology*, 48, 2907-2912.
- Manova R and Mladenova R, 2009. Incidence of zearalenone and fumonisins in Bulgarian cereal production. *Food Control*, 20, 362-365.

- Marin DE, Taranu I, Burlacu R and Tudor DS, 2010. Effects of zearalenone and its derivatives on the innate immune response of swine. *Toxicon*, 56, 956-963.
- Marin ML, Murtha J, Dong WM and Pestka JJ, 1996. Effects of mycotoxins on cytokine production and proliferation in EL-4 thymoma cells. *Journal of Toxicology and Environmental Health*, 48, 379-396.
- Marques MF, Martins HM, Costa JM and Bernardo F, 2008. Co-occurrence of deoxynivalenol and zearalenone in crops marketed in Portugal. *Food Additives & Contaminants, Part B-Surveillance*, 1, 130-133.
- Martin LM, Wood KM, McEwen PL, Smith TK, Mandell IB, Yannikouris A and Swanson KC, 2010. Effects of feeding corn naturally contaminated with *Fusarium* mycotoxins and/or a modified yeast cell wall extract on the performance, immunity and carcass characteristics of grain-fed veal calves. *Animal Feed Science and Technology*, 159, 27-34.
- Martos PA, Thompson W and Diaz GJ, 2010. Multiresidue mycotoxin analysis in wheat, barley, oats, rye and maize grain by high-performance liquid chromatography-tandem mass spectrometry. *World Mycotoxin Journal*, 3, 205-223.
- Massart F and Saggese G, 2010. Oestrogenic mycotoxin exposures and precocious pubertal development. *International Journal of Andrology*, 33, 369-376.
- Matsuura Y, Yoshizawa T and Morooka N, 1981. Effect of food additives and heating on the decomposition of zearalenone in wheat flour. *Journal of the Food Hygienic Society of Japan*, 22, 293-298.
- Meister U, 2009. *Fusarium* toxins in cereals of integrated and organic cultivation from the Federal State of Brandenburg (Germany) harvested in the years 2000-2007. *Mycotoxin Research*, 25, 133-139.
- Metzler M, Pfeiffer E and Hildebrand AA, 2010. Zearalenone and its metabolites as endocrine disrupting chemicals. *World Mycotoxin Journal*, 3, 385-401.
- Miles CO, Erasmuson AF, Wilkins AL, Towers NR, Smith BL, Garthwaite I, Scahill BG and Hansen RP, 1996. Ovine metabolism of zearalenone to alpha-zearalanol (zeranol). *Journal of Agricultural and Food Chemistry*, 44, 3244-3250.
- Minervini F, Dell'Aquila ME, Maritato F, Minoia P and Visconti A, 2001. Toxic effects of the mycotoxin zearalenone and its derivatives on *in vitro* maturation of bovine oocytes and 17 beta-estradiol levels in mural granulosa cell cultures. *Toxicology In Vitro*, 15, 489-495.
- Minervini F, Lacalandra GM, Filannino A, Nicassio M, Visconti A and Dell'Aquila ME, 2010. Effects of *in vitro* exposure to natural levels of zearalenone and its derivatives on chromatin structure stability in equine spermatozoa. *Theriogenology*, 73, 392-403.
- Mirocha CJ, Pathre SV and Robison TS, 1981. Comparative metabolism of zearalenone and transmission into bovine-milk. *Food and Cosmetics Toxicology*, 19, 25-30.
- Nair R and Shaha C, 2003. Diethylstilbestrol induces rat spermatogenic cell apoptosis *in vivo* through increased expression of spermatogenic cell Fas/FasL system. *The Journal of Biological Chemistry*, 278, 6470-6481.
- Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N and Tsubura A, 2004. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reproductive Toxicology*, 18, 803-811.
- Nikaido Y, Yoshizawa K, Pei RJ, Yuri T, Danbara N, Hatano T and Tsubura A, 2003. Prepubertal zearalenone exposure suppresses N-methyl-N-nitrosourea-induced mammary tumorigenesis but causes severe endocrine disruption in female Sprague-Dawley rats. *Nutrition and Cancer*, 47, 164-170.
- Nordic Council of Ministers, 1998. *Fusarium* toxins in cereals - a risk assessment. *TemaNord*, 502, 22-27.

- NTP (National Toxicology Program), 1982. NTP Carcinogenesis Bioassay of Zearalenone in F 344/N Rats and F6C3F1 Mice (Technical Report Series No. 235), Research Triangle Park, North Carolina, Department of Health and Human Services.
- Nuryono N, Noviandi CT, Bohm J and Razzazi-Fazeli E, 2005. A limited survey of zearalenone in Indonesian maize-based food and feed by ELISA and high performance liquid chromatography. *Food Control*, 16, 65-71.
- Obremski K, Gajecki M, Zwierzchowski W, Bakula T, Apoznaniski J and Wojciechoeski J, 2003a. The level of zearalenone and a-zearalenol in the blood of gilts with clinical symptoms of toxicosis, fed diets with a low zearalenone content. *Journal of Animal and Feed Sciences*, 12, 529-538.
- Obremski K, Gajecki M, Zwierzchowski W, Zielonka L, Otrocka-Domagala I, Rotkiewicz T, Mikolajczyk A, Gajecka M and Polak M, 2003b. Influence of zearalenone on reproductive system cell proliferation in gilts. *Polish Journal of Veterinary Science*, 6, 239-245.
- OECD (Organisation for Economic Co-operation and Development), 1997. OECD Guideline for the Testing of Chemicals 475, Mammalian Bone Marrow Chromosome Aberration Test, Adopted: 21st July 1997. Organisation for Economic Co-operation and Development (OECD)/Organisation de Coopération et de Développement Économiques (OCDE). Available from <http://www.oecd.org/dataoecd/18/11/1948450.pdf>
- Oliver WT, Miles JR, Diaz DE, Dibner JJ, Rottinghaus GE and Harrell RJ, 2010. Zearalenone Increases Reproductive Tract Development, but not Skeletal Muscle Signaling in Prepubertal Gilts *Journal of Animal Science Supplement*, 88,
- Olsen M, 1989. Metabolism of zearalenone in farm animals. In: *Fusarium: Mycotoxins, Taxonomy and Pathogenicity*. Ed Chelkowski J. Elsevier, Amsterdam, 167-177.
- Olsen M, Pettersson H and Kiessling KH, 1981. Reduction of zearalenone to zearalenol in female rat liver by 3 alpha-hydroxysteroid dehydrogenase. *Acta Pharmacologica et Toxicologica*, 48, 157-161.
- Ouanes Z, Abid S, Ayed I, Anane R, Mobio T, Creppy EE and Bacha H, 2003. Induction of micronuclei by Zearalenone in Vero monkey kidney cells and in bone marrow cells of mice: protective effect of Vitamin E. *Mutation Research*, 538, 63-70.
- Ouanes Z, Ayed-Boussema I, Baati T, Creppy EE and Bacha H, 2005. Zearalenone induces chromosome aberrations in mouse bone marrow: preventive effect of 17beta-estradiol, progesterone and Vitamin E. *Mutation Research*, 565, 139-149.
- Ouanes-Ben Othmen Z, Essefi SA and Bacha H, 2008. Mutagenic and epigenetic mechanisms of zearalenone: prevention by Vitamin E. *World Mycotoxin Journal*, 1, 369-374.
- Palpacelli V, Beco L and Ciani M, 2007. Vomitoxin and zearalenone content of soft wheat flour milled by different methods. *Journal of Food Protection*, 70, 509-513.
- Palyusik M, Harrach B, Mirocha CJ and Pathre SV, 1980. Transmission of zearalenone and zearalenol into porcine milk. *Acta Veterinaria Academiae Scientiarum Hungaricae*, 28, 217-222.
- Pérez Comas A, 1982. Precocious sexual development: clinical study in the western region of Puerto Rico. *Boletín de la Asociación Médica de Puerto Rico*, 74, 245-251.
- Pestka JJ, Tai JH, Witt MF, Dixon DE and Forsell JH, 1987. Suppression of immune response in the B6C3F1 mouse after dietary exposure to the *Fusarium* mycotoxins deoxynivalenol (vomitoxin) and zearalenone. *Food and Chemical Toxicology*, 25, 297-304.
- Pfeiffer E, Hildebrand A, Damm G, Rapp A, Cramer B, Humpf HU and Metzler M, 2009. Aromatic hydroxylation is a major metabolic pathway of the mycotoxin zearalenone in vitro. *Molecular Nutrition & Food Research*, 53, 1123-1133.

- Pfeiffer E, Hildebrand A, Mikula H and Metzler M, 2010. Glucuronidation of zearalenone, zearanol and four metabolites in vitro: formation of glucuronides by various microsomes and human UDP-glucuronosyltransferase isoforms. *Molecular Nutrition & Food Research*, 54, 1468-1476.
- Pfohl-Leschkowitz A, Chekir-Ghedira L and Bacha H, 1995. Genotoxicity of zearalenone, an estrogenic mycotoxin: DNA adduct formation in female mouse tissues. *Carcinogenesis*, 16, 2315-2320.
- Pillay D, Chuturgoon AA, Nevines E, Manickum T, Deppe W and Dutton MF, 2002. The quantitative analysis of zearalenone and its derivatives in plasma of patients with breast and cervical cancer. *Clinical Chemistry and Laboratory Medicine*, 40, 946-951.
- Plasencia J and Mirocha CJ, 1991. Isolation and characterisation of zearalenone sulfate produced by *Fusarium* spp. *Applied and Environmental Microbiology*, 57, 146-150.
- Prelusky D, Warner RM and Threnholm HL, 1989. Sensitive analysis of the mycotoxin zearalenone and its metabolites in biological fluids by high-performance liquid chromatography alpha. *Journal of Chromatography*, 494, 267-277.
- Prelusky DB, Scott PM, Trenholm HL and Lawrence GA, 1990. Minimal transmission of zearalenone to milk of dairy cows. *Journal of Environmental Science and Health. Part B*, 25, 87-103.
- Pung OJ, Luster MI, Hayes HT and Rader J, 1984. Influence of steroidal and nonsteroidal sex hormones on host resistance in mice: increased susceptibility to *Listeria monocytogenes* after exposure to estrogenic hormones. *Infection and Immunity*, 46, 301-307.
- Pussemier L, Pierard JY, Anselme M, Tangni EK, Motte JC and Larondelle Y, 2006. Development and application of analytical methods for the determination of mycotoxins in organic and conventional wheat. *Food Additives & Contaminants*, 23, 1208-1218.
- Rainey MR, Tubbs RC, Bennett LW and Cox NM, 1990. Prepubertal exposure to dietary zearalenone alters hypothalamohypophyseal function but does not impair postpubertal reproductive function of gilts. *Journal of Animal Science*, 68, 2015-2022.
- Ranzenigo G, Caloni F, Cremonesi F, Aad PY and Spicer LJ, 2008. Effects of *Fusarium* mycotoxins on steroid production by porcine granulosa cells. *Animal Reproduction Science*, 107, 115-130.
- Rasmussen PH, Ghorbani F and Berg T, 2003. Deoxynivalenol and other *Fusarium* toxins in wheat and rye flours on the Danish market. *Food Additives & Contaminants*, 20, 396-404.
- Riley RT, 1998. Mechanistic interaction of mycotoxins: theoretical considerations. In: *Mycotoxins in Agriculture and Food Safety*. Eds Sinha KK and Bhatnagar D, Marcel Dekker, New York. 227-253.
- Romagnoli B, Ferrari M and Bergamini C, 2010. Simultaneous determination of deoxynivalenol, zearalenone, T-2 and HT-2 toxins in breakfast cereals and baby food by high-performance liquid chromatography and tandem mass spectrometry. *Journal of Mass Spectrometry*, 45, 1075-1080.
- Ryu D, Hanna MA and Bullerman LB, 1999. Stability of zearalenone during extrusion of corn grits. *Journal of Food Protection*, 62, 1482-1484.
- Santos L, Marin S, Sanchis V and Ramos AJ, 2010. Co-occurrence of aflatoxins, ochratoxin A and zearalenone in Capsicum powder samples available on the Spanish market. *Food Chemistry*, 122, 826-830.
- SCF (Scientific Committee on Food), 2000. Opinion of the Scientific Committee on Food on *Fusarium* toxins. Part 2: Zearalenone (ZEA). 12 pp.
- Schmidt K, Stachel C and Gowik P, 2008. Development and in-house validation of an LC-MS/MS method for the determination of stilbenes and resorcylic acid lactones in bovine urine. *Analytical and Bioanalytical Chemistry*, 391, 1199-1210.
- Schneweis I, Meyer K, Engelhardt G and Bauer J, 2002. Occurrence of zearalenone-4-D-glucopyranoside in wheat. *Journal of Agricultural and Food Chemistry*, 50, 1736-1738.

- Schollenberger M, Jara HT, Suchy S, Drochner W and Muller HM, 2002. *Fusarium* toxins in wheat flour collected in an area in southwest Germany. *International Journal of Food Microbiology*, 72, 85-89.
- Schollenberger M, Muller HM, Rufle M and Drochner W, 2008. Natural occurrence of 16 *Fusarium* toxins in edible oil marketed in Germany. *Food Control*, 19, 475-482.
- Schollenberger M, Muller HM, Rufle M, Suchy S, Planck S and Drochner W, 2005. Survey of *Fusarium* toxins in foodstuffs of plant origin marketed in Germany. *International Journal of Food Microbiology*, 97, 317-326.
- Schollenberger M, Muller HM, Rufle M, Suchy S, Plank S and Drochner W, 2006. Natural occurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany. *Mycopathologia*, 161, 43-52.
- Schollenberger M, Muller HM, Rufle M, Terry-Jara H, Suchy S, Plank S and Drochner W, 2007. Natural occurrence of *Fusarium* toxins in soy food marketed in Germany. *International Journal of Food Microbiology*, 113, 142-146.
- Schollenberger M, Suchy S, Jara HT, Drochner W and Muller HM, 1999. A survey of *Fusarium* toxins in cereal-based foods marketed in an area of southwest Germany. *Mycopathologia*, 147, 49-57.
- SCOOP (European Commission, Directorate-General Health and Consumer Protection - Scientific Co-Operation on Questions relating to Food), 2003. SCOOP, task 3.2.10. Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU Member States. European Commission, Directorate-General Health and Consumer Protection, Reports on tasks for scientific co-operation, April 2003, Available from <http://ec.europa.eu/food/fs/scoop/task3210.pdf>.
- Scudamore KA, Guy RC, Kelleher B and MacDonald SJ, 2008b. Fate of *Fusarium* mycotoxins in maize flour and grits during extrusion cooking. *Food Additives & Contaminants. Part A*, 25, 1374-1384.
- Scudamore KA, Guy RC, Kelleher B and MacDonald SJ, 2008a. Fate of the *Fusarium* mycotoxins, deoxynivalenol, nivalenol and zearalenone, during extrusion of wholemeal wheat grain. *Food Additives & Contaminants. Part A*, 25, 331-337.
- Scudamore KA and Patel S, 2000. Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom. *Food Additives & Contaminants*, 17, 407-416.
- Scudamore KA and Patel S, 2009. Occurrence of *Fusarium* mycotoxins in maize imported into the UK, 2004-2007. *Food Additives & Contaminants. Part A*, 26, 363-371.
- Seeling K, Dänicke S, Ueberschär KH, Lebzien P and Flachowsky G, 2005. On the effects of *Fusarium* toxin-contaminated wheat and the feed intake level on the metabolism and carry over of zearalenone in dairy cows. *Food Additives & Contaminants*, 22, 847-855.
- Shephard GS, Berthiller F, Dorner J, Krska R, Lombaert GA, Malone B, Maragos C, Sabino M, Solfrizzo M, Trucksess MW, van Egmond HP and Whitaker TB, 2009. Developments in mycotoxin analysis: an update for 2007-2008. *World Mycotoxin Journal*, 2, 3-21.
- Shier WT, Shier AC, Xie W and Mirocha CJ, 2001. Structure-activity relationships for human estrogenic activity in zearalenone mycotoxins. *Toxicol*, 39, 1435-1438.
- Shim WB, Kim KY and Chung DH, 2009. Development and Validation of a Gold Nanoparticle Immunochromatographic Assay (ICG) for the Detection of Zearalenone. *Journal of Agricultural and Food Chemistry*, 57, 4035-4041.
- Shin BS, Hong SH, Bulitta JB, Hwang SW, Kim HJ, Lee JB, Yang SD, Kim JE, Yoon HS, Kim do J and Yoo SD, 2009. Disposition, oral bioavailability, and tissue distribution of zearalenone in rats at various dose levels. *Journal of Toxicology and Environmental Health. Part A*, 72, 1406-1411.
- Siegel D, Andrae K, Proske M, Kochan C, Koch M, Weber M and Nehls I, 2010. Dynamic covalent hydrazine chemistry as a selective extraction and cleanup technique for the quantification of the

- Fusarium* mycotoxin zearalenone in edible oils. *Journal of Chromatography. Part A*, 1217, 2206-2215.
- Smith JE, Lewis CW, Anderson JG and Solomons GL, 1994. Mycotoxins in human nutrition and health. *Mycotoxins in human nutrition and health.*, EUR 16048 EN, xiii + 16300 pp.
- Songsermsakul P, Sontag G, Cichna-Markl M, Zentek J and Razzazi-Fazeli E, 2006. Determination of zearalenone and its metabolites in urine, plasma and faeces of horses by HPLC-APCI-MS. *Journal of Chromatography. Part B*, 843, 252-261.
- Sørensen LK and Elbaek TH, 2005. Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. *Journal of Chromatography. Part B*, 820, 183-196.
- Spanjer MC, Rensen PM and Scholten JM, 2008. LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. *Food Additives & Contaminants. Part A*, 25, 472-489.
- Stob M, Baldwin RS, Tuite J, Andrews FN and Gillette KG, 1962. Isolation of an anabolic, uterotrophic compound from corn infected with *Gibberella zea*. *Nature*, 196, 1318.
- Sulyok M, Krska R and Schuhmacher R, 2007. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. *Analytical and Bioanalytical Chemistry*, 389, 1505-1523.
- Suproniene S, Justesen A, Nicolaisen M, Mankeviciene A, Dabkevicius Z, Semaskiene R and Leistrumaite A, 2010. Distribution of trichothecene and zearalenone producing *Fusarium* species in grain of different cereal species and cultivars grown under organic farming conditions in Lithuania. *Annals of Agricultural and Environmental Medicine*, 17, 79-86.
- Sutkevičienė N, Riskeviciene V, Januskauskas A, Zilinskas H and Andersson M, 2009. Assessment of sperm quality traits in relation to fertility in boar semen. *Acta Veterinaria Scandinavica*, 51, 53.
- Suzuki T, Munakata Y, Morita K, Shinoda T and Ueda H, 2007. Sensitive detection of estrogenic mycotoxin zearalenone by open sandwich immunoassay. *Analytical Sciences*, 23, 65-70.
- Sypecka Z, Kelly M and Brereton P, 2004. Deoxynivalenol and zearalenone residues in eggs of laying hens fed with a naturally contaminated diet: effects on egg production and estimation of transmission rates from feed to eggs. *Journal of Agricultural and Food Chemistry*, 52, 5463-5471.
- Szuets P, Mesterhazy A, Falkay G and Bartok T, 1997. Early telarche symptoms in children and their relations to Zearalenon contamination in foodstuffs. *Cereal Research Communications*, 25, 429-436.
- Tabuc C, Marin D, Guerre P, Sesan T and Bailly JD, 2009. Molds and mycotoxin content of cereals in southeastern Romania. *Journal of Food Protection*, 72, 662-665.
- Tajima O, Schoen ED, Feron VJ and Groten JP, 2002. Statistically designed experiments in a tiered approach to screen mixtures of *Fusarium* mycotoxins for possible interactions. *Food and Chemical Toxicology*, 40, 685-695.
- Takagi M, Mukai S, Kuriyagawa T, Takagaki K, Uno S, Kokushi E, Otoi T, Budiyo A, Shirasuna K, Miyamoto A, Kawamura O, Okamoto K and Deguchi E, 2008. Detection of zearalenone and its metabolites in naturally contaminated follicular fluids by using LC/MS/MS and in vitro effects of zearalenone on oocyte maturation in cattle. *Reproductive Toxicology*, 26, 164-169.
- Tanaka H, Takino M, Sugita-Konishi Y and Tanaka T, 2006. Development of a liquid chromatography/time-of-flight mass spectrometric method for the simultaneous determination of trichothecenes, zearalenone and aflatoxins in foodstuffs. *Rapid Communications in Mass Spectrometry*, 20, 1422-1428.
- Tanaka H, Takino M, Sugita-Konishi Y, Tanaka T, Leeman D, Toriba A and Hayakawa K, 2010. Determination of *Fusarium* mycotoxins by liquid chromatography/tandem mass spectrometry

- coupled with immunoaffinity extraction. *Rapid Communications in Mass Spectrometry*, 24, 2445-2452.
- Tanaka T, Yoneda A, Inoue S, Sugiura Y and Ueno Y, 2000. Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography-mass spectrometry. *Journal of Chromatography. Part A*, 882, 23-28.
- Tangni EK, Waegeneers N, Van Overmeire I, Goeyens L and Pussemier L, 2009. Mycotoxin analyses in some home produced eggs in Belgium reveal small contribution to the total daily intake. *Science of the Total Environment*, 407, 4411-4418.
- Thongrussamee T, Kuzmina NS, Shim WB, Jiratpong T, Eremin SA, Intravong J and Chung DH, 2008. Monoclonal-based enzyme-linked immunosorbent assay for the detection of zearalenone in cereals. *Food Additives & Contaminants. Part A*, 25, 997-1006.
- Thurst R, Kneist S and Huhne V, 1983. Genotoxicity of *Fusarium* mycotoxins (nivalenol, fusarenon-X, T-2 toxin, and zearalenone) in Chinese hamster V79-E cells in vitro. *Archives für Geschwulstforschung*, 53, 9-15.
- Tiemann U, Brüssow KP, Dänicke S and Vanselow J, 2008a. Feeding of pregnant sows with mycotoxin-contaminated diets and their non-effect on foetal and maternal hepatic transcription of genes of the insulin-like growth factor system. *Food Additives & Contaminants. Part A*, 25, 1365-1373.
- Tiemann U, Brüssow KP, Dannenberger D, Jonas L, Pöhland R, Jäger K, Dänicke S and Hagemann E, 2008b. The effect of feeding a diet naturally contaminated with deoxynivalenol (DON) and zearalenone (ZON) on the spleen and liver of sow and fetus from day 35 to 70 of gestation. *Toxicological Letters*, 179, 113-117.
- Tiemann U, Brüssow KP, Küchenmeister U, Jonas L, Pöhland R, Reischauer A, Jäger K and Dänicke S, 2008c. Changes in the spleen and liver of pregnant sows and full-term piglets after feeding diets naturally contaminated with deoxynivalenol and zearalenone. *Veterinary Journal*, 176, 188-196.
- Tiemann U and Dänicke S, 2007. In vivo and in vitro effects of the mycotoxins zearalenone and deoxynivalenol on different non-reproductive and reproductive organs in female pigs: a review. *Food Additives & Contaminants*, 24, 306-314.
- Tiemann U, Tomek W, Schneider F and Vanselow J, 2003a. Effects of the mycotoxins alpha- and beta-zearalenol on regulation of progesterone synthesis in cultured granulosa cells from porcine ovaries. *Reproductive Toxicology*, 17, 673-681.
- Tiemann U, Viergutz T, Jonas L and Schneider F, 2003b. Influence of the mycotoxins alpha- and beta-zearalenol and deoxynivalenol on the cell cycle of cultured porcine endometrial cells. *Reproductive Toxicology*, 17, 209-218.
- Tomaszewski J, Miturski R, Semczuk A, Kotarski J and Jakowicki J, 1998. Tissue zearalenone concentration in normal, hyperplastic and neoplastic human endometrium. *Ginekologia Polska*, 69, 363-366.
- Toyama Y and Yuasa S, 2004. Effects of neonatal administration of 17beta-estradiol, beta-estradiol 3-benzoate, or bisphenol A on mouse and rat spermatogenesis. *Reproductive Toxicology*, 19, 181-188.
- Trenholm HL, Warner RM and Farnworth ER, 1981. High performance liquid chromatographic method using fluorescence detection for quantitative analysis of zearalenone and alpha-zearalenol in blood plasma. *Journal - Association of Official Analytical Chemists*, 64, 302-310.
- Tsakmakidis IA, Lymberopoulos AG, Alexopoulos C, Boscios CM and Kyriakis SC, 2006. In vitro effect of zearalenone and alpha-zearalenol on boar sperm characteristics and acrosome reaction. *Reproduction in Domestic Animals*, 41, 394-401.

- Tsakmakidis IA, Lymberopoulos AG, Vainas E, Boscós CM, Kyriakis SC and Alexopoulos C, 2007. Study on the in vitro effect of zearalenone and alpha-zearalenol on boar sperm-zona pellucida interaction by hemizona assay application. *Journal of Applied Toxicology*, 27, 498-505.
- Tsakmakidis IA, Lymberopoulos AG, Khalifa TA, Boscós CM, Saratsi A and Alexopoulos C, 2008. Evaluation of zearalenone and alpha-zearalenol toxicity on boar sperm DNA integrity. *Journal of Applied Toxicology*, 28, 681-688.
- Underhill KL, Rotter BA, Thompson BK, Prelusky DB and Trenholm HL, 1995. Effectiveness of cholestyramine in the detoxification of zearalenone as determined in mice. *Bulletin of Environmental Contamination and Toxicology*, 54, 128-134.
- Urraca JL, Marazuela MD, Merino ER, Orellana G and Moreno-Bondi MC, 2006. Molecularly imprinted polymers with a streamlined mimic for zearalenone analysis. *Journal of Chromatography. Part A*, 1116, 127-134.
- Urry WH, Wehrmeister HL, Hodge EB and Hidy PH, 1966. The structure of zearalenone. *Tetrahedron Letters*, 7, 3109-3114.
- Valenta H, Dänicke S and Blüthgen A, 2002. Mycotoxins in Soybean Feedstuffs Used in Germany. *Mycotoxin Research*, 18, 208-211.
- Vendl O, Berthiller F, Crews C and Krska R, 2009. Simultaneous determination of deoxynivalenol, zearalenone, and their major masked metabolites in cereal-based food by LC-MS-MS. *Analytical and Bioanalytical Chemistry*, 395, 1347-1354.
- Vendl O, Crews C, MacDonald S, Krska R and Berthiller F, 2010. Occurrence of free and conjugated *Fusarium* mycotoxins in cereal-based food. *Food Additives & Contaminants. Part A*, 27, 1148-1152.
- Vlata Z, Porichis F, Tzanakakis G, Tsatsakis A and Krambovitis E, 2006. A study of zearalenone cytotoxicity on human peripheral blood mononuclear cells. *Toxicology Letters*, 165, 274-281.
- Warner R, Ram BP, Hart LP and Pestka JJ, 1986. Screening for zearalenone in corn by competitive direct enzyme-linked-immunosorbent-assay. *Journal of Agricultural and Food Chemistry*, 34, 714-717.
- Wasowicz K, Gajecka M, Calka J, Jakimiuk E and Gajecki M, 2005. Influence of chronic administration of zearalenone on the processes of apoptosis in the porcine ovary. *Veterinari Medicina*, 50, 531-536.
- WHO (World Health Organization), 2000. Zearalenone. Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In: *Safety Evaluation of Certain Food Additives and Contaminants*, WHO Food Additives Series 44. International Programme on Chemical Safety, World Health Organization, Geneva.
- WHO (World Health Organization), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food, International Programme on Chemical Safety, Environmental Health Criteria 240. Chapter 6: Dietary Exposure Assessment of Chemicals in Food. Available from <http://www.who.int/ipcs/food/principles/en/index1.html>
- WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2008. Uncertainty and Data Quality in Exposure Assessment. Part 1: Guidance document on characterizing and communicating uncertainty in exposure assessment. Part 2: Hallmarks of data quality in chemical exposure assessment. Available from http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf.
- Wolff J, 2005. Effects of handling and processing on deoxynivalenol and zearalenone content of cereals and cereal products. *Mycotoxin Research*, 21, 246-250.
- Wolff J, Blüthgen A, Brüggemann J, Dänicke S, Hecht H, Jira W, Sender I, Rabe E, Schenkel H, Schwind K-H, Ubben E-H, Uberschär K-H and Valenta H, 2004. Untersuchungen an Nebenprodukten der Müllerei auf unerwünschte Stoffe und deren futtermittelrechtliche Bewertung.

- Schriftenreihe des Bundesministeriums für Verbraucherschutz, Ernährung und Landwirtschaft - Angewandte Wissenschaft, Schifflleitung: J Wolff und A Blüthgen, Landwirtschaft GmbH Münster-Hiltrup Heft 496:.
- Wollenhaupt K, Dänicke S, Brüßow KP and Tiemann U, 2006. In vitro and in vivo effects of deoxynivalenol (DNV) on regulators of cap dependent translation control in porcine endometrium. *Reproductive Toxicology*, 21, 60-73.
- Wollenhaupt K, Jonas L, Tiemann U and Tomek W, 2004. Influence of the mycotoxins alpha- and beta-zearalenol (ZOL) on regulators of cap-dependent translation control in pig endometrial cells. *Reproductive Toxicology*, 19, 189-199.
- Yang JY, Wang GX, Liu JL, Fan JJ and Cui S, 2007b. Toxic effects of zearalenone and its derivatives alpha-zearalenol on male reproductive system in mice. *Reproductive Toxicology*, 24, 381-387.
- Yang JY, Zhang YF, Wang YQ and Cui S, 2007a. Toxic effects of zearalenone and alpha-zearalenol on the regulation of steroidogenesis and testosterone production in mouse Leydig cells. *Toxicology In Vitro*, 21, 558-565.
- Yonghang Z, Shaobing Z, Weijun T et al., 1995. Isolation of *Fusarium* and extraction of its toxin from buckwheat grown in an area with "endemic breast enlargement" disease (article in Chinese). *Chung Hua Yu Fang Hsueh Tsa Chih*, 29, 273-275.
- Yu Z, Zhang L, Wu D and Liu F, 2005. Anti-apoptotic action of zearalenone in MCF-7 cells. *Ecotoxicology and Environmental Safety*, 62, 441-446.
- Yuan H, Deng Y, Yuan L, Wu J, Yuan Z, Yi J, Zhang M, Guo C, Wen L, Li R, Zhu L and He Z, 2010. *Gynostemma pentaphyllum* protects mouse male germ cells against apoptosis caused by zearalenone via Bax and Bcl-2 regulation. *Toxicology Mechanisms and Methods*, 20, 153-158.
- Yumbe-Guevara BE, Imoto T and Yoshizawa T, 2003. Effects of heating procedures on deoxynivalenol, nivalenol and zearalenone levels in naturally contaminated barley and wheat. *Food Additives & Contaminants*, 20, 1132-1140.
- Zhang Y, Zhu S and Tong W, 1995. Isolation of *Fusarium* and extraction of its toxin from buckwheat grown in an area with "endemic breast enlargement" disease (article in Chinese). *Zhonghua Yu Fang Yi Xue Za Zhi* (Chinese Journal of Preventive Medicine), 29, 273-275.
- Zinedine A, Soriano JM, Molto JC and Manes J, 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. *Food and Chemical Toxicology*, 45, 1-18.
- Zöllner P, Jodlbauer J, Kleinova M, Kahlbacher H, Kuhn T, Hochsteiner W and Lindner W, 2002. Concentration levels of zearalenone and its metabolites in urine, muscle tissue, and liver samples of pigs fed with mycotoxin-contaminated oats. *Journal of Agricultural and Food Chemistry*, 50, 2494-2501.
- Zwierzchowski W, Przybyłowicz M, Obremski K, Zielonka L, Skorska-Wyszyńska E, Gajecka M, Polak M, Jakimiuk E, Jana B, Rybarczyk L and Gajecki M, 2005. Level of zearalenone in blood serum and lesions in ovarian follicles of sexually immature gilts in the course of zearalenone micotoxicosis. *Polish Journal of Veterinary Sciences*, 8, 209-218.

APPENDIXES

A. MEAN AND 95TH PERCENTILE BREAKFAST CEREAL CONSUMPTION IN EUROPEAN COUNTRIES

Mean and 95th percentile breakfast cereal consumption in European countries are presented in Tables A1 and A2.

Table A1: Mean breakfast cereal consumption (g/day) in total population by European country, dietary survey and age classes.

Dietary survey ^(a)	Average breakfast cereal consumption (g/day)					
	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
BE/1			13	5.0	1.9	2.0
BE/2	5.8	9.3				
BG	0.3	1.1				
CY			17			
CZ		7	6.9	3.5		
DK		6.1	6.8	5.0	4.7	3.1
FI/1	4.0	6.8				
FI/2				10	17	
FI/3		29				
FR		10	13	4.0	2.1	0.6
DE/1	4.9	14				
DE/2	4.1	13				
DE/3	2.9	13				
DE/4			8.1	4.7	3.8	3.5
GR		5.5				
HU				1.8	0.47	0.7
IE				19		
IT	0.4	4.4	3.2	1.6	0.7	0.7
LV		28	15	12		
NL/1				4.1		
NL/2	3.7	3.8				
ES/1				7.0		
ES/2			4.0	3.1		
ES/3		11	12			
ES/4	5.3	12	8			
SE/1				7.2		
SE/2		17	19			
UK				25		

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 9.

Table A2: 95th percentile of breakfast cereal consumption (g/day) in total population by European country, dietary survey and age classes.

Dietary survey ^(a)	95 th percentile breakfast cereal consumption (g/day)					
	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
BE/1			62	30	10	7
BE/2	43 ^(b)	40				
BG		0.0 ^(c)	0.0 ^(c)			
CY			50			
CZ		50	50	20		
DK		24	27	26	26	21 ^(b)
FI/1	22	23				
FI/2				41	50	
FI/3		117				
FR		41	54	28	11	0.0 ^(c)
DE/1	18	61				
DE/2	21	55				
DE/3	11	52				
DE/4			40	30	30	30
GR		30				
HU				15	0.0 ^(c)	0.0 ^(c)
IE				64		
IT	7 ^(a)	23	23	13	0.0 ^(c)	0.0 ^(c)
LV		140	100	100		
NL/1				25		
NL/2	23	25				
ES/1				43		
ES/2			20	25		
ES/3		60	45			
ES/4	60 ^(b)	40	60			
SE/1				34		
SE/2		84	93			
UK				91		

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 9.

(b): If the number of subjects is < 60 then the calculated 95th percentile should be considered as an indicative value only due to the limited number of data (EFSA, 2011b).

(c): In certain dietary surveys, more than 95 % of the subjects did not report breakfast cereal consumption thus the 95th percentile equals zero.

B. MEAN AND 95TH PERCENTILE DIETARY EXPOSURE TO ZEARALENONE IN EUROPEAN COUNTRIES

Mean and 95th percentile dietary exposure to zearalenone (ng/kg b.w. per day) for average and high consumers (95th percentile) in European countries are presented in Table B1 (lower bound (LB) scenario) and Table B2 (upper bound (UB) scenario).

Table B1: Mean and 95th percentile dietary exposure to zearalenone (ng/kg body weight (b.w.) per day) for average and high consumers (95th percentile) in lower-bound (LB) scenario.

Dietary survey ^(a)	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95
BE/1							6.2	15	4.4	12	2.8	8.1	2.6	7.7
BE/2			9.3	23 ^(b)	9	19								
BG	9.4	33	23	43	22	42								
CY							5.2	12						
CZ					17	35	12	26	6.7	14				
DK					5.7	9.9	3.6	7.5	2.4	4.7	2.0	3.5	2.3	5.1 ^(b)
FI/1			20	50	16	35								
FI/2									3.0	6.6	2.5	5.2		
FI/3					7.6	16								
FR					15	30	7.6	17	4.5	10	3.2	7.5	2.9	7.1
DE/1			13	33	10	21								
DE/2			11	25	11	20								
DE/3			12	24	11	22								
DE/4							3.9	11	3.4	8.7	2.9	7.2	2.8	7.0
GR					12	26								
HU									7.2	13	6.4	12	7.1	13
IE									4.7	9.5				
IT	3.3	27 ^(b)	16	42 ^(b)	14	30	8.5	19	5.5	12	4.6	10	4.7	10
LV					7.1	19	4.6	14	2.6	9.0				
NL/1									3.3	7.8				
NL/2			14	28	13	25								
ES/1									4.3	10				
ES/2							5.0	12	3.8	8.7				
ES/3					14	31	8.6	19						
ES/4			10	30 ^(b)	9.5	21	6.3	15						

Table B1: Continued.

Dietary survey ^(a)	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95
SE/1									3.4	7.7				
SE/2					9.0	19	6.0	14						
UK									5.0	11				

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 9.

(b): If the number of subjects < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b).

Table B2: Mean and 95th percentile dietary exposure to zearalenone (ng/kg body weight (b.w.) per day) for average and high consumers (95th percentile) in upper-bound (UB) scenario.

Dietary survey ^(a)	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95
BE/1							31	63	25	54	18	39	16	35
BE/2			51	86 ^(b)	46	83								
BG	87	217	83	134	75	12								
CY							20	42						
CZ					56	10	42	76	24	45				
DK					38	59	23	43	17	29	15	25	15	28 ^(b)
FI/1			56	130	46	81								
FI/2									15	30	13	25		
FI/3					37	68								
FR					48	90	27	55	18	35	16	31	15	30
DE/1			100	241	42	74								
DE/2			89	229	42	73								
DE/3			99	277	42	80								
DE/4							17	38	15	33	13	30	12	26
GR					32	66								
HU									29	49	26	42	29	47
IE									24	44				
IT	88	268 ^(b)	83	143 ^(b)	61	105	37	66	25	43	22	38	23	39
LV					29	68	21	49	14	33				
NL/1									19	39				
NL/2			57	104	50	89								
ES/1									18	35				
ES/2							22	50	17	32				
ES/3					51	95	34	61						
ES/4			62	210 ^(b)	44	75	31	66						

Table B2: Continued.

Dietary survey ^(a)	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95	Average	P95
SE/1									15	28				
SE/2					36	77	24	49						
UK									22	43				

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 9.

(b): If the number of subjects < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b).

C. STATISTICAL ANALYSIS OF THE IMPACT ON DIETARY EXPOSURE OF INCREASING THE MAXIMUM LEVEL (ML) FOR ZEARALENONE IN BREAKFAST CEREALS

The change in the average occurrence level of zearalenone in breakfast cereals due to an increase in the maximum level (ML) was evaluated against simulated samples (occurrence scenarios) that were generated applying a random sampling from statistical distributions.

The basic assumption under which hypothetical occurrence samples are simulated is that, by changing the ML, the range will change according to the increased ML, but the shape of the distribution of occurrence data will remain the same. The type of distribution and implicitly its shape is determined by the behaviour of breakfast cereal producers and represents the main limitation of the evaluation.

The assumption that the type of distribution will not change requires knowledge of the statistical distribution that best fits the original occurrence data set. The occurrence of zearalenone in breakfast cereals (UB) is described by 1,377 samples included in the assessment (see Section 4.2.4). Distribution fitting was tested over 55 statistical distributions and the results were ranked according to the Kolmogorov-Smirnov test, which does not show a robust fitting for any of the 55 distributions. However, the best fitting distribution in rank is a Lognormal distribution with $\sigma = 1.262$ and $\mu = 0.984$ (Figure C1).

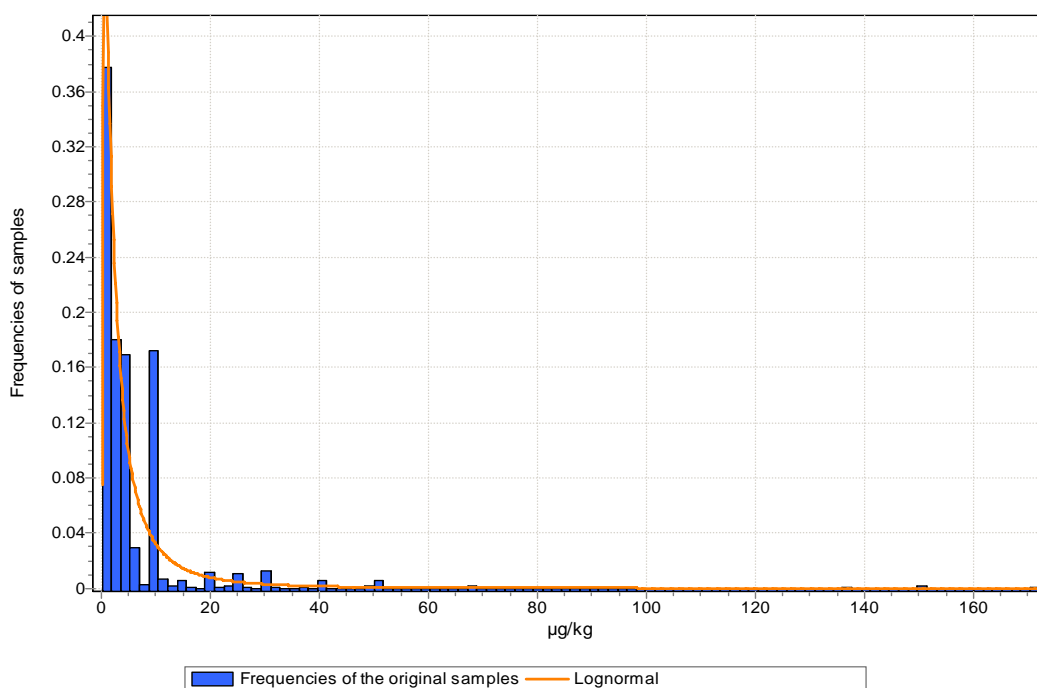


Figure C1: Occurrence of zearalenone in breakfast cereals (range upper-bound (UB) 0.05-172 µg/kg). Distribution is best fitted by a lognormal distribution with $\sigma = 1.262$ and $\mu = 0.984$.

The simulated data sets were generated applying a sequence of random samplings from a lognormal distribution with the same standard deviation as the original data and an average that changes at any sequence. The average increases up to a limit defined by the constraint that the percentage of data exceeding the new threshold should be maintained as close as possible to the percentage of exceedances calculated in the original samples. The constraint is respected when the difference among the two limits is below 1 %.

C1. Simulation assuming a change in the ML from 50 µg/kg to 75 µg/kg

Randomly sampled data are distributed as a lognormal with a constant standard deviation ($\sigma = 1.262$) and the average changing at each simulation step. After 1000 simulations, only a subset of these were in line with the constraint (difference of the percentiles < 1 %). The maximum of the acceptable averages was 1.67 (1.8 times the original average when the potential maximum level is 1.5 times the current one). The average of the acceptable averages was 1.40 (1.51 times the original average) (Figure C2).

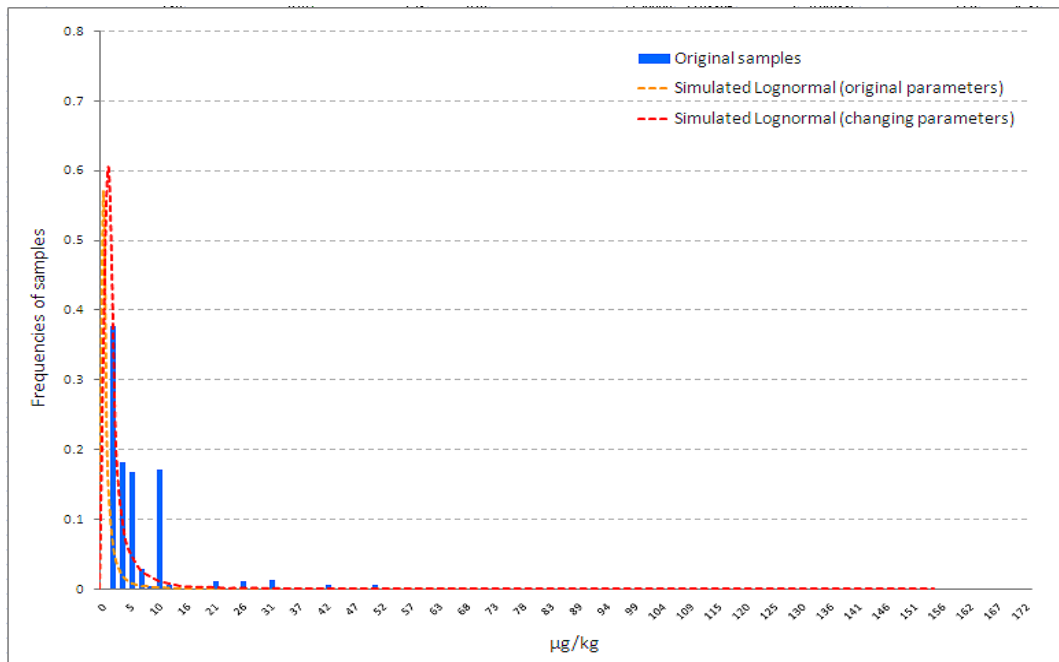


Figure C2: Frequency distribution of the original data set and simulated lognormal distributions. The random simulated lognormal has $\sigma = 1.262$ and $\mu = 1.67$.

C2: Simulation assuming a change in the ML from 50 µg/kg to 100 µg/kg

Randomly sampled data are distributed as a lognormal with a constant standard deviation ($\sigma = 1.2615$) and the average changing at each simulation step. After 1000 simulations, only a subset of these were in line with the constraint (difference of the percentiles < 1 %). The maximum of the acceptable averages was 1.97 (2.1 times the original average when the potential ML is 2 times the current one). The average of the acceptable averages was 1.53 (1.64 times the original average) (Figure C3).

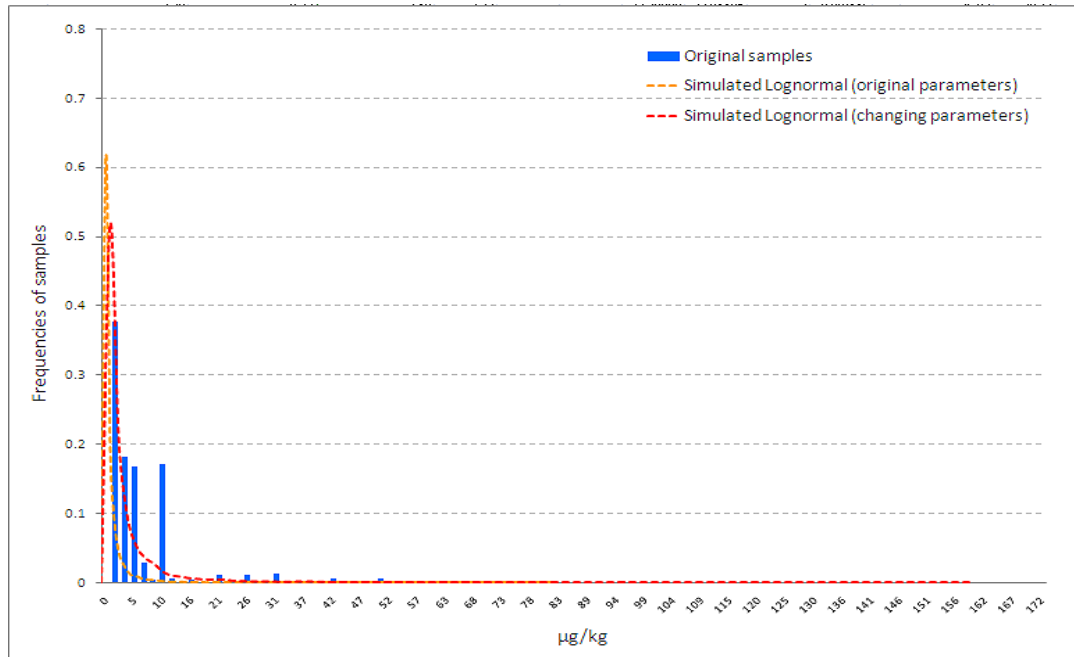


Figure C3: Frequency distribution of the original data set and simulated lognormal distributions. The random simulated lognormal has $\sigma = 1.262$ and $\mu = 1.97$.

C3. Simulation assuming a change in the ML from 50 µg/kg to 125 µg/kg

Randomly sampled data are distributed as a lognormal with a constant standard deviation ($\sigma = 1.262$) and the average changing at each simulation step. After 1000 simulations, only a subset of these were in line with the constraint (difference of the percentiles < 1 %). The maximum of the acceptable averages was 2.16 (2.3 times the original average when the potential ML is 2.5 times the current one). The average of the acceptable averages was 1.67 (1.8 times the original average) (Figure C4).

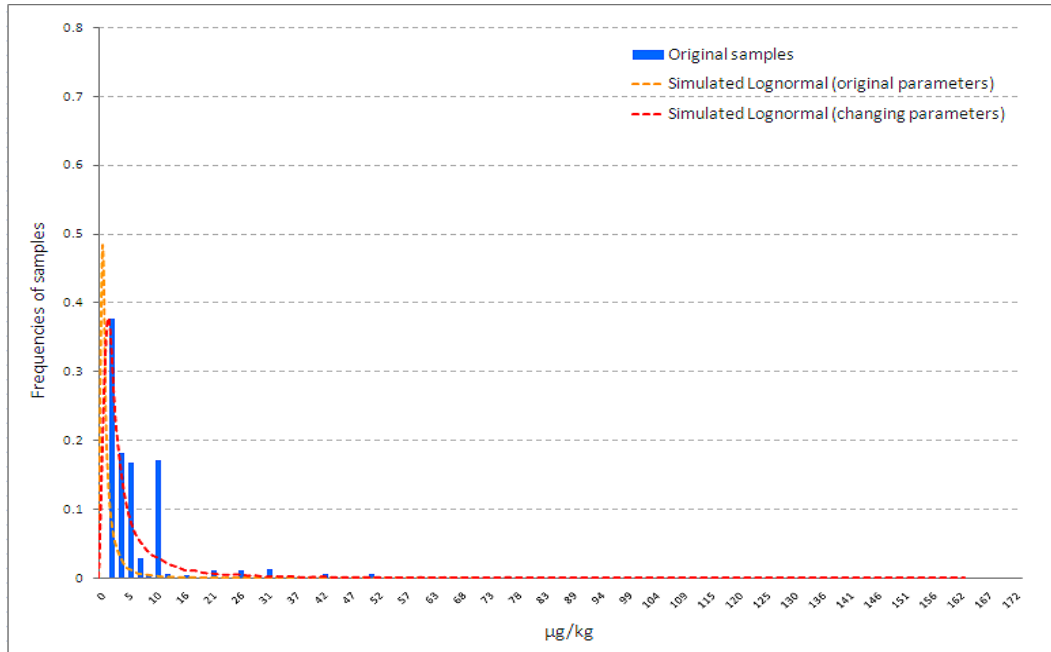


Figure C4: Frequency distribution of the original data set and simulated lognormal distributions. The random simulated lognormal has $\sigma = 1.262$ and $\mu = 2.16$.

C4. Simulation assuming a change in the ML from 50 µg/kg to 150 µg/kg

Randomly sampled data are distributed as a lognormal with a constant standard deviation ($\sigma = 1.262$) and the average changing at each simulation step. After 1000 simulations, only a subset of these were in line with the constraint (difference of the percentiles < 1 %). The maximum of the acceptable averages was 2.36 (2.5 times the original average when the potential ML is 3 times the current one). The average of the acceptable averages was 1.78 (1.9 times the original average) (Figure C5).

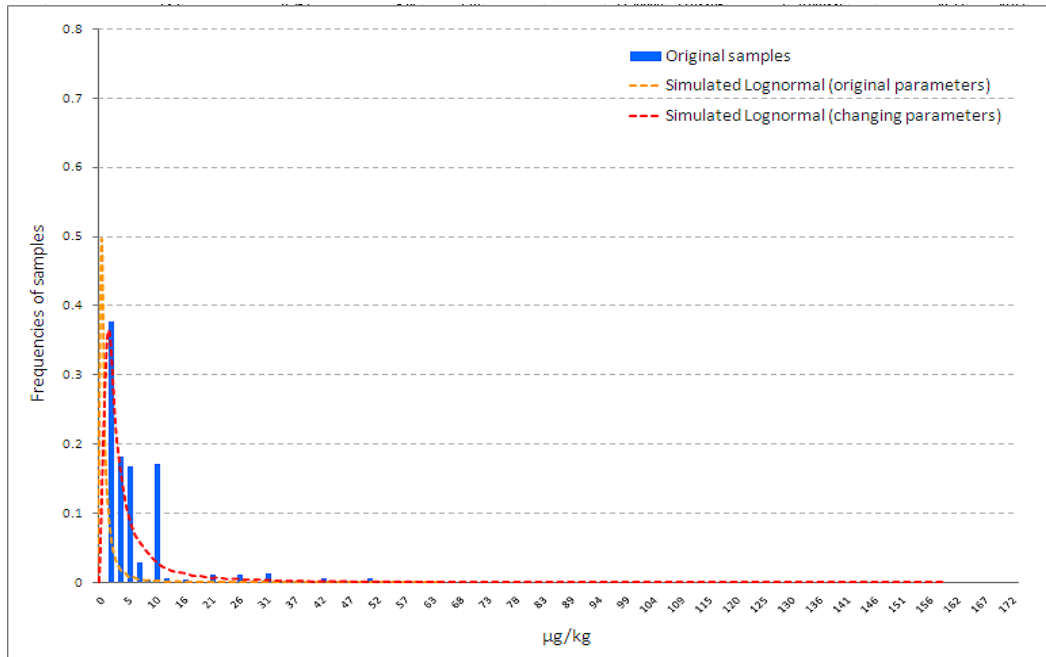


Figure C5: Frequency distribution of the original data set and simulated lognormal distributions. The random simulated lognormal has $\sigma = 1.262$ and $\mu = 2.36$.

In conclusion, changing the ML for zearalenone in breakfast cereals from 50 $\mu\text{g}/\text{kg}$ to 75, 100, 125 or 150 $\mu\text{g}/\text{kg}$ would most likely lead to an increase in the mean concentration of zearalenone by a factor of 1.51, 1.64, 1.8 or 1.9 with a maximum factor of 1.8, 2.1, 2.3 and 2.5.

D. DOSE RESPONSE MODELLING

The incidence of pituitary adenomas in male B6C3F1 mice (NTP, 1982) were selected for modelling of the benchmark dose (BMD) for a 10 % extra risk of pituitary adenomas. Zearalenone was administered in the diet resulting in doses of approximately 0, 8 and 17 mg/kg b.w. per day. The incidence of pituitary adenomas was 0/40, 4/45 and 6/44 (in Section 7.2.3.1. expressed as 0, 9 and 14 %, respectively).

All quantal dose response models in the US EPA's benchmark dose software²¹ (BMDS) 2.1.2 were used, using both unrestricted and restricted options where provided (Table D1). Acceptability of a model was assessed using the log-likelihood value associated with the fitted model (when tested vs the full model). When unrestricted, the Log-Logistic, LogProbit and Weibull models all resulted in a computation failure with the 95 % lower confidence limit of the benchmark dose (BMDL₁₀) including zero. The Gamma model did not give an acceptable fit when unrestricted. The results of running the Multistage model unrestricted resulted in an error in computing chi-square, a p-value of 2, and error messages regarding the BMDL₁₀. Therefore the results of this model were also considered not acceptable.

The remaining models all provided acceptable fits with BMD₁₀ values ranging from 10.95-14.67 mg/kg b.w. per day, and BMDL₁₀ values ranging from 6.39-11.32 mg/kg b.w. per day. The results of the Log-Logistic model, which provided the lowest BMDL₁₀ of 6.39 mg/kg b.w. per day are shown in Figure D1.

Table D1: Results of dose-response modeling for pituitary adenomas in male B6C3F1 mice.

Model	Number of parameters	Log-likelihood	Accepted p > 0.05	BMD ₁₀ (mg/kg b.w. per day)	BMDL ₁₀ (mg/kg b.w. per day)
Reduced	1	-35.17	No	-	-
Full	3	-31.02	-	-	-
Gamma unrestricted	2	-31.02	No	9.83	3.68e-016
Gamma restricted	1	-31.13	Yes	10.95	6.78
Logistic	2	-32.34	Yes	14.67	11.32
Log-Logistic unrestricted	2	-31.02	No	-	Failed
Log-Logistic restricted	1	-31.10	Yes	10.77	6.39
LogProbit unrestricted	2	-31.02	No	-	Failed
LogProbit restricted	1	-32.00	Yes	11.54	8.67
Multistage unrestricted	2	-32.00	> 1, no	9.45	3.26
Multistage restricted	1	-31.13	Yes	10.95	6.78
Probit	2	-32.20	Yes	14.15	10.72
Weibull unrestricted	2	-31.02	1	-	Failed
Weibull restricted	1	-31.13	Yes	10.95	6.78
Quantal Linear	1	-31.13	Yes	10.95	6.78

b.w.: body weight; BMD: benchmark dose; BMDL₁₀: 95 % lower confidence limit of the benchmark dose with 10 % extra risk.

²¹ <http://www.epa.gov/ncea/bmds/about.html>

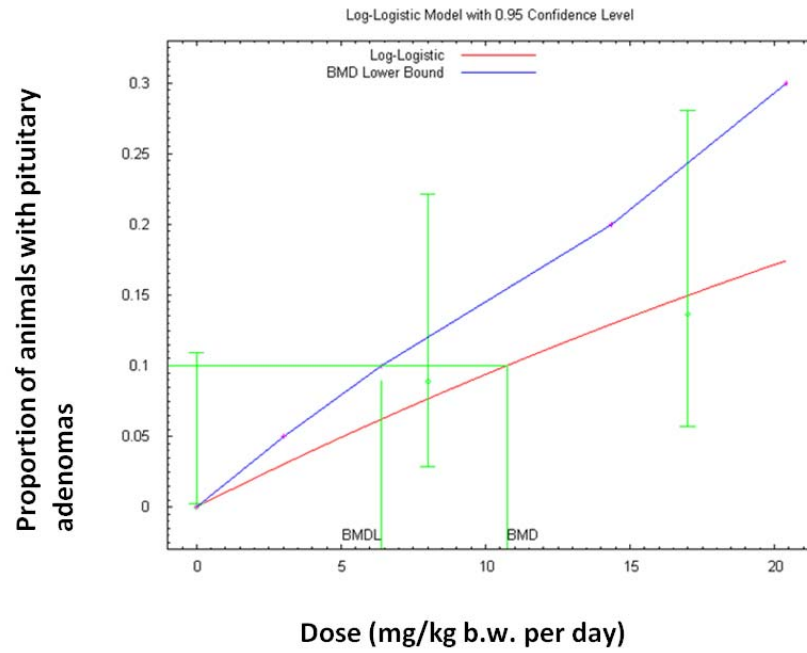


Figure D1: Illustration of dose-response modeling for pituitary adenomas in male B6C3F1 mice using the restricted Log-Logistic model.

ABBREVIATIONS

3 β -HSD-1	3 β -hydroxysteroid dehydrogenase/isomerase
ABC	ATP-binding cassette
ADP	Adenosine diphosphate
ADI	Acceptable Daily Intake
AESAN	Spanish Food and Drink Industry Federation
AESAN_FIAB	Spanish Food and Drink Industry Federation – Spanish dietary survey
AhR	Aryl hydrocarbon receptor
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the time-concentration curve
b.w.	Body weight
BE	Belgium
BG	Bulgaria
BMD	Benchmark dose
BMDL	The 95 % lower confidence limit of the benchmark dose
BMDS	Benchmark dose software
BMR	Benchmark response
CA	Chromosome aberration
Ca ²⁺	Calcium ion
CAR	Constitutive androstane receptor
CAS	Chemical Abstracts Service
CC α	Decision limits
CC β	Detection capabilities
CEEREAL	The European Breakfast Cereal Association
CEN	European Committee for Standardization
Childhealth	Childhealth (Cyprus, Dietary survey)
CHO	Chinese hamster ovary
CN	Combined Nomenclature
COC	Cumulus-oocyte complex
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
CY	Cyprus
CYP	Cytochrome
CZ	Czech Republic
Danish_Dietary_Survey	Danish Dietary Survey
DATEX	Data Collection and Exposure Unit (EFSA), currently DCM Unit (EFSA)
DE	Germany
Diet_National_2004	Diet_National_2004 (Belgium, Dietary survey)
DIPP	DIPP (Finland, Dietary survey)
DK	Denmark
DNFCS_2003	Dutch National Food Consumption Survey
DONALD_2006	DONALD 2006 (Germany, Dietary survey)
DONALD_2007	DONALD_2007 (Germany, Dietary survey)
DONALD_2008	DONALD_2008 (Germany, Dietary survey)
EC	European Commission
EC ₅₀	Effective concentration
EFSA	European Food Safety Authority
EFSA_TEST	EFSA_TEST (Latvia, Dietary survey)
ELISA	Enzyme-linked immunosorbent assay
enKid	Food preferences of Spanish children and young people

ER	Oestrogen receptor
ES	Spain
ESI	Electrospray ionization
EU	European Union
EXPOCHI	Article 36 project “Individual food consumption data and exposure assessment studies for children”
FAO	Food and Agriculture Organization of the United Nations
FAPAS	Food Analysis Performance Assessment Scheme
FI	Finland
FINDIET_2007	FINDIET 2007 (Finland, Dietary survey)
Regional Flanders	Regional Flanders (Belgium, Dietary survey)
FLD	Fluorescence detection
FR	France
FSH	Follicle stimulating hormone
FSA	Food Standards Agency, UK
GC	Gas chromatography
GD	Gestation day
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GMA	Glucomannan mycotoxin adsorbent
GR	Greece
hGC	Human chorionic gonadotropin
HPLC	High-performance liquid chromatography
HSCAS	Hydrated sodium calcium aluminosilicate
HSD	Hydroxysteroid dehydrogenase
HU	Hungary
IARC	International Agency for Research on Cancer
IE	Ireland
IGF	Insulin-like growth factor
Ig	Immunoglobulin
IL	Interleukin
INCA2	Enquête Individuelle et Nationale sur les Consommations Alimentaires
INRAN_SCAI_2005_06	Italian National Food Consumption Survey
<i>i.p.</i>	Intraperitoneal
IRMM	Institute for Reference Materials and Measurements
IT	Italy
<i>i.v.</i>	Intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LB	Lower bound
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LD	Lactate dehydrogenase
LD ₅₀	Lethal dose
LH	Luteinising hormone
LOD	Limit of detection
LOEL	Lowest-observed-effect-level
LOQ	Limit of quantification
LV	Latvia
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
ML	Maximum level
MS	Mass spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

N	Number of samples/subjects
National_Nutrition_Survey_II	National_Nutrition_Survey_II (Germany, Dietary survey)
National_Repr_Surv	National_Repr_Surv (Hungary, Dietary survey)
NDNS	National Diet and Nutrition Survey (United Kingdom)
NFA	National Food Administration (Sweden, Dietary survey)
NH ₄ Cl	Ammonium chloride
NL	The Netherlands
NOAEL	No-observed- adverse-effect-level
NOEL	No-observed-effect-level
NSIFCS	North/South Ireland Food Consumption Survey
NUT_INK05	NUT_INK05 (Spain, Dietary survey)
NUTRICHILD	Bulgarian dietary survey
NZFSA	New Zealand Food Safety Authority
OECD	Organisation for Economic Co-operation and Development
P450 _{scc}	Cytochrome P450 side chain cleavage enzyme
PARP	poly (ADP-ribose) polymerase
PBMC	Peripheral blood mononuclear cells
PCNA	Proliferating cell nuclear antigen
PMN	Polymorphonuclear
PMSF	Phenylmethyl sulfonyl fluoride
PMTDI	Provisional maximum tolerable daily intake
PND	Postnatal day
PXR	Pregnane X receptor
Regional_Crete	Regional_Crete (Greece, Dietary survey)
Riksmaten_1997_98	Swedish national food survey_1997_98
<i>s.c.</i>	Subcutaneous
SCE	Sister chromatid exchanges
SCF	Scientific Committee on Food
SE	Sweden
StAR	Steroidogenic acute regulatory protein
SULT	Sulfotransferase
t-TDI	Temporary TDI
UB	Upper bound
UGT	Uridinediphosphate- glucuronosyl transferase
TDI	Tolerable daily intake
TRE	Tunisian radish extract
WHO	World Health Organization
ZEA	Zearalenone
ZOL	α -zearalenol