

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

(Question N° EFSA-Q-2003-037)

Adopted on 28 July 2004

SUMMARY

Zearalenone is a mycotoxin produced by several field fungi, including *Fusarium graminearum* and *Fusarium culmorum*. The toxin is common in maize and maize products, but can be found in soybeans and various cereals and grains, and their by-products as well. Moreover, zearalenone seems to occur on grass, hay and straw resulting in additional exposure of animals from roughage and bedding. Co-occurrence with other *Fusarium* toxins, particularly deoxynivalenol, nivalenol, and fumonisins is regularly observed. In domestic animals, like in all mammalian species, zearalenone interacts with oestrogen receptors, resulting in an apparent hyperoestrogenism, including reduced fertility. Female pigs of all age groups are considered to be the most sensitive animal species, but the hormonal effects vary in intensity according to age and reproductive cycle. Ruminants and poultry show a lower responsiveness to zearalenone. However, monitoring of feedingstuffs are needed to improve exposure assessment and dose-response studies are essential to establish safe levels of exposure for zearalenone in feed materials for all individual farm animal species, including minor species such as rabbits and small ruminants. Due to the rapid biotransformation and excretion of zearalenone in animals, secondary human exposure resulting from meat, milk and eggs is expected to be low, contributing only marginally to the daily intake.

Key words: Zearalenone, animal feeds, toxicity, estrogenic effects, tissue accumulation.

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BACKGROUND

1. General Background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed¹ replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition².

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination (“early warning system”) and to take measures to reduce or eliminate the contamination (“pro-active approach”).

In particular the introduction of the principle of non-dilution is an important and far-reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful mean to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)³.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003⁴ provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health section Animal Nutrition that additional detailed risks assessments are necessary to enable a complete review of the provisions in the Annex, including the establishment of maximum levels for undesirable substances currently not listed.

¹ OJ L140, 30.5.2002, p. 10

² OJ L 115, 4.5.1999, p. 32

³ Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)

⁴ Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)

2. Specific Background

Zearalenone is an oestrogenic compound produced by several fungi species, primarily by *Fusarium graminearum* and by *Fusarium culmorum*. These fungi infect grains normally during blooming. zearalenone is usually produced pre-harvest but can also be produced under bad storage conditions.

No maximum levels for zearalenone in animal feed have been established in EU legislation. Some Member States have established national orientation values for the presence of zearalenone in feed⁵.

Maximum levels for zearalenone in foodstuffs are currently under discussion at EU level.

SCAN concluded⁶ that zearalenone has a potent estrogenic effect and consequently causes physiological disturbances and fertility problems in mammals. SCAN recommended therefore that a full risk assessment should be undertaken as a priority.

TERMS OF REFERENCE

The European Commission requests the EFSA to provide a detailed scientific opinion on the presence of zearalenone in animal feed.

This detailed scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of zearalenone for the different animal species of relevance (difference in sensitivity between animal species) above which
 - signs of toxicity can be observed (animal health / impact on animal health) or
 - the level of transfer/carry-over of zearalenone from the feed to the products of animal origin results in unacceptable levels of zearalenone or of its metabolites in the products of animal origin in view of providing a high level of public health protection.
- identification of feed materials which could be considered as sources of contamination by zearalenone and the characterisation, insofar as possible, of the distribution of levels of contamination
- assessment of the contribution of the different identified feed materials as sources of contamination by zearalenone
 - to the overall exposure of the different relevant animal species to zearalenone,
 - to the impact on animal health
 - to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry-over rates⁷.

⁵ Germany and Austria: orientation values established ranging from 50 µg/kg for feedingstuffs for gilts up to 500 µg/kg for dairy cattle.

⁶ Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 7.6. Conclusions and recommendations.

⁷ Importance of the human exposure to zearalenone from foods of animal origin compared to overall human dietary zearalenone exposure can be assessed making use of the information contained in the report on a task on human exposure assessment to zearalenone which has been finalised in July 2003 at EU level within the framework of co-operation by Member States in the scientific examination of questions related to food (EC, 2003) http://europa.eu.int/comm/food/fs/scoop/index_en.html

- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

ASSESSMENT

1. Introduction

Zearalenone is a mycotoxin that can be produced by several field fungi including *Fusarium graminearum* (*Gibberella zeae*), *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. Many countries have gathered data on occurrence of zearalenone in (mainly grain-based) foods. A SCOOP report on *Fusarium* toxins has recently become available, which includes data on zearalenone in foodstuffs in 9 European countries (EC, 2003).

Zearalenone (formerly denoted F2-toxin) is a resorcylic acid lactone chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)- β -resorcylic acid lactone (C₁₈H₂₂O₅, MW: 318.36, CAS 17924-92-4) (see figure 1).

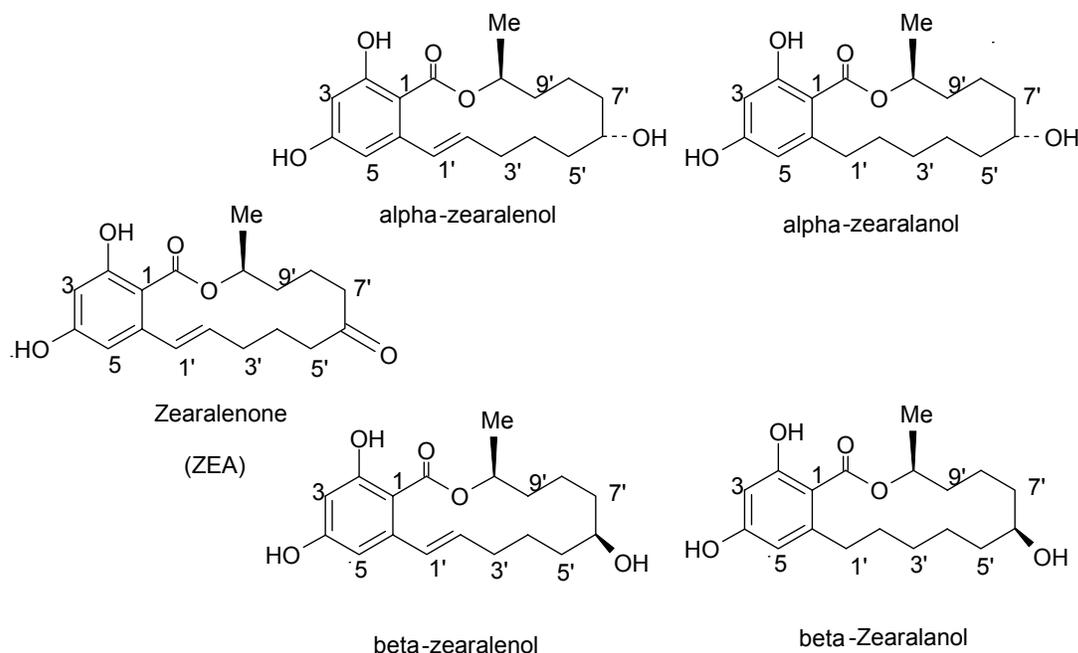


Figure 1. Chemical structure of zearalenone and its major metabolites

Zearalenone is a white, crystalline compound with a melting point of 164 - 165°C. Maximum UV absorption is at 236 nm. Zearalenone is insoluble in water, but soluble in aqueous alkali and various organic solvents. Zearalenone is a stable compound, both during storage, milling, processing, and cooking of food, and it does not degrade at high temperatures.

The structure of zearalenone allows its binding to mammalian oestrogen receptors. Subsequently, zearalenone induces oestrogenic effects in mammals and interferes with conception, ovulation, implantation, foetal development and viability of newborn animals (Kuiper-Goodman *et al.* 1987; IARC, 1993; Kennedy *et al.*, 1998). Zearalenone causes alterations in the reproductive tract of laboratory animals (mice, rats, guinea pigs) and farm

animals. Decreased fertility, increased number of resorptions, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and change in serum levels of progesterone and oestradiol have been observed, but no teratogenic effects were found. Occurrence in mixed feeds associated with hyperoestrogenism has been reported in farm animals, particularly in pigs (Kuiper-Goodman *et al.* 1987).

Zearalenone has been implicated as causative agents in epidemics of premature thelarche in girls in Puerto Rico between 1978 and 1981 (SCF 2000) and an increased incidence of early thelarche has also been reported from South-eastern Hungary (Szuetz *et al.*, 1997). However, adequate information on exposures is lacking.

Zearalenone was evaluated by the International Agency for Research on Cancer. Based on inadequate evidence in humans and limited evidence in experimental animals, zearalenone was placed, together with other *Fusarium* toxins, in Group 3 (not classifiable as to their carcinogenicity to humans) (IARC, 1993).

Risk assessments of zearalenone have been performed by the EC Scientific Committee on Food (SCF, 2000), the FAO/WHO Joint Expert Committee on Food Additives (WHO, 2000) and a Nordic Working Group (Eriksen and Alexander, 1998). A temporary tolerable daily intake (t-TDI) of 0.2 µg/kg body weight was established by the SCF, whereas the provisional maximum tolerable daily intake (PMTDI) established by JECFA was 0.5 µg/kg body weight.

2. Analytical methods

Various methods of analysis exist for the monitoring of the presence of zearalenone in food and feed commodities, all allowing to detect zearalenone with sufficient sensitivity (for review see Krska and Josephs, 2001). Most quantitative methods applied for determination of zearalenone include immunoaffinity (IA) cleanup in combination with liquid chromatography (LC) followed by fluorescence (FLD) or mass spectrometric (MS) detection. Other methods include techniques as gas chromatography (GC) with flame ionisation (FID) or MS detection, whereas for screening purposes thin layer chromatographic (TLC) procedures and enzyme-linked immunosorbent assays (ELISA) are available. Some methods have been validated through AOAC collaborative studies for maize (TLC, LC) and maize, wheat and feed (ELISA) (AOAC, 2000). Chromatography-based interlaboratory-validated methods for animal feedingstuffs are not yet available through AOAC and they have not been standardized yet by the European Standardization Committee (CEN).

Certified reference materials and a certified calibrant for zearalenone have become available in 2003. They consist of naturally contaminated maize flour and blank maize flour. Through adequate mixing at certain ratios, materials for analytical quality assurance can be obtained at other desired levels. This certified reference calibrant is a solution of zearalenone in acetonitrile. The certified reference materials are available through the European Commission's Joint Research Centre/Institute for Reference Materials and Measurements (see <http://www.irmm.jrc.be>). The Food Analysis Performance Assessment Scheme (FAPAS®) organises proficiency tests for zearalenone twice yearly. Recent FAPAS studies (FAPAS, 2000; FAPAS, 2002; FAPAS, 2003) indicate that LC-FLD is the preferred analytical technique. ELISA is used to a much lesser extent, and GC and TLC are also used. The studies showed that satisfactory scores for the participants ranged from 72 % to 82 % for various wheat and maize test materials, contaminated at zearalenone levels with assigned values ranging from 112 µg/kg to 285 µg/kg. Comparable proficiency tests have not yet been conducted for the analysis of zearalenone in feedingstuffs.

3. Current legislation

In 1996 zearalenone was only regulated in 6 countries (FAO, 1997), but the toxin has gained increasing interest amongst regulatory authorities during the last years. By 2003, approximately 16 countries around the world had set regulatory or guideline levels for zearalenone in food and animal feeds (FAO, 2004). Limits for zearalenone in maize and other cereals, ranging from 50-1000 µg/kg, have been set in several countries in Europe, Asia, Africa and Latin America (FAO, 2004). Most limits have been set at the higher end of this range. *Codex Alimentarius* is also in the process of developing maximum levels for zearalenone in cereals and cereal products, designated for human consumption, including infant foods.

Specific limits for zearalenone in animal feedingstuffs have not been proposed yet in the EU, but worldwide they have been established in 13 countries, among which are 5 EU Member States (Austria, Cyprus, Estonia, Lithuania, and Slovenia), where limits exist for zearalenone in feedingstuffs for pigs and incidentally for cattle (FAO, 2004).

4. Occurrence of zearalenone in feed commodities in Europe

Zearalenone is almost exclusively associated with cereals (particularly maize), and the levels of occurrence are of the order of tens to hundreds of µg/kg upwards. Zearalenone occurs as a field (pre-harvest rather than a storage) contaminant and almost always co-occurs with other *Fusarium* toxins such as deoxynivalenol. As seasonal variations significantly influence the extent of *Fusarium* infections, levels of zearalenone tend to vary from year-to-year, making it difficult to generalise as to typical levels of occurrence. Studies of wheat and maize, infected with *Fusarium graminearum* in the field, indicate that maize is invariably contaminated with both deoxynivalenol and zearalenone whereas wheat can have high levels of deoxynivalenol but usually little or no zearalenone.

An extensive compilation of occurrence data for zearalenone worldwide prior to 1987 is included in the risk assessment paper by Kuiper-Goodman *et al.* (1987), and an overview of data from 19 countries has been published by Tanaka *et al.* (1988). These papers indicate the occurrence of zearalenone in wheat, barley, maize, oats, rye, sorghum and rice with samples originating from Argentina, Canada, China, Germany, Italy, Japan, Korea, India, Poland and UK. Surveillance of Canadian grain samples for the presence of zearalenone over the period 1978-1993 found that of 919 samples analysed the highest incidence (69 %) and the highest level (647 µg/kg) was in maize (Scott, 1997). A more recent review of the worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins has tabulated the findings of zearalenone in wheat, oats, barley, rye and feeds in Bulgaria, Finland, Germany, Netherlands, Norway and Poland, at levels ranging from a few µg/kg to 8000 µg/kg (Placinta *et al.*, 1999). The highest reported levels in European cereals were in wheat from Germany, although worldwide levels of zearalenone as high as 10,500 µg/kg were reported in maize from New Zealand. In most cases deoxynivalenol and nivalenol were found to co-occur with zearalenone. The most recent compilation of data for zearalenone in cereals can be found in the SCOOP Report on *Fusarium* toxins, and some of these data are presented in Annex 1 (EC, 2003). As the SCOOP report primarily focussed on assessing human exposure to zearalenone and other toxins, inevitably the analysis centred on grain and grain products presumably destined for human food. Table 1 of the Annex therefore takes only data on unprocessed grains, presuming that - as none of the samples are specifically indicated as being intended for animal feeds - this compilation represents a 'best case' situation. If surveillance had been focussed on poorer quality grain that is frequently diverted to animal feeds, a higher incidence and higher levels of zearalenone would undoubtedly have been found. This table indicates a general picture of relatively low incidence of zearalenone in wheat, oats, rye and barley but frequent and sometimes very high levels of zearalenone in samples of maize. The only exception seems to be

high incidence of zearalenone in oats from Finland (47 % of samples containing > 200 µg/kg with a maximum level of 1310 µg/kg being reported) and high levels of zearalenone in wheat from France (16 % of samples containing > 200 µg/kg with a maximum level of 1817 µg/kg being reported). For maize a total of 740 samples from five countries indicates an incidence of 14 % of samples containing > 200 µg/kg the highest level reported being 6492 µg/kg in a maize sample from Italy.

Surveys carried out in Hungary were specifically targeted towards cereals intended for use as animal feeds (Rafai *et al.*, 2000). Between 1991 and 1998 analyses of maize, wheat, barley, oats, triticale, rye, bran, soybean and sunflower were carried out (1681 samples in total). These data cannot be compared directly with the SCOOP results as in this case ranges of levels found and % incidence are reported, but no detailed numbers of samples within specified ranges of zearalenone contamination are provided. For maize, wheat and barley zearalenone contamination were reported to range between 60 - 1350, 50 - 890 and 50 - 840 µg/kg, respectively. This paper also reports zearalenone in bran (50 - 1560 µg/kg), soybean (50 - 520 µg/kg), rye (80 - 520 µg/kg) and oats (50 - 290 µg/kg) intended for animal feeds. The occurrence of zearalenone in soybean meal has been confirmed in Germany with zearalenone found at levels up to 363 µg/kg. Mycotoxin surveillance in the UK focussed on maize and maize products as ingredients of animal feeds (Scudamore *et al.*, 1998). Out of 8 samples of corn gluten, 6 samples (75 %) contained zearalenone at levels between 100 - 480 µg/kg. In 27 other maize products, zearalenone levels above 500 µg/kg (with the highest level of 1800 µg/kg) were found in 8 samples (30 %). A more recent survey of 140 samples of maize imported into the UK showed zearalenone and fumonisins in almost every sample with 42 % containing more than 100 µg/kg of zearalenone and 48 % containing more than 1000 µg/kg of total fumonisins (Scudamore and Patel, 2000). These samples were not specifically destined for animal feed, and following processing elevated concentrations of the toxins in animal feed materials, such as maize bran, germ and gluten could be anticipated. In another investigation of 330 samples of UK animal feed zearalenone was found in one sample of rice bran (44 µg/kg) but was not found in cottonseed (21 samples), sunflower (20 samples), palm products (15 samples), soy bean products (20 samples) or dried peas and beans (15 samples) (Scudamore *et al.*, 1997). Zearalenone can be found in crude maize oil at levels from a few hundred µg/kg to above 1000 µg/kg. This can be reduced to below 250 µg/kg on refining⁸. Zearalenone has been reported in mustard and linseed cakes from India presumably intended for animal consumption at levels up to 3290 µg/kg (Kaushai *et al.*, 1994).

The data reported on *Fusarium* toxins in cereals is extensive, both from the SCOOP report (EC, 2003) and in the scientific literature, and whilst it is apparent that co-occurrence in individual samples of zearalenone with trichothecenes in wheat, and with aflatoxins, trichothecenes and fumonisins in maize is common, it is not always easy to assess from data in published papers how many toxins and at what levels they were found in specific samples. The available data on maize products in animal feeds indicate that in addition to zearalenone, deoxynivalenol, nivalenol, 15-acetyl-deoxynivalenol, and fumonisins were also found at levels above 500 µg/kg in the same samples, as mentioned above (Scudamore *et al.*, 1998). For example, one sample of corn meal containing 1500 µg/kg of zearalenone also contained 4900 µg/kg of deoxynivalenol, 12,000 µg/kg of fumonisin B1, 1600 µg/kg of nivalenol and 3200 µg/kg of moniliformin. It should be pointed out that although this case is extreme, the sample was not visibly mouldy, and it was a commercial sample being traded as an animal feed material (Scudamore *et al.*, 1998). There are a number of reports on the occurrence of zearalenone in maize silage, corncob mix and forage maize from surveys and case studies, which confirm the high occurrence of zearalenone in maize (Hochsteiner and Schuh 2001; Dieber and Köfer 1999; Oldenburg *et al.* 1996; Oldenburg 1993),

⁸ According to letter from the EC Seed Crushers' and Oil Processors' Federation to the Commission of the European Communities, DG SANCO dated 27th October 2003.

In addition to cereals and mixed feeds, there is some evidence that straw, hay and silage may be occasionally sources of zearalenone exposure. Recently, the occurrence of zearalenone in straw from farms with health problems in sows has been reported (Swedish Veterinary Institute, 2004, unpublished data). Three out of four wheat straw samples contained 92 - 2840 µg zearalenone per kg, and were also contaminated with high amounts of DON (250 - 6600 µg/kg).

Zearalenone is common in pasture grass in New Zealand and is causing fertility problems in sheep (Towers 1993). Zearalenone occurrence in grass and pasture in Europe can also be expected but very few studies have been performed. There is, however, one report by Engels and Krämer (1996) on the occurrence of zearalenone in ryegrass. They reported that 67 % of more than 832 samples were positive using an ELISA method and that concentrations were between 40 and 2780 µg/kg dry matter. One percent of the samples were above 1000 µg/kg. Golinski (2003) reported the occurrence of zearalenone in pasture from Poland in 50 - 65 % of pasture samples from three locations. The concentrations reported were between 2 and 12 µg/kg dry matter.

There are only a few reports on the occurrence of zearalenone in grass and grass-clover silage, particularly relating to aerated silage in big bales or towers. Benham (1981) reported the occurrence of zearalenone in 4 of 211 silage samples from UK, and Lacey (1991) mentioned the potential risk, but did not provide any occurrence data. The occurrence of zearalenone in grass silage is probably much lower and of less importance as compared to maize silage.

The occurrence of zearalenone in hay has been reported incidentally (Mirocha *et al.* 1968; Benham 1981, Drochner *et al.* 1984). Mirocha *et al.* (1968) found zearalenone at a concentration of 14,000 µg/kg in hay in a case of infertility in cattle in England. Benham (1981) found zearalenone in 1 out of 74 hay samples from UK in 1980, but no concentrations were reported. Drochner *et al.* (1984) found zearalenone in several hay samples from Germany. There is also one report on the occurrence of DON and zearalenone in hay from USA (Raymond *et al.*, 2000).

In conclusion, available data indicate that maize is the most prominent cereal at risk, in terms of a high incidence and occasional high levels of contamination with zearalenone. In addition, wheat and oats as well as soybean products have been found to be contaminated occasionally. Data on the occurrence of zearalenone in straw, hay, grass and silage are less well documented, but there are indications that this exposure route needs to be considered.

5. Estimating zearalenone intake by farm livestock

In most cases, pigs and poultry are fed with completed (compounded) diets, which are manufactured commercially or prepared at the farm level. These diets consist of 40 - 80 % cereal grains, 0 - 50 % protein concentrates, 0 to 10 % oil and 0 to 10 % minerals, vitamins and other supplements, depending on the region and manufacturer. This indicates that no general formula exists for pig and poultry diets. Straw from bedding is also taken up by the animals (particularly sows) and might be an additional source of exposure.

The cereal fraction of the diets principally contains all type of cereal grains and their by-products, which are cultivated under European conditions, but maize, wheat and barley are incorporated in higher proportions for several reasons. Soybean meal is the most used protein concentrate. Other European protein concentrates such as rape products, sunflower meal, peas, field beans and lupines are used at much lower proportions, but might be of local importance in some cases.

In ruminants, the concentrate portion of the diet is principally composed of the same materials as used for monogastric animals. The amount of the individual feed materials, however, might be different from those normally used in diets for monogastric animals, as for example protein concentrates others than soybean meal might be used. The total amount of concentrate (cereal

grains plus protein concentrates) in diets for dairy cows varies between 0 and 70 %, depending on the milk production levels. The remaining 30 – 100 % of the diet are composed of roughage such as fresh green fodder and/or conserved feedstuffs (silages, hay, straw). Maize and grass silage are the most widely used roughage.

In conclusion, the great variability in diet composition for the major farm animal species precludes the calculation of actual exposure levels based on the occurrence of zearalenone in individual feed materials.

6. Toxicological properties of zearalenone

6.1. Mode of action

Numerous *in vivo* and *in vitro* studies have indicated that zearalenone binds to oestrogen receptors, generating an oestrogen-like response. Following oral exposure, zearalenone is metabolised in various tissues, particularly in the liver, the major metabolites being α -zearalenol and β -zearalenol (Mirocha *et al.*, 1981, Olsen and Kiessling, 1983, Kuiper-Goodman, 1987). The rate of conversion, and the ratio between α : β zearalenol varies, and may account for the recognized species differences in the sensitivity to zearalenone, as α -zearalenol has a higher binding affinity to the oestrogen receptors (Fitzpatrick *et al.*, 1989, Biehl *et al.*, 1993). Following hydroxylation, all metabolites (as well as the parent compound) can be glucuronidated. Again, considerable species differences exist with respect to the glucuronidation capacity. Studies with radio-labelled zearalenone in mice showed that zearalenone and its metabolites are distributed to oestrogen target tissues such as the uterus, interstitial cells of the testes, and ovarian follicles. Some radiolabel was also found in adipose tissues, indicating that deposition in fat may occur.

Zearalenone passively crosses the cell membrane and binds to the cytosolic oestrogen receptor. The receptor-zearalenone complex is rapidly transferred to the nucleus, where it binds to oestrogen-responsive elements, thereby activating gene transcription (Riley, 1998). The binding affinity of zearalenone to ER in target tissues and cells is 1 – 10 % of that of 17- β -oestradiol, whereas α -zearalenol showed a higher binding affinity. In turn, β -zearalenol shows a significantly lower binding affinity than zearalenone. In a comparison of the potency of zearalenone and 17- β -oestradiol in MCF-7 cells the relative response of zearalenone was 2.5 - 5 % (Mayr *et al.*, 1992). The binding to and activation of ER- α and ER- β by zearalenone have also been examined in cells transfected with human recombinant ER- α and ER- β complementary DNA in the presence of an oestrogen-dependent reporter plasmid. In the presence of 17- β -oestradiol, zearalenone was found to be a competitive agonist for ER- α , and a mixed agonist-antagonist for ER- β (Kuiper *et al.*, 1998). The relative potency of zearalenone compared to 17- β -oestradiol and diethylstilboestrol in the uterotrophic assay after subcutaneous or oral administration was about 0.001, whereas the potency relative to that of 17- β -oestradiol in the vaginal cornification assay was 0.001 after subcutaneous injection, and 0.01 after topical administration. α -Zearalanol had about the same potency in this assay but is usually several times more active in the uterotrophic assay. *In vitro* experiments conducted with porcine endometrial cells confirmed the binding of α -zearalanol to cytoplasmic oestrogen receptors (Tiemann *et al.*, 2003). The relative binding affinity to the rat uterine cytoplasmic ER receptors can be ranked in the following order: α -zearalanol > α -zearalenol > β -zearalanol > β -zearalenol (Erikson and Alexander, 1998). Finally, β -zearalenol (as well as DON), but not α -zearalenol, was found to impair the cell cycle, arresting cells in the G₀/G₁ phase (Tiemann *et al.*, 2003).

6.2. Toxicological studies

Zearalenone has low acute toxicity after oral administration, with oral LD50 values of > 4000 mg/kg b.w. (Kuiper-Goodman *et al.* 1987). Although high concentrations of zearalenone can alter several immunological parameters *in vitro* (Eriksen and Alexander 1998, WHO, 2000) no convincing immunotoxic potency have been reported following *in vivo* administration of zearalenone to mice (Pestka *et al.* 1987, Pung *et al.* 1984, Forsell *et al.* 1986).

In B6C3F1 mice, the effects produced by high doses of zearalenone in a 13-week study were atrophy of the seminal vesicles and testes, cytoplasmic vacuolization of the adrenals, and squamous metaplasia of the prostate in male mice and endometrial hyperplasia of the uterus in females. Osteoporosis and myelofibrosis of the bone marrow was observed in animals of each sex (NTP 1982). In a long-term carcinogenicity study in B6C3F1 mice fed diets containing 0, 50 or 100 mg zearalenone per kg feed for 103 weeks (corresponding to 0, 8, 17 mg/kg b.w./day in males and 0, 9, 18 mg/kg b.w. in females) hepatocellular adenomas were found in 8, 6, and 14 % of the males, and 0, 4 and 14 % of the females, respectively. A statistically significant trend in the incidence of pituitary adenomas was observed in both males (0, 9, and 14 %) and females (7, 5, and 31 %). However, the incidences of pituitary carcinomas in treated and control mice were not different (NTP 1982).

In a 13-week feeding study in Fischer 344/N rats, high doses of zearalenone induced hyperplasia and inflammation of the prostate and testicular atrophy in males and endometrial hyperplasia of the uterus in females. Ductular hyperplasia of the mammary gland and hyperplasia of the pituitary was observed in animals of each sex. Osteoporosis was observed in males (NTP 1982). Indications of hepatic toxicity and impairment of blood coagulation processes by zearalenone administration have also been observed in the rat (NTP 1982, Maaroufi *et al.* 1996). In one long-term carcinogenicity study, where Fischer 344/N rats were fed 0, 1 or 2 mg zearalenone/kg b.w./day for 103 weeks the mean body-weight gain of treated rats was lower than that of controls. No treatment-related increase in tumour incidence was found (NTP 1982). Similarly, in another long-term carcinogenicity study, where FDRL Wistar rats were fed 0, 0.1, 1 or 3 mg zearalenone/kg b.w./day for 104 weeks no treatment-related increase in tumour incidence was found. The NOEL deduced from this study was 0.1 mg zearalenone/kg b.w./day (Becci *et al.*, 1982a).

In a combined two-generation reproduction and teratogenicity study in rats (daily dietary zearalenone doses of 0, 0.1, 1, or 10 mg/kg b.w.) zearalenone reduced the number of liveborn F1 pups per litter only at the highest dose while the doses of 1 and 10 mg/kg body weight/day reduced the number of liveborn F2 pups per litter. Fertility was significantly decreased at the highest dose in both the F1 and F2 generations. No teratogenic effect was observed and zearalenone had no effect on the rate of survival of liveborn pups (Becci *et al.*, 1982b). Similarly, no teratogenic effects were observed in three experiments in guinea-pigs given oral doses of zearalenone (Long & Diekman, 1989). In male rats, apoptosis of germ cells was described (Kim *et al.*, 2003)

In vitro tests of zearalenone for gene mutations were generally negative whereas test for chromosomal aberration and sister chromatid exchange have provided equivocal results (WHO, 2000). DNA adducts have been detected by 32P-postlabelling in liver, kidney and ovary of female mice, but not in organs from male and female rats given zearalenone (Li *et al.*, 1992; Pfohl-Leszkowicz *et al.*, 1995; Grosse *et al.*, 1997). Recently, DNA fragmentation and apoptotic bodies have been described to occur in different cell lines, following exposure to zearalenone. Moreover, micronuclei have been found in bone marrow cells, indicating a clastogenic pathway in the potential genotoxicity of zearalenone (Abid-Essefi *et al.*, 2003; Ouanes *et al.*, 2003). In contrast, zearalenone exerted an anti-apoptotic effect in oestrogen-dependent MCF-7 cells (Ahamed *et al.*, 2001).

In summary, hepatocellular adenomas and pituitary adenomas were observed in long-term studies of carcinogenicity in mice. However, these tumours were observed only at doses greatly

in excess of the concentrations that have hormonal effects, i.e. at levels of 8 - 9 mg/kg b.w./day or more and it is concluded that these tumours are a consequence of the estrogenic effects of zearalenone. In the rat there was no treatment-related increase in the incidence of tumours at doses of 0.1 - 3 mg/kg b.w./day.

7. Adverse effects on livestock

The effects seen in livestock following experimental or feed-related ingestion of zearalenone are dependent on interactions of zearalenone or its metabolites with the oestrogen receptors (ER). The most sensitive adverse effects to be expected are therefore related to hormonal effects on reproductive and developmental parameters.

7.1. Pigs

Various studies have addressed the effects of zearalenone in pigs (see table 2 of the Annex). The source of zearalenone that have been used is either crystalline zearalenone, or highly contaminated zearalenone-containing feedingstuffs. The use of pure, crystalline zearalenone, often in high concentration, will not adequately reflect the normal feeding conditions in agricultural practice, as zearalenone-contaminated feed will be co-contaminated in most cases with several other *Fusarium* toxins, including deoxynivalenol, in varying quantities and compositions. However, when naturally contaminated feedingstuffs are used in experimental approaches, the outcome of the study may reflect the practical situation (co-exposure to various *Fusarium* toxins) but seldom provide the opportunity to identify a no-effect-level (NOEL). Thus, for the studies presented in the Annex, an indication is given whether the tested zearalenone concentrations in the test materials used are likely to be representative of the levels occurring under normal feeding conditions.

The majority of the experiments available with adult or cycling sows have used rather high concentrations of crystalline zearalenone. Initial studies suggested that an effective dietary zearalenone concentration producing clear estrogenic effects would be higher than 1 mg/kg feed (Mirocha and Christensen, 1974). In more recent studies using crystalline zearalenone estrogenic effects have been reported at concentrations ranging from 0.05 to 0.4 mg zearalenone/kg feedingstuff (Bauer *et al.*, 1987; Lusky *et al.*, 1997; Coenen and Boyens, 2001; Obremski *et al.*, 2003). In a study from Edwards *et al.* (1987a), conducted with non-pregnant gilts exposed to 0, 0.04, 0.2 and 0.4 mg/kg b.w. during days 5 - 20 of oestrus, a no-effect level of 0.04 mg/kg b.w./day was discussed.

Several studies have indicated that the female pre-pubertal (growing) pig is particularly sensitive to the estrogenic effects of zearalenone. In an experiment that used naturally contaminated wheat providing a dietary concentration of 0.2 mg zearalenone/kg feed clear toxic effects were reported but interfering effects from a high DON content (2.5 mg DON/kg diet) seemed likely (Jadamus and Schneider, 2002). In a comprehensive and well-conducted study using naturally contaminated maize providing dietary concentrations of zearalenone from 0.01 to 0.42 mg/kg and of DON from 0.2 to 3.9 mg/kg feed clear estrogenic effect (increased swelling of cervix and increased mean relative uterus weight) was observed at the highest dose level of 0.42 mg zearalenone/kg feed. A slight decrease in mean serum FSH concentration in the dosed groups was considered influenced by the co-occurrence of deoxynivalenol (DON) in the feed (Döll *et al.*, 2003a). Although only a few studies have been conducted, boars seem to be quite resistant to the effects of zearalenone (see Annex).

In conclusion, pigs seem to be the most sensitive animals species (followed by sheep as described below) being even more sensitive than rodents (JECFA, 2000, SCF, 2002).

7.2 Sheep

Smith *et al.* (1990) fed ewes with 1.5, 3, 6, 12, or 24 mg zearalenone/animal (0.03, 0.06, 0.11, 0.23, 0.45 mg/kg b.w./day) for 10 days from day 7 in oestrus before mating. It was found that 0.03 mg/kg body weight/day reduced the relative ovulation rate (pre-treatment vs. post-treatment); 0.06 mg/kg body weight/day increased duration of oestrus and increased uterine weight; 0.11 mg/kg body weight/day increased liver and ovarian weights; 0.23 mg/kg body weight/day reduced the incidence of the ovulation and reduced fertilization; 0.45 mg/kg body weight/day did not affect live weight, number of ovulating ewes yielding ova or the number of ewes with ova yielding fertilized ova. When given for 10 days from day 5 after mating, similar dosages did not affect the number of ovulations, the ovulation and conception rates, the incidence of gestation, the number of lambs born and embryo or ova wastage. Feeding of a diet containing 12 mg zearalenone/kg diet to rams did neither influence the volume of ejaculate or semen concentration, nor semen motility or the percentage of semen abnormalities (Milano *et al.* 1991).

7.3 Poultry

Chi *et al.* (1980b) administered crystalline zearalenone, either orally or intramuscularly, at doses of 50, 200, 400 and 800 mg/kg body weight/day for seven days to broilers. A dose-response related increase of the oviduct weights was found but the potency of zearalenone was estimated to be only 1.37 % of that of oestradiol propionate. In another study, the feeding of diets containing 50, 200, 400 and 800 mg of zearalenone/kg to broilers resulted in an increased incidence of ovarian cystic development, but increased oviduct weights were only seen in some birds at the highest zearalenone concentration (Chi *et al.*, 1980a).

Oviduct weights of broilers remained unaffected whereas testes weights were reduced at higher zearalenone concentrations (50, 100, 200, 400 and 800 mg crystalline zearalenone/kg diet). In turkeys, the oviducts and testes were unaffected at dietary zearalenone concentrations of 10, 25, 50, 100, 200, 400 and 800 mg/kg diet (week 4 to 7 of age). Dewlaps and combs showed an increased development and a considerable strutting behaviour were observed at 400 and 800 mg zearalenone/kg diet (Allen *et al.*, 1981b). No negative effects on laying performance and reproductive performance of hens and cocks was found when diets with 10, 25, 50, 100, 200, 400 and 800 mg zearalenone/kg were fed from week 30 to 41 of age (Allen *et al.*, 1981a). A hyper-androgenic response was observed in male turkeys fed a diet with 800 mg crystalline zearalenone/kg diet (Olsen *et al.*, 1986). Maryamma *et al.* (1992) reported cystic alterations of the lamina epithelialis of the oviduct of female Leghorn chicks, hyperactivity of secreting cells of the oviduct, and atrophy of testes in male chicks exposed to 10 mg zearalenone/kg body weight/day for 20 days. Khajarern *et al.* (2003) attributed the reduced testes weights and the increased weight of the oviducts of ducks to the presence of 0.15 mg zearalenone/kg diet. However, other mycotoxins were also present in the diet in this case.

From the experiments conducted with the poultry species, so far poultry can be regarded as quite tolerant to zearalenone as symptoms of hormonal effects were only observed at high zearalenone doses, which will hardly occur under practical feeding conditions.

7.4 Dairy cattle

The effects of zearalenone on heifers and dairy cows have only been examined in a few studies. Two experiments with heifers were reported by Weaver *et al.* (1986a, b) who found the conception rate over 3 oestrus cycles to be decreased when 250 mg crystalline zearalenone (approximately 50 mg/kg diet) were fed per day. No effects on the reproductive organs and no changes in the progesterone concentrations in the blood could be detected when 500 mg crystalline zearalenone was fed per day. Moeser (2001) fed contaminated oats with a dietary concentration of zearalenone at 1.25 mg/kg diet to heifers which were found to be clinically inconspicuous. No deviations in the oestrus cycle and no pathologic and histological alterations

of the reproductive organs were detected. There are several field or case reports where the observed symptoms of oestrogenic effects could not be related to the zearalenone concentrations found, which might reflect the variability in rumen (microflora) degradation of zearalenone. The zearalenone concentrations in these studies ranged between 0.1 and 75 mg/kg diet (Mirocha *et al.*, 1968; Roine *et al.*, 1971; Vanyi *et al.*, 1974; Bloomquist *et al.*, 1982; Schuh, 1981, 1983; Drochner, 1990).

8. Toxicokinetics

Zearalenone is rapidly and extensively absorbed after oral administration (Kuiper-Goodman *et al.*, 1987). In one pig administered a single oral dose of 10 mg zearalenone/kg body weight the uptake was estimated to be 80 – 85 % (Biehl *et al.*, 1993). Zearalenone and its metabolites were found in the plasma of a pig < 30 minutes after the beginning of feeding (Olsen *et al.*, 1991; Biehl, 1993).

The main metabolites of zearalenone are α - and β -zearalenol and the glucuronide conjugates of both, the parent compound and its metabolites. Various tissues including intestinal mucosa and liver tissues are able to metabolise zearalenone (Kuiper-Goodman *et al.*, 1987). Olsen *et al.*, (1987) reported that zearalenone was reduced to α - and β -zearalenol when incubated with homogenized intestinal mucosa samples (duodenum and jejunum) from sows in the presence of NADPH. However, in the presence of UDPGA the rate of glucuronic acid conjugation of zearalenone was about 30-fold higher than that of reduction (Olsen *et al.*, 1987).

Significant differences between species were found in the metabolic profile in urine and faeces. A higher proportion of the administered zearalenone dose was metabolized to α -zearalenol in pigs than in rats or cows. In both humans and pigs, zearalenone was found mainly as glucuronide conjugates of zearalenone and α -zearalenol in urine (Mirocha *et al.*, 1981).

In gilts given zearalenone in feed, the concentrations of α -zearalenol in plasma exceeded those of zearalenone in some studies, while the concentrations of the parent compound exceeded those of α -zearalenol in others (Bauer *et al.*, 1987; Kuiper-Goodman *et al.*, 1987). In some studies, all of the zearalenone detected in pigs was in the form of conjugated metabolites, while free zearalenone was also found in others. A significant fraction of the zearalenone in the urine of rabbits and pigs was in the form of α -zearalenol or its glucuronide conjugate (Kuiper-Goodman *et al.*, 1987; Biehl *et al.*, 1993).

In one prepubertal gilt, fed 192 μ g zearalenone/kg body weight/day for 4 days, the plasma concentration of α -zearalenol was 3 - 4 times higher than that of the parent compound during the treatment. A maximum circulating amount of zearalenone plus α -zearalenol (10.4 ng/ml plasma) was found on the 4th day of treatment followed by an urinary excretion of 305 ng/ml urine. Zearalenone and α -zearalenol in plasma and urine were entirely bound to glucuronic acid (Olsen *et al.*, 1985a). In another experiment, where a pig was fed 80 μ g zearalenone/kg body weight (1 single feeding occasion: 1.03 kg feed containing 5 mg zearalenone/kg) Olsen *et al.* (1985b) also reported that almost all zearalenone and α -zearalenol were found conjugated with glucuronic acid in both blood and urine (only approx. 10 % were found free).

Zearalenone and its metabolites undergo enterohepatic cycling. This was shown by Biehl *et al.*, (1993) in immature pigs given radiolabelled zearalenone, either as a single intravenous dose of 5 mg/kg body weight or a single oral dose of 10 mg/kg body weight. The estimated biological half-life of total radiolabel was 87 hours in the intact pig, whereas it was reduced to 3.3 hours when the bile was removed through a cannula. In the pigs dosed orally, 45 % of the administered dose was recovered in the urine and 22 % was recovered in the faeces during the first 48 hours.

Dänicke *et al.* (2004a) studied the fate of a single intravenous dose of 1 mg zearalenone/kg body weight in a pig. A rapid distribution phase with a half-life of 12 minutes ($t_{1/2\alpha}$) was followed by a slower elimination phase corresponding to a half-life of 2.63 hours ($t_{1/2\beta}$). α -Zearalenol was the only detectable metabolite of zearalenone. The cumulative recovery of zearalenone plus α -zearalenol in urine and duodenal digesta after 72 hours, expressed as percentage of the total zearalenone dose given, was 70 % and 35 %, respectively. Fourteen days after the bolus injection, zearalenone and α -zearalenol concentrations in bile, liver and urine were below the limit of detection.

Further reduction of the C11-C12 double bond leading to α - and β -zearalanol has been demonstrated in sheep. It was suggested that the failure to detect zearalanols in other species might be due to the use of high-performance liquid chromatography with fluorescence detection in those studies. Reduction of the C11 - C12 double bond of zearalenone leads to loss of fluorescence and the method is therefore much less sensitivity for zearalanols than for the fluorescent zearalenols (Miles *et al.*, 1996).

9. Carry-over and residues

Several studies have been conducted to assess the carry-over of zearalenone into edible tissues, milk and eggs. A summary of these studies is presented in Table 3 of the Annex. These data show that there is only limited tissue deposition of zearalenone in meat and other edible tissue, and a low transmission rate into milk and eggs.

In accordance, low levels of zearalenone in cattle and sheep liver and in meat, milk and cheese have been reported. In the UK, for example, zearalenone was detected in 3 percent of conventional retail milk samples at levels ranging from 1.2 to 5.5 $\mu\text{g/L}$. (EC, 2003).

10. Human dietary exposure

Estimates of average dietary intakes of zearalenone based on individual diet records have been presented by FAO, indicating an exposure of 0.03 to 0.06 $\mu\text{g/kg}$ b.w. per day, thus remaining below the PMTDI of 0.5 $\mu\text{g/kg}$ body weight/day set by JECFA and the t-TDI of 0.2 $\mu\text{g/kg}$ body weight/day established by the SCF. Data from the EU SCOOP task (EC, 2003) showed that the mean intake of zearalenone, estimated from various European countries, might range from 1 ng/kg b.w. to 420 ng/kg b.w.. Bread and other cereal product were the most prominent sources of exposure.

Thus, although only few analyses have been performed on residues of zearalenone in animal derived products, the available information indicates that due to rapid metabolism and excretion of zearalenone in farm animals, the contribution of products from animal origin to human dietary exposure to zearalenone is very limited.

CONCLUSIONS

- The mycotoxin zearalenone, produced by different *Fusarium* species, is a frequently found contaminant of maize and maize by-products, but may occur in other cereals and grains as well. Mould invasion and subsequent toxin production occurs mainly at the pre-harvest stage and cannot readily be avoided under the conditions of current agricultural practice.
- The actual toxin concentration in feed materials varies considerably depending on climatic, seasonal and geographic conditions, genetic predisposition of crops, use of fungicides and actual crop cultivation practice. Concentrations ranging from less than 0.05 mg/kg to a few mg/kg feedingstuff have been reported.
- At present, feed materials are not monitored routinely for the presence of zearalenone, and validation studies for feed materials and composed feeds have not been conducted yet. Moreover, within the EU member states feeding regimes for farm animals vary considerably. Thus, a reliable estimate of the common exposure of farm animals to zearalenone cannot be presented.
- The most prominent effects of zearalenone result from its interaction with oestrogen receptors resulting in apparent hyperoestrogenism, including reduced fertility.
- Pigs are generally considered as the most sensitive animal species to zearalenone, and males are less sensitive than females. The outcome of clinical trials in this target animal species, however, vary considerably and seem to reflect differences in the application of the toxin, being administered in experimental studies either in its crystalline form, or via contaminated feed materials. Under practical conditions, these feed materials will be co-contaminated with other *Fusarium* toxins in most cases, which alter the biological effects related to zearalenone exposure and preclude the establishment of no-effect levels for pigs from these studies,
- Susceptibility varies considerably amongst species. Limited experimental studies indicate that next to pigs sheep are rather sensitive to the adverse effects of zearalenone, whilst cattle are less sensitive. Poultry (chicken and turkey) are the least sensitive to the hormonal effects of zearalenone. There are no reliable data from other species (rabbits, horses, cats and dogs).
- There is only limited tissue deposition of zearalenone in meat and other edible tissues, and a low transmission rate into milk and eggs. Subsequently, it can be expected that animal derived foods contribute only marginally to total human exposure, as compared to cereals and grain products.

RECOMMENDATIONS

- More data on occurrence of zearalenone in feed materials (as opposed to cereals intended for human consumption) and bedding are needed to improve exposure assessment for farm animals.
- Sensitive analytical methods for feedingstuffs should be validated by collaborative trials.
- Studies in farm animals should be initiated that allow the establishment of a safe level of zearalenone in feed materials and compounded feeds, particularly for pigs of different age groups, as they are considered to be the most sensitive animal species, followed by dose-effect studies in other (farm) animals.

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ANNEX

Table 1. Collection of data for zearalenone in unprocessed cereals taken from the SCOOP Report (EC, 2003)

Country	Sample type	Survey Year	Total no Samples	Numbers of samples containing ZEA in range µg/kg			
				< 50	50-100	100-200	> 200
Austria	Maize	1996-98	198	153	18	22	5
	oats	1999-00	192	192	0	0	0
Finland	wheat	1998-01	138	138	0	0	0
	barley	1998-01	77	76	1	0	0
	oats	1998-01	68	27	3	6	32
	rye	1998-01	36	36	0	0	0
France	maize	2000-01	330	203	55	54	52
	wheat	1994-02	200	197	2	1	0
Germany	wheat	2000-02	77	76	1	0	0
	barley	2001	18	18	0	0	0
	rye	2000-01	20	20	0	0	0
Netherlands	wheat	2000-01	31	30	1	0	0
	maize	2000	5	5	0	0	0
Italy	wheat	2000-01	53	53	0	0	0
	barley	1999	1	1	0	0	0
	maize	1998-2001	68	24	9	10	25
Norway	wheat	1990-98	346	344	2	0	0
	barley	1990-98	20	20	0	0	0
	oats	1990-98	113	112	1	0	0
	rye	1990-98	28	28	0	0	0
Portugal	wheat	2002	1	1	0	0	0
UK	maize	1998-99	139	43	36	40	20

Table 2. Literature findings on the reproductive toxicity of zearalenone in pigs

Age, weight	Dosage, duration (ZEA-source)	Findings	Realistic feeding conditions? ¹	Reference
Sows				
Adult	2.2 mg/kg diet (0.09 mg/kg b.w./day); exposure over the whole gravidity (naturally contaminated wheat)	No effects on reproductive performance, no signs of hyperoestrogenism; piglets were clinically inconspicuously but had reduced relative pituitary, thyroid and kidney weights and increased relative spleen and spinal cord weights	+	Shreeve <i>et al.</i> , 1978
Adult	25, 50, 10 mg/kg diet (1, 2, 4 mg/kg b.w./day); various exposure lengths	Infertility, pseudogestation, nymphomania, constant oestrus, decreased offspring weight, juvenile hyperoestrogenism	-	Chang <i>et al.</i> , 1979
247 days (±3)	3.6 mg/kg diet (1st experiment), 4.3 mg/kg diet (2nd experiment), puberty until insemination (naturally contaminated maize)	45 % of the sows were apparently gravid	-	Etienne and Jemmali, 1982
247 days (±3)	3.6 mg/kg diet (1st experiment), 4.3 mg/kg diet (2nd experiment), during gravidity (naturally contaminated maize)	Reduction of the weights of uterus, placenta and fetus	-	Etienne and Jemmali, 1982
Adult	7, 38 and 64 mg ZEA/kg diet (0.28, 1.5, 2.6mg/kg b.w./day) and 0.5, 2.5 and 4.5 mg DON/kg diet from 3 to 34 days after breeding (corn culture of <i>Fusarium roseum</i>)	Decreased number of foetuses and decreased average foetal weight, hyperoestrogenism and feed refusal in some of the sows exposed to the highest dosage	-	Long <i>et al.</i> , 1982
~ 210 days (1st oestrus)	0, 2.1, 3.7, 4.8 mg/kg diet, 1st cycle, 2 gravidities and lactations (330- 340 days) (naturally contaminated maize)	No effects on oestrus and duration of gravidity. 4.8 mg/kg: tendency for anoestrus. Number of piglets born and alive 14 days p.p. decreased; rate of death births increased	+	Young <i>et al.</i> , 1982

Age, weight	Dosage, duration (ZEA-source)	Findings	Realistic feeding conditions? ¹	Reference
Adult	107 mg ZEA/kg diet and 8 mg DON/kg diet from days 7 to 17 post oestrus to bred and non-bred sows (corn culture of <i>Fusarium roseum</i>)	Hyperoestrogenism in bred and non-bred sows; non of the bred gilts farrowed	-	Long <i>et al.</i> , 1983
Adult	0, 5, 15, 30, 60, 90 mg/kg diet (1.8 kg diet) day 2 to 15 post mating (crystalline)	No foetuses present 40 d post mating in gilts fed 60 to 90 mg/kg, serum progesterone in these groups decreased at 2, 3 and 6 wk and estradiol-17(decreased at 4 wk post mating); No effect on LH, FSH or PRL secretory pattern	-	Long and Diekman, 1984
Pubertal gilts	3, 6, 9 mg/kg diet (0.12, 0.24, 0,36 mg/kg b.w./day) (throughout gestation)	Decreased breeding and live litters, increased pseudo-gestation, no swollen vulvas or abortions	-	Young and King, 1984
Adult	60 mg/kg diet (1.1 mg/kg b.w./day) on post mating days 2 to 6, 7 to 10 and 11 to 15 (crystalline)	3 out of 4 gilts treated between days 7 and 10 post mating were not pregnant after 30 to 32 days post mating; no relationship between altered serum concentration pattern of LH and FSH and embryonic mortality	-	Long and Diekman, 1986
Adult	1, 5, 10 mg/kg diet, 15 days (5 -20 day of cycle), (crystalline)	No signs of hyperoestrogenism. 5 and 10 mg/kg: cycle duration prolonged	+	Edwards <i>et al.</i> , 1987b
Adult	10 mg/kg diet, 14 days, (14 -28 day of lactation) (crystalline)	Sows and piglets: no hyperoestrogenism; 1st heat delayed (~1 day), no effects on the following gravidity	-	Edwards <i>et al.</i> , 1987a
Adult	1 mg/kg b.w./day, day 7 to 10 post mating (crystalline)	No effect on number of blastocysts or the position in the uterus on d 9 or 11; concentrations of intrauterine cations were altered; no effect on intrauterine concentrations of progesterone and oestradiol	-	Long <i>et al.</i> , 1988
Adult	5 and 10 mg/kg diet without and with 15 % alfalfa hay, diets were fed from day 7 of lactation until 40 days after the last breeding or 40 days post weaning (no oestrus);(Exp. 1: parity 1 sows, exp. 2: parity 2 sows) (crystalline)	No effects on the proportion of sows returning to oestrus; tendency for increasing weaning-to-oestrus intervals; embryonic mortality increased in exp.2; increased numbers of sows which were not pregnant at slaughter (exp. 1); no preventive effects of alfalfa	-	Young <i>et al.</i> , 1990

Age, weight	Dosage, duration (ZEA-source)	Findings	Realistic feeding conditions? ¹	Reference
Adult (1st litter)	1 mg/kg b.w./day from day 7 to 10 after mating (crystalline)	Blastocysts collected from days 11 and 13 after mating showed degenerative changes in the embryonic disk, retarded development and increased number of necrotic cells; no morphological changes in the endometrium that could be associated with hyperoestrogenism	-	Long <i>et al.</i> , 1992
Adult	2 mg ZEA/kg diet alone or in combination with 10 mg tamoxifen/kg diet from day 30 of gravidity until weaning of piglets (day 21 of age) (crystalline)	No effects on reproductive performance, no signs of hyperoestrogenism; combined feeding of ZEA and tamoxifen resulted in an increase of the weights of the uteri and a decrease of the testes - later reproductive performance unaffected	+	Yang <i>et al.</i> , 1995
Adult	0.2 mg ZEA/kg diet and 2.5 mg deoxynivalenol/kg diet over 3 reproduction cycles, mycotoxin diet tested without or with a commercial detoxifying agent (naturally contaminated wheat)	Decreased number of weaned piglets, increased percentage of splay-legged piglets, piglets with necroses of tail and ears and hyperoestrogenism; all effects prevented by the detoxifying agent	++	Jadamus and Schneider, 2002
Growing pigs				
~ 60 days; ~ 15kg (piglet)	50 mg/kg diet for 28 days; Exp. 1 at various levels of dietary protein; Exp.2 without or with the addition of 15 or 25% alfalfa (crystalline)	Increased uterus weights in both experiments; neither the level of dietary protein nor alfalfa prevented the effect	-	Smith, 1980
Piglets	10, 20 and 40 mg/kg diet without or with 15 and 25 % alfalfa hay (crystalline)	Increased weights of the uteri independently of ZEA-dosage, partial preventive effects of alfalfa hay	-	James and Smith, 1982
20-30 kg	70, 150, 230 mg/kg diet, single bolus (crystalline)	Hyperoestrogenism 24 h after the bolus	-	Farnworth and Trenholm, 1983
20 kg	0.25 mg/kg diet (11 days), 0.05 mg/kg diet (21 days) (crystalline)	0.25 mg/kg: hyperoestrogenism; 0.05 mg/kg: intensified formation of tertiary follicles	++	Bauer <i>et al.</i> , 1987

Age, weight	Dosage, duration (ZEA-source)	Findings	Realistic feeding conditions? ¹	Reference
145 days	10 mg/kg diet, 48 days (crystalline)	Delayed puberty; no effects on cycle length	-	Edwards <i>et al.</i> , 1987a
178 days	10 mg/kg diet (0.26 mg/kg b.w./day), 14 days (crystalline)	No delayed puberty; no deleterious effects on reproductive performance	-	Green <i>et al.</i> , 1990
70 days	1.5-2.0 mg/kg diet, 45 and 90 days (crystalline)	No effects on ovulation and conception rate and on reproductive performance	+	Rainey <i>et al.</i> , 1990
Growing pigs	0.25 mg ZEA/kg diet alone or in combination with 0.1 mg ochratoxin A/ kg diet for 90 days (crystalline)	No effects on growth rate; histopathological signs of hyperoestrogenism in ZEA- and combination group	++	Lusky <i>et al.</i> , 1997
~ 41 kg	0.18 and 0.36 mg/kg diet in absence and presence of zeolithe; ovariectomy at 5 to 7 weeks of age; 66 days of exposure (crystalline)	Increased weights of the uteri independent on the ZEA-dosage; inconsistent preventive effects of zeolithe	++	Coenen and Boyens, 2001
~ 75 days	0.01, 0.06, 0.15, 0.22 and 0.42 mg ZEA and 0.2, 0.8, 1.0, 1.9 and 3.9 mg DON/kg diet (0.5, 3.0, 7.4, 10.4 and 17.6 µg ZEA/kg b.w./day; and 9.8, 39.6, 49.1, 90.2 and 163.5 µg DON/kg b.w./day); gross macroscopically inspection of reproductive tract after 35 days of exposure (contaminated maize)	Dose-response related increase in the number of piglets with swollen and reddened vulva and cervix; increased weights of the uteri only at the highest dosage; no effects on LH, decrease in FSH independently on dosage	++	Döll <i>et al.</i> , 2003b
~ 75 days	0.01, 0.06, 0.15, 0.22 and 0.42 mg ZEA and 0.2, 0.8, 1.0, 1.9 and 3.9 mg DON/kg diet (0.5, 3.0, 7.4, 10.4 and 17.6 µg ZEA/kg b.w./day; and 9.8, 39.6, 49.1, 90.2 and 163.5 µg DON/kg b.w./day); morphometric evaluation (hight of luminal epithelium and glandular area per range of vision) of uterus sections after 35 days of exposure (contaminated maize)	No marked effects on the general appearance of the uterus sections; no conspicuous alterations in morphometric parameters	++	Döll <i>et al.</i> , 2003a

Age, weight	Dosage, duration (ZEA-source)	Findings	Realistic feeding conditions? ¹	Reference
~ 49 kg b.w.	0.2 and 0.4 mg/kg b.w./day for 7 days	Hyperoestrogenism more pronounced at the highest dosage, no correlations between plasma ZEA and α -ZOL-concentrations and signs of hyperoestrogenism	-	Obremski <i>et al.</i> , 2003
~ 75 days	0.35 mg ZEA and 2.5 mg DON/kg diet in absence and presence of an inorganic adsorbent for 35 days (contaminated maize)	No effect on the weight of the uterus without adsorbent; increased uterus weight in the presence of the adsorbent	++	Döll <i>et al.</i> , 2003a
Boars				
Young male	30 mg/kg diet (1.2 mg/kg b.w./day) for various time periods	Precocious spermatogenesis, damage to germinal epithelium, interstitial-cell hyperplasia	-	Vanyi and Szekey, 1980
Boars	40 mg/kg diet from week 14 to 18 of age	Decreased testosterone concentration in plasma; decreased libido - no adverse effects detectable at week 36 of age	-	Berger <i>et al.</i> , 1981
Boars	0, 2, 20 and 200 mg/kg diet for 56 days	No effects on copulatory behaviour or male reproduction	+	Ruhr <i>et al.</i> , 1983
Boars	3, 6, 9 mg/kg diet (0.12, 0.24, 0.36 mg/kg b.w./day) for 330 days	No effect on growth rate, libido, puberty, or indications of reduction in sperm concentration, testicular weight, or epididymal weight	-	Young and King, 1984
Boars	0.2 and 1 mg ZEA/kg diet for 7 weeks	No effects on libido and semen quality	++	Stolla <i>et al.</i> , 1987
Boars	Feeding of mixed contaminated diets (DON: 0.28 - 3.14 mg/kg diet, NIV: 3.27 - 12.9 mg/kg diet, ZEA: 0.08 - 4.79 mg/kg diet) for 6 to 8 weeks (naturally contaminated corn-cob-mix)	Inconsistent effects on chromosomal aberrations	+	Lusky <i>et al.</i> , 1991

b.w. - body weight, ZEA - zearalenone, α -ZOL - α -zearalenol, DON - deoxynivalenol, NIV - nivalenol

¹) Probability of exceeding tested ZEA concentrations under feeding conditions in agricultural practice: "-" seldom, "+" sometimes, "++" frequently

Table 3. Carry-over of zearalenone into animal tissues and foodstuffs of animal origin

Species/category	ZEA-dosage (mg/kg diet) ¹⁾	Duration (days)	ZEA and metabolites in tissues and foodstuffs (µg/kg or µg/l)	Remarks	Reference
Broiler	3H-ZEA: 5 mg/kg b.w. (appr. 50 mg/kg diet)	Single bolus	Liver: Σ ZEA, α - and β -ZOL: 17-2540; rapid clearance Muscle: ZEA max. 111 (no ZOL)	Conjugates n.d.	Mirocha <i>et al.</i> , 1982
Laying hen	14C-ZEA: 10 mg/kg b.w.	Single bolus	Tissue: very low radioactivity Yolk: 2000 µg ZEA-equivalents/kg after 72 h	94 % of radioactivity excreted after 72h	Dailey <i>et al.</i> , 1980
Laying hen	1.1	112	Liver: α -ZOL 3.5 -3.8 (36 % free, 28 % conjugated with glucuronic acid, and 36 % with sulphate); ZEA < 1 - 3.2 (46 % free, 54 % conjugated with glucuronic acid, and <5 % with sulfate); no residues in yolk, albumen, breast muscle, abdominal fat, ovary and follicles, magnum		Dänicke <i>et al.</i> , 2002b
Chicken	10 mg/kg b.w.	20	Liver: ZEA 207; Kidney: ZEA 416; Muscle: ZEA 170	Metabolites and conjugates n.d.	Maryamma <i>et al.</i> , 1992
Turkey	800	14	Liver: ZEA 280 ²⁾ ; α -ZOL 2720 ²⁾ Kidney: ZEA 120 ²⁾ ; α -ZOL 480 ²⁾ , β -ZOL traces in liver and kidney		Olsen <i>et al.</i> , 1986
Peking duck	Up to 0.06	49	Liver ²⁾ : ZEA, α - u. β -ZOL < d.l.	Dose-response related increase in ZEA, α - and β -ZOL-concentrations in bile; mean proportions of ZEA, α -ZOL and β -ZOL of the sum of all three metabolites were 80 %, 16 % and 4 %, respectively	Dänicke <i>et al.</i> , 2004b

Species/c ategory	ZEA-dosage (mg/kg diet) ¹⁾	Duration (days)	ZEA and metabolites in tissues and foodstuffs (µg/kg or µg/l)	Remarks	Reference
Rabbit	0.3	21	Liver, kidney, muscle, adipose tissue: ZEA, α - and β -ZOL: < 1-2 ³⁾		Ueberschär, 1999
Female piglet (8-11 kg b.w.)	40	28	Liver: ZEA 128; α -ZOL 94; β -ZOL < d.l.	Conjugates n.d.	James and Smith, 1982
Piglet (appr. 18 kg b.w.)	0.5 mg/kg b.w.	Single bolus	Liver, kidney, muscle: ZEA, α - and β -ZOL < d.l. (after incubation with glucuronidase)	ZEA: d.l., α - and β -ZOL: 0.8-9.2 µg/kg	Enders, 1984
Pig (appr. 50 kg b.w.)	a) ZEA: 0.25 b) ZEA: 0.25 + OTA: 0.1	90	a) Liver, kidney, muscle, adipose tissue: ZEA and α -ZOL < d.l. b) Liver, kidney: α -ZOL-traces (max. 4 µg/kg after incubation with glucuronidase), ZEA < d.l.; muscle and adipose tissue : ZEA and α -ZOL < d.l.		Lusky <i>et al.</i> , 1997
Pig (appr. 70 kg b.w.)	0.7	18	Liver: ZEA < d.l. - 3.1; α -ZOL 3.6 - 12; β -ZOL 1.9 - 4.8 Muscle: α -ZAL up to 13.3; α -ZOL up to 14.5; traces of ZEA and β -ZAL; ZEA and ZAN < d.l.		Zöllner <i>et al.</i> , 2002
Piglet (appr. 33 kg b.w.)	0.01 0.06 0.15 0.22 0.42	35	Liver ²⁾ : 1.8 ZEA + 0.3 α -ZOL 0.2 ZEA + 0.1 α -ZOL 2.1 ZEA + 1.1 α -ZOL 2.9 ZEA + 1.7 α -ZOL 5.3 ZEA + 2.8 α -ZOL		Döll <i>et al.</i> , 2003a

Species/category	ZEA-dosage (mg/kg diet) ⁴⁾	Duration (days)	ZEA and metabolites in tissues and foodstuffs (µg/kg or µg/l)	Remarks	Reference
Piglet (appr. 33 kg b.w.)	1 mg/kg b.w.	Single bolus	Liver (14 days after the bolus) ²⁾ : ZEA, α - u. β -ZOL < d.l.		Dänicke <i>et al.</i> , 2004a
Lactating cow	0.39-1.93 mg/kg concentrate	49	Muscle, liver, kidney, milk: ZEA < 4		Shreeve <i>et al.</i> , 1979
Lactating cow	5000 mg/animal	Single bolus	Milk: ZEA and β -ZOL: traces (< 1)	Incubation with β -glucuronidase	Hagler <i>et al.</i> , 1980
Lactating ewe	1800 mg/animal	Single bolus	Milk: ZEA and β -ZOL: 1-2	Incubation with β -glucuronidase	Hagler <i>et al.</i> , 1980
Lactating cow	25	7	Milk: 1360 µg/l Total residues (ZEA, α - and β -ZOL; conjugated and free)	0,7 % of consumed ZEA recovered with milk	Mirocha <i>et al.</i> , 1981
Lactating cow	50 or 165 mg/day 545 mg/day 1800 or 6000 mg/animal	21 21 Single bolus	Milk: ZEA, α - and β -ZOL and conjugates < d.l. Milk: ZEA max. 2.5; α -ZOL max. 3.0 ⁴⁾ Milk: ZEA max. 4.0 or 6.1; α -ZOL max. 1.5 or 4.0; β -ZOL max. 4.1 or 6.64	d.l. in milk: ZEA, α -ZOL: 0.5 µg/l, β -ZOL: 1.5 µg/l	Prelusky <i>et al.</i> , 1990
Lactating cow	25 or 100 mg/day	6	Milk: ZEA-equivalents ⁵⁾ max. 0.4 or 1.2 µg/l		Usleber <i>et al.</i> , 1992
Lactating cow	0.02-0.05 mg/kg dry matter	63	Milk: ZEA and α -ZOL < 0.5 (after incubation with β -glucuronidase)		Goll <i>et al.</i> , 1995

Species/category	ZEA-dosage (mg/kg diet) ¹	Duration (days)	ZEA and metabolites in tissues and foodstuffs (µg/kg or µg/l)	Remarks	Reference
Heifer	158 µg/animal	84	Liver: β-ZOL < 0.5	Urine: ZEA and β-ZOL: < 0.5 µg/l	Kleinova et al., 2002
	12740 µg/animal	84	Liver: ZEA and α-ZOL < 0.5 – 1.2, β-ZOL 5-11.5 No residues in muscle	Urine: ZEA 5-8, α-ZOL 3-5, β-ZOL 20-65, α-ZAL 2-3, β-ZAL < 0.5 µg/l	
Growing bull (approximately 460 kg b.w.)	0.1 mg/kg at a dry matter content of 88 % of the total daily ration	152-160	Muscle, liver, kidney, back fat ² : ZEA, α-ZOL, β-ZOL, ZAN, α-ZAL, β-ZAL < 1, < 0.5, < 5, < 100, < 50, < 200	Bile: ZEA 7-24, α-ZOL 2-11, β-ZOL 23-53, ZAN < 100, α-ZAL < 50, β-ZAL < 200	Dänicke et al., 2002a

b.w. - body weight; n.d. - not determined; d.l. - detection limit; ZEA - zearalenone; α-ZOL - α-zearalenol; β-ZOL - β-zearalenol; ZAN - zearalanone; α-ZAL - α-zearalanol; β-ZAL - β-zearalanol; OTA - ochratoxin A

- 1) Air dry basis, if not otherwise stated
- 2) After incubation with β-glucuronidase and sulfatase; Proportions of free and conjugated metabolites n.d.
- 3) After incubation with β-glucuronidase
- 4) Only as conjugates (incubation with β-glucuronidase/aryl sulfatase); free metabolites not found
- 5) Determination with ELISA (partial co-detection of α- and β-ZOL) after incubation with β-glucuronidase