Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the Commission related to ochratoxin A (OTA) as undesirable substance in animal feed

Request N° EFSA-Q-2003-039

Adopted on 22 September 2004

SUMMARY

Ochratoxin A is a mycotoxin produced by several fungi of the genera *Aspergillus* and *Penicillium*, principally *P. verrucosum* in temperate climates and *A. ochraceus* in warm regions. In animal feed materials the toxin is found most commonly in cereals (rye, barley, maize, and wheat) and to a lesser degree in peanuts and soybean. Toxin production usually occurs during storage and control of formation of the toxin requires adequate drying of grains prior to storage under conditions where water activity does not rise in localised "hot spots". The distribution of ochratoxin A in stored grains is very heterogeneous, making analysis and dietary exposure assessment of farm animals difficult. However, exposure of animals may also be assessed from analysis of blood levels. Ochratoxin A is a potent renal toxin in all animal species investigated as of yet and has been causally associated with nephropathy in pigs and poultry. Exposure to nephrotoxic doses is associated with renal tumours in laboratory rodents but no data on carcinogenicity are available in other animal species. Ochratoxin A is also immunotoxic and teratogenic but usually at higher than nephrotoxic doses. Pigs, dogs and poultry are particularly sensitive to the nephrotoxicity and a no-observed-adverse-effect level has not been established in pigs or dogs; ruminants are less sensitive due to degradation of ochratoxin A to the less toxic ochratoxin α by the rumen microflora. Upon absorption from the gastro-intestinal tract, ochratoxin A binds to serum proteins resulting in considerable variation in elimination half-lives across species depending on the affinity and degree of protein binding. It is only metabolised to a small extent systemically. Accumulation occurs in blood, liver and kidney, and significant lower residue concentrations are found in muscle tissue, fat and milk. Carry-over into eggs has been demonstrated under experimental conditions using high toxin concentrations. Multiple source exposure assessment indicates that the overall contribution of animal products to human exposure does generally not exceed 3 – 10 %.

KEY WORDS

Ochratoxin A, animal feed, toxicity, tissue accumulation, residues

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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 feed[1] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[2].

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 2003[4] provides a comprehensive overview on the possible risks for

[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)

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.

2. Specific background

Ochratoxin A is produced mainly by the *Penicillium verrucosum* fungi and is commonly found in cereals in Europe.

No maximum levels for ochratoxin A in animal feed has been established in EU legislation. Sweden has national provisions on ochratoxin A in animal feed[5].

Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs[6], as amended by Commission Regulation (EC) No 472/2002 of 12 March 2002[7] establishes a maximum level for ochratoxin A in following foodstuffs: raw cereal grains (5 µg/kg), all products derived from cereals (3 µg/kg) and dried vine fruit (10 µg/kg).

SCAN concluded[8] that ochratoxin A contamination of crops is undesirable both because of its known effects on animal health and its possible significance as a human carcinogen. SCAN recommended therefore that a 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 ochratoxin A in animal feed.
This detailed scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of ochratoxin A 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

[5]Maximum level for ochratoxin A in complete feedingstuffs for poultry: 200 µg/kg and in complete feedingstuffs for pigs: 100 µg/kg
[6]OJ L 77, 16.3.2001, p. 1
[7]OJ L 75, 16.3.2002, p. 18
[8]Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 7.6. Conclusions and recommendations.

- the level of transfer/carry over of ochratoxin A from the feed to the products of animal origin results in unacceptable levels of ochratoxin A 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 ochratoxin A 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 ochratoxin A

  - to the overall exposure of the different relevant animal species to ochratoxin A

  - 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 rates[9].

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

ASSESSMENT

1. Introduction

Ochratoxin A is produced by several fungi of the genera *Aspergillus* and *Penicillium*, including *Penicillium verrucosum (*previously referred to as *P. viridicatum II), P. nordicum (*previously denoted *P. viridicatum III)*[10]*, Aspergillus ochraceus, A. niger, A. sulphureus, A. carbonarius, Neopetromyces muricatus* and *Petromyces alliaceus* (Frisvad and Thrane, 2000, Frisvad and Viuf, 1986, Larsen *et al.*, 2001, Frisvad and Samson, 2000).

In temperate climate zones ochratoxin A is produced by *P. verrucosum* at below 30^0C and down to 0.8 a_w most notably in maize, wheat, barley and rye. Infections can occur at the pre-harvest and post-harvest stage, but post-harvest ochratoxin A formation predominates.

In warm regions, *Aspergillus ochraceus* is found predominantly on peanuts and soy beans, as well as sporadically on other stored food commodities, but this fungal species is seldom the cause of a substantial contamination with ochratoxin A. *Aspergillus ochraceus* as well as *A. carbonarius* are related to ochratoxin A contamination of green coffee beans and grapes (WHO/FAO, 2001).

[9]Importance of the human exposure to ochratoxin A from foods of animal origin compared to overall human dietary ochratoxin A exposure can be assessed making use of the information contained in the report on a task on human exposure assessment to ochratoxin A which has been finalised in January 2002 at EU level within the framework of co-operation by Member States in the scientific examination of questions related to food (SCOOP – Task 3.2.7) http://europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf

[10]*Nordicum* is found frequently on fermented meat products and ochratoxin A formation on these products can not be excluded.

Ochratoxin A consists of a chlorinated dihydroisocoumarin moiety linked through a 7-carboxyl group by an amide bond to one molecule of L-β-phenylalanine. Ochratoxin A is chemically described as N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl) carbonyl]-,(R)-L-Phenylalanine ($C_{20}H_{18}ClNO_6$, MW:403.8, CAS:303-47-9). The chemical structure of ochratoxin A is given in figure 1.

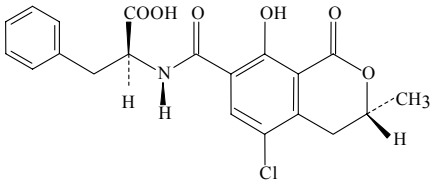

Figure 1. Chemical structure of ochratoxin A

Ochratoxin A is relatively stable and the toxin is only partially degraded under normal cooking, roasting and fermenting processes, and thus the toxin can be detected in various manufactured food products. Many countries have generated data on occurrence of ochratoxin A in foods and SCOOP reports on ochratoxin A have been published in 1997 (EC, 1997) and in 2002 (EC, 2002). The 2002 report summarises detailed data on ochratoxin A in foodstuffs from 12 European countries.

After oral ingestion with foods or feed, ochratoxin A is slowly absorbed from the upper small intestine. Reaching the systemic circulation, it binds extensively to serum proteins, and translocates to and accumulates in the kidney resulting in measurable residues, whereas lower residual concentrations are found in the liver, muscle and fat. Transfer to milk has been demonstrated in rats, rabbits and humans, but the percentage of ochratoxin A excreted with milk of ruminants is limited, owing to the degradation of ochratoxin A by the rumen microflora (Jonker *et al*., 1999). Despite the relatively long half life of ochratoxin A in blood serum and several tissues of food-producing animals, it has been estimated that within the EU, the overall contribution of products of animal origin to human exposure has been estimated to be not more than 3 % of the total ingested ochratoxin A, but may reach 10 % in certain regions with distinct preferences for traditional meat products, like blood puddings (EC, 2002).

Risk assessments for ochratoxin A have been performed several times. Ochratoxin A is a potent nephrotoxin in all animal tested as yet. It is carcinogenic in laboratory rodents, but the mechanism of the renal carcinogenicity remains unresolved. In rodents, tumours are generally only observed at dose levels in excess of nephrotoxic doses. The minimum nephrotoxic dose in rats (karyomegaly of proximal tubule cells) is 15 µg/kg b.w./day while renal tumours arise at doses of 70 µg/kg b.w./day for two years. However, at present, a genotoxic mechanism cannot be excluded unequivocally[11].

A Nordic expert Group proposed a Tolerable Daily Intake (TDI) of 5 ng/kg b.w./day (NNT, 1991). The Canadian Authorities proposed a provisional TDI of 1.2 - 5.7 ng/kg b.w./day (Kuiper-Goodman, 1996). The Scientific Committee on Food expressed the opinion that exposure should be below 5 ng/kg b.w./day (EC, 1998). The Joint Expert Committee on Food

[11] The final report of an EU Concerted Action (QLK1-2001-01614), devoted to the identification of the potential genotoxicity of ochratoxin A is not yet available.

Additives, in 2001, retained its previously established provisional Tolerable Weekly Intake of 100 ng/kg b.w. per week, pending the results of on-going studies on the mechanisms of nephrotoxicity and carcinogenicity (WHO/FAO, 2001). The International Agency for Research on Cancer (IARC) had evaluated ochratoxin A in 1993 (IARC, 1993), and classified it as possibly carcinogenic to humans (group 2B), based on sufficient evidence for carcinogenicity in animal studies and inadequate evidence in humans.

2. Methods of analysis

For the monitoring of the presence of ochratoxin A in food and feed materials, various methods of analysis exist, which are discussed in an exhaustive review by Scott in 2002. Most methods of analysis for ochratoxin A include immunoaffinity (IA) cleanup in combination with liquid chromatography (LC), followed by fluorescence detection (FLD). Detection can also be done with mass spectrometry (MS). In addition to LC, thin layer chromatographic (TLC) methods and enzyme-linked immunosorbent assays (ELISA) are also available. ELISAs are very sensitive; however the uncertainty of the results is generally higher than for the chromatographic procedures. Newer methods have been developed based on biosensor technology[12], but these are not yet commonly used. Several methods of analysis (TLC, LC) for ochratoxin A have been validated through formal (AOAC) collaborative studies (AOAC International, 2000), and several LC methods for the determination of ochratoxin A in foodstuffs (e.g. grains) have been standardised by the European Committee for Standardisation (CEN) (De Vreeze, 2003). CEN has also established general performance criteria for methods of analysis for ochratoxin A (CEN, 1999). Interlaboratory-validated and standardised methods, specifically tested for ochratoxin A in animal feedstuffs, are not yet available.

Certified reference materials and ochratoxin A calibrants are available. The certified reference materials consist of naturally contaminated wheat flour and a blank wheat flour. Through adequate mixing at certain ratios, materials for analytical quality assurance can be obtained at other desired levels. The reference calibrants are commercially available, and they are supplied as solutions of ochratoxin A e.g. in benzene-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 ochratoxin A several times a year. Recent FAPAS studies (FAPAS, 2003/2004) show that IA cleanup, in combination with LC and FLD is, by far, the most often used procedure to determine ochratoxin A. Only incidentally determination is done with TLC, ELISA or a fluorimeter. MS and UV detectors are sporadically used. The studies showed that satisfactory scores for the participants ranged from 83 % to 100 % for various test materials. Animal feedingstuffs were not included, but three of the studies involved cereals. For these cereals the assigned levels tested ranged from 6.4 – 23.4 µg/kg, and the satisfactory scores ranged from 85 - 100 %. The results of these FAPAS studies indicate that participating laboratories generally had their methods of analysis for ochratoxin A well under control. The reference materials and the proficiency studies are indispensable for providing a sound quality assurance framework for measurements of ochratoxin A.

[12] MycoSens 2003, Alpha Bioverfahrenstechnik GmbH, http://www.alphabvt.com/homeengl.htm

3. Current legislation

Approximately 40 countries around the world had set regulatory or guideline levels for ochratoxin A in food and animal feed in 2003 (FAO, 2004), whereas in 1996 ochratoxin A was regulated in only 10 countries (FAO, 1997). In the EU, harmonised regulations exist for ochratoxin A in raw cereal grains including rice and buckwheat, all products derived from cereals, dried vine fruit and baby food[13]. EU-harmonised limits for ochratoxin A in coffee, wine and grape juices are close to be set. Several other countries in Europe, Asia, North and Latin America have set limits for ochratoxin A in cereals and cereal products, ranging from 3 – 50 µg/kg (FAO, 2004). Codex Alimentarius is also in the process of developing maximum levels for ochratoxin A in wheat, barley, rice and derived products, and has issued a Code of Practice for the prevention and reduction of ochratoxin A in cereals (Codex Alimentarius, 2003). EU-harmonised specific limits for ochratoxin A in animal feedstuffs have not been proposed yet, but limits have been established at the national level in 8 countries. These countries include Estonia, Lithuania, Slovenia and Sweden, reporting the existence of limits for ochratoxin A in various feedstuffs (including feeds for cattle, pigs, poultry and other farm animals; see table 1) (FAO, 2004). In addition to legislation on ochratoxin A in animal feed, national legislation exists on ochratoxin A in products of animal origin in three countries: Denmark (for pig kidney), Estonia (for pig liver) and Italy (for pig meat and derived products).

4. Occurrence of ochratoxin A in feed materials

Ochratoxin A occurrence in animal feed and feed components is predominantly a problem of poor or inadequate drying of cereals prior to storage, or poor storage conditions leading to 'hot-spots' of contamination. There is extensive published literature concerning ochratoxin A contamination of cereals, although whether they are destined for animal feed or human consumption is not always indicated from surveys. Where cereals are known to be destined for feeds, there is rarely a distinction made as to whether destined for cattle, pigs, sheep or poultry.

There has been substantial surveillance data generated by EU Member States (EC, 2002), which is collated in Table 2. This shows that for wheat, oats, rye, barley and maize, out of 1500 samples analysed, only 61 samples (4 %) contained ochratoxin A above 1 µg/kg with the highest level reported being 27 µg/kg in a rye sample from Sweden. These SCOOP data were aimed at assessing human exposure and data on ochratoxin A in cereals is probably weighted towards the better quality grains destined for human consumption. UK data from 1997 on 306 samples of farm-stored grain samples destined for animal feed (Scudamore *et al.,* 1999) showed ochratoxin A as being detectable in 45 samples (15 %) above 1 µg/kg with the highest level being 17.8 µg/kg in a sample of barley. There was a significant correlation between ochratoxin A concentration, and moisture content, storage time and geographical

[13]Commission Regulation No 472/2002 of 12 March 2002 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs. OJ L 75, 18-20, 2002.

area. Further work in 1999 (MacDonald *et al.,* 2004), indicated that of, 320 samples, ochratoxin A was detectable in 35 samples (11 %) above 1 µg/kg with the highest level being 231 µg/kg in a sample of feed wheat. Jonsson and Pettersson (1999) studied the effect on ochratoxin A levels of different grain preservation methods at the farm level in Sweden (1986 - 89). They found ochratoxin A (> 1 µg/kg) in 28 % and 15 % of samples from cereals dried with ambient air (n = 82) and heated air (n = 91), respectively. Highest concentrations were 1383 µg/kg and 680 µg/kg in the cereals from the two methods. Cereals from other preservation methods, such as acid treatment and air-tight storage, did not contain detectable levels of ochratoxin A.

Data world-wide on ochratoxin A occurrence prior to 1994 has been summarised (Van Egmond and Speijers, 1994) and for Europe showed a picture of significantly higher incidence of contamination with maximum levels of contamination ranging up to several thousand µg/kg in some instances. Particularly high levels of ochratoxin A contamination (> 1000 µg/kg) were reported in samples of maize, wheat, rye, oats and barley from Austria, Bulgaria, Poland, and the Czech Republic (Van Egmond and Speijers, 1994), all countries which notably were not included in the SCOOP data in Table 2. Data from Hungary (Rafai *et al.,* 2000) reporting results from 1681 cereal samples obtained between 1991 - 1998 found ochratoxin A in maize, wheat, barley, oats, triticale, rye, soybean and sunflower at incidence levels from 2.5 % to 18 %, at levels ranging up to 1850 µg/kg and with average levels from 76 to 350 µg/kg again being significantly higher than data in the SCOOP report (EC, 2002). Apart from cereals as animal feed components, ochratoxin A has also been found in palm kernel, rice bran, dried peas and beans (Scudamore *et al.,* 1997) and sunflower seeds (Juric *et al.,* 1999).

Relatively high occurrence and concentrations of ochratoxin A can be obtained in the final mixed feeds, as results from Lithuania indicate. Ochratoxin A (> 10 µg/kg) was found in 92 % and 93 % of mixed feeds produced 1999 in Lithuania for pigs (n = 25) and poultry (n = 27), respectively (Garaleviciene *et al*., 2002). The highest concentration of ochratoxin A was 68 µg/kg for both types of mixed feed.

The fact that ochratoxin A is heterogeneously distributed in a contaminated lot of feed material makes the sampling problematic. Unless surveys encompass large sample sizes and statistically representative sample numbers, such surveys may well be flawed into the insight they can provide as to the real situation with animal feed contamination with ochratoxin A. It has been shown that an alternative method to monitor the ochratoxin A contamination in the feed is to analyse blood samples from pigs, which reflect the toxin content of the ingested feed (Hult *et al.,* 1979, 1980, 1984). However, this method is not suitable to use for animals with high plasma clearance, such as poultry or fish, or animals with low absorption, such as ruminants. Holmberg and co-workers (1990a,b) further developed this and collected pig blood samples (> 2500) over six years to study the contamination of cereal grain in Sweden. Ochratoxin A (≥ 2 ng/ml blood) was found in 18 % of the samples and the yearly incidence varied between 11 – 35 %. The average ochratoxin A concentration in positive samples varied between 6.7 and 9.4 ng/ml blood, and the highest concentration found was 400 ng/ml. The annual variation was primarily a function of the moisture content of the grain at the time of harvest, showing increased contamination levels of during years with wet harvest conditions. Seasonal variations were also observed with an increased incidence and higher blood levels in pigs of ochratoxin A after prolonged storage of the grain. Regional differences were also seen, with higher occurrence for example in the province of Gotland, Sweden. Finally, it has been demonstrated that managerial measures, such as the use of heated air for drying the grain lead

to significantly lower toxin levels in the finished feed, and subsequently the prevalence of ochratoxin A in the blood samples from pigs.

In a study conducted in Romania, blood serum, kidney, liver and muscle samples were collected from slaughtered pigs. A total of 98 % of serum samples were ochratoxin A positive in the range 0.05 to 13.4 ng/ml (85 % were < 5 ng/ml) indicating a high incidence of feed contamination. Occurrence of ochratoxin A in kidney and liver were similar (79 - 75 %, respectively) with corresponding mean levels of 0.54 and 0.16 ng/g. The lowest incidence (17 %) and the lowest mean level contamination (0.15 ng/g) occurred in muscle samples. Mean distribution followed the pattern serum > kidney > liver > muscle (100 %; 26 %; 8.5 %; 2.6 %). No ochratoxin B could be detected. (Curtui *et al*., 2001).

The SCOOP report (EC, 2002) contains fairly limited data on occurrence of ochratoxin A in meat and meat products across all Member States but does have extensive data in some instances For example: for pork offal from France (2 % of 1011 samples contained ochratoxin A above 1 µg/kg – maximum level = 6 µg/kg), pork, ham and sausages from Germany (1 % of 686 samples contained ochratoxin A above 1 µg/kg – maximum level = 9 µg/kg), and pig kidneys, black pudding and sausage/pate products from the UK (123 samples with mean values 0.6 - 1.1 µg/kg and a maximum level of 9.3 µg/kg). These results are consistent with other published work e.g. for pigs in Romania, where in kidneys mean levels of 0.5 µg/kg, and levels around 0.1 µg/kg in the muscle were found (Curtui *et al.,* 2001). It is difficult to correlate this data to feed intake, but, for example, for the UK where average levels in pork products are around 1 µg/kg, this finding suggest a higher rate of exposure than indicated from animal feed surveillance data (notwithstanding that there is ochratoxin A accumulation and long half-life).

Several reports have been published on the occurrence of porcine nephropathy and ochratoxin A in damaged kidneys, collected at slaughterhouses in north European countries (Rosseau and van Peteghem, 1989; Büchmann and Hald, 1985; Golinski *et al*., 1984, 1985 and review by Krogh, 1991). Ochratoxin A concentrations above 150 µg/kg have occasionally been found in kidneys with signs of ochratoxicosis. However, the prevalence of damaged pig kidneys has not always been given, and may vary considerably. In Denmark, the reported frequency of affected kidneys can vary between 10 and 80 cases per 100 000 slaughtered pigs (Büchmann and Hald 1985, and review by Krogh, 1991). These findings suggest that pig feed may contain relatively often ochratoxin A, resulting in tissue accumulation and subsequently kidney damage.

In conclusion, the SCOOP data probably underestimates ochratoxin A exposure via animal feed as it focuses primarily on food grade materials. The data are also deficient in that they do not include results from the new EU member states where there are some indications from the literature that incidence of occurrence of ochratoxin A in cereals and levels might be significantly higher than elsewhere. This might be related to differences in practices in drying and storing grains, and in climatic conditions, which make cereals more prevalent to ochratoxin A contamination. Limited data on animal products indicates the occurrence of ochratoxin A in pork and pork products. Although, these data cannot be directly extrapolated to feed contamination, they suggest a higher or more frequent exposure than estimated form the animal feed data.

5. Estimating ochratoxin A intake by farm livestock

In most cases, pigs and poultry are fed with complete diets, which are manufactured commercially or prepared at the farm level. Roughly, the diets consist of 40 - 80 % cereal grains including by-products, 0 - 50 % protein concentrates, 0 - 10 % oil and 0 - 10 % minerals, vitamins and other supplements. In general, the proportion of protein concentrates is higher and the cereal proportion is lower in diets for growing animals, and for poultry as compared to pigs.

In ruminants, the concentrate portion of the diet is principally composed of the same materials as used for monogastric animals. The total amount of concentrate (cereal grains plus protein concentrates) in diets for dairy cows varies between 0 and 70 %, depending on the milk yield. The remaining 30 – 100 % of the diet are composed of roughage.

The great variability in diet composition for the major farm animal species and the heterogeneity in distribution of ochratoxin A in animal feeds preclude a calculation of actual exposure levels based on the occurrence of ochratoxin A in individual feed materials.

Potential intakes that would arise at different levels of contamination of complete diet may be calculated on the basis that young, rapidly growing animals consume approx. 10 % of body weight daily, declining to approx. 5 % in adult animals. If the complete diets contained the commonly observed levels of contamination in cereals of up to 30 µg/kg feed, intakes in young animals fed exclusively on such diets would be less than 3 µg/kg b.w., thus remaining below the LOEL for nephrotoxicity in the pig (see below). However, the highest observed levels of contamination (> 1000 µg/kg) could give rise to nephrotoxic levels of intake (see below). Contamination levels of 100 µg/kg for complete pig feed would lead to intakes close to the LOEL in young animals fed exclusively on such diets.

As indicated above, exposure to ochratoxin A may also be estimated from analysis of blood levels that provide an indication of the overall intake during the past weeks. Comparison of such estimates with analytical data on feed suggests that calculations based on observed levels in feed may underestimate exposure.

6. Adverse effects on livestock

6.1. Pigs

The pig is one of the most sensitive species to the adverse effects of ochratoxin A and much more sensitive than most laboratory animal species except the dog. The oral LD_{50} in the pig is about 1 mg/kg b.w. (Harwig *et al.,* 1983).

Nephrotoxicity

The presence of ochratoxin A in feed was considered to be the most important cause of spontaneous porcine mycotoxic nephropathy (Krogh, 1978).

In female pigs, dietary levels as low as 0.2 mg/kg diet (equivalent to 8 µg/kg b.w./day) for 90 days caused a reduction in renal activity of cytosolic phosphoenolpyruvate carboxykinase and *gamma*-glutamyl transpeptidase accompanied by decreased kidney function as indicated by reduced tubular excretion of *p*-aminohippurate and increased glucosuria. In chronic toxicity studies, progressive nephropathy but no renal failure was observed at dietary levels of 1

mg/kg diet (40 µg/kg b.w./day) for two years. (Krogh and Elling, 1977; Elling, 1979, 1983; Meisner and Krogh, 1986; Krogh *et al.,* 1988). Based on the effects on enzymes and kidney function, a NOEL could not be established from these studies, and 8 µg/kg b.w./day must be considered as a LOEL.

In a Belgian study (Rosseau. and van Peteghem, 1989) two groups of pigs (95 pigs with suspected ochratoxicosis (I) and 385 pigs (II, selected at random), both groups showing macroscopic lesions, were examined for ochratoxin A in kidneys. In group I, 28 kidneys had ochratoxin A concentration > 0.2 µg/kg. Follow-up studies showed feed concentrations up to 7.2 µg/kg. In group II, 68 samples had ochratoxin A levels > 0.2 µg/kg.

Immunotoxicity

Ochratoxin A has been described to affect humoral and cellular immunity in several species. However, the only systematic study in the pig was by subcutaneous injection (and not via the diet) of pure or crude ochratoxin A (Müller *et al.,* 1999). The animals were given doses of 7 - 50 µg/kg b.w./day for 19 - 39 days, and were then challenged by inhalation with *Pasteurella.* A reduction in relative lymphocyte counts and an increase in total leukocyte, relative neutrophil and eosinophil counts were found. Ochratoxin A decreased the phagocytotic index of individual cells and decreased expression of the lymphocyte surface marker, SWC1, but did not affect lymphocyte proliferation, an effect that had been described in *ex vivo* experiments (Holmberg *et al.,* 1988).

Reproductive and developmental toxicity

Ochratoxin A is teratogenic in laboratory species (rat, mouse and hamster) (Arora and Frölen, 1981, Singh and Hood, 1985, Moré and Galtier, 1974, 1975, Hood *et al.* 1976) at high experimental doses, but there are no data available on these aspects in pigs. This is remarkable in that it is expected that increased malformations in piglets would have been reported because of the economic consequences. However, the doses that produced these effects in rodents were significantly higher than the nephrotoxic doses.

Ochratoxin A has been reported to have an adverse effect on sperm production and semen quality in boars given daily doses of 20 µg per animal. The effects were seen only after a lag period following withdrawal of exposure, suggesting that they were caused at an early stage of spermatogenesis. No histological effects were observed on Leydig cells or epididymal structures (Biro *et al.* 2003).

6.2. Poultry

The oral LD_{50} in chickens is about 3.3 mg/kg b.w., demonstrating that chickens are less sensitive than pigs. In groups of broiler chickens given ochratoxin A at a dietary concentration of 4 mg/kg for 2 months, the mortality rate was 42 %. When the feed was supplemented with 0.8 or 2.4 % phenylalanine, the mortality rate was decreased to 12 and 15 % respectively (Gibson *et al.,* 1990).

When laying hens were given feed naturally contaminated at levels of 1.3, 2.6 or 5.2 mg/kg feed, egg production decreased in a dose-dependent manner, being as low as 28.4 % of normal values in the highest dose group (Scholtyssek *et al.,* 1987; Bauer *et al.,* 1988)

Nephrotoxicity

The presence of ochratoxin A in feed is thought to be the most important cause of poultry nephropathy (Elling *et al.,* 1975) but quantitative dose-response data are lacking and the NOEL for nephropathy has not been established.

When broiler chickens were given feed containing ochratoxin A at 2.5 mg/kg diet, there was a significant reduction in weight gain and an increase in relative kidney weight associated with increases in serum uric acid and triglycerides, and decreases in total protein and albumin (Gentles *et al.,* 1999). In a gavage experiment, in which broilers were given ochratoxin A at a dose of 350 µg/kg b.w. over a period of 28 days, no adverse clinical effects were observed, but histological examination of the kidneys revealed mild glomerulonephrosis with enlarged glomeruli and swollen capillary and mesangial cells (Biro *et al*., 2002).

Immunotoxicity

The lymphoid cell population was decreased in chickens fed diets containing ochratoxin A at a concentration of 2 - 4 mg/kg for 20 days and the levels of IgG, IgA and IgM were depressed in lymphoid tissues and serum (Dwivedi and Burns, 1984a,b). Complement activity was slightly affected in birds fed such diets for 5 - 6 weeks (Campbell *et al.,* 1983). Immunosuppression was observed when chickens were fed diets containing ochratoxin A at levels of 0.5 or 2 mg/kg.; the treated birds had reduced lymphocyte counts and weights of thymus, spleen and bursa of Fabricius (Singh *et al.,* 1990).

These data do not allow a NOEL to be established for immunotoxicity in chickens.

6.3. Dogs

When beagle dogs were given doses of ochratoxin A equivalent to feed levels of 5 – 10 mg/kg feed, necrotic changes were seen in the intestine, liver, kidney, spleen and lymphoid tissue (Szczech *et al*., 1973a,b).

Nephrotoxicity

When dogs were given doses of ochratoxin A of 0.1 or 0.2 mg/kg b.w./day for 14 days, no changes in renal function were observed but kidney tubular necrosis and ultrastructural changes in proximal tubules were seen at both dose levels (Kitchen *et al.,* 1977a,b,c).

Immunotoxicity

In the above study by Kitchen *et al.* (1977a,b,c) necrosis of lymphoid tissue was seen in the thymus and tonsils of dogs exposed to ochratoxin A.

A NOEL for nephrotoxicity or immunotoxicity in the dog could not be established from the above studies.

6.4. Ruminants

Cows, sheep and goats are considered to be less sensitive to the toxicity of ochratoxin A due to an extensive pre-absorptive metabolism in the rumen, where the rumen flora converts ochratoxin A into the less toxic ochratoxin α (see below).

In contrast, if pre-ruminant calves obtained feed containing 0.1 - 2 mg ochratoxin A/kg b.w. for 30 days, polyuria and mild enteritis was observed; at termination there was mild renal

tubular degeneration (Pier *et al.*, 1976). Mature cows given oral doses of ochratoxin A ranging from 0.2 – 1.66 mg/kg b.w. for 4 days remained clinically normal; a single cow given one dose of 13.3 mg/kg b.w. developed diarrhoea, anorexia and cessation of milk production one day later (Ribelin *et al.,* 1978).

6.5. Equidae

The available data are confined to individual case reports of suspected ochratoxin A intoxications, in which no quantitative assessment of toxin exposure is presented.

7. Toxicokinetics, metabolism and tissue distribution

There is extensive literature on the toxicokinetics, metabolism and tissue distribution of ochratoxin A, and this has been reviewed in the toxicological monographs published by the JECFA and IARC (WHO, 1990, 1991, WHO/FAO, 2001). In most species, ochratoxin A is absorbed primarily from the small intestine, maximally from the proximal jejunum. In ruminants there is extensive metabolism to ochratoxin α in the rumen prior to absorption. Following absorption, ochratoxin A binds to serum albumin and other macromolecules, which retards elimination by limiting glomerular filtration and thus renal excretion. The serum half-life of ochratoxin A depends *inter alia* on the avidity of binding to serum proteins and there are large interspecies differences in half-lives. For example, ochratoxin A has a long half-life in non-ruminant mammals, e.g. 24 - 39 hours in mice, 55 - 120 hours in rats, 72 - 120 hours in pigs, 510 hours in one macaque and 840 hours in a human volunteer, whereas in poultry, the half-life is rather short (3.5 - 4 hours) (Hagelberg *et al*., 1989, WHO/FAO, 2001).

There is little metabolism of ochratoxin A in mammalian tissues and only about 1 - 1.5 % of the dose undergoes oxidative metabolism to the 4-hydroxy derivative in the rat (Storen *et al.,* 1982), whereas 33 % is excreted via bile (Suzuki *et al*., 1977). Part of the dose is converted by the gut microflora to ochratoxin α, which is absorbed and excreted in urine as well. The extent of enterohepatic circulation also influences the serum half-life. Excretion occurs both in bile and urine, the relative amount via either route depending on species and dose.

7.1 Pigs

The overall absorption of ochratoxin A in pigs is about 66 % (Galtier *et al.,* 1981). Upon absorption, ochratoxin A is extensively bound to serum proteins (predominantly to albumin). The serum half-life is 72 – 120 hours (Galtier *et al.,* 1981; Mortensen *et al.,* 1983a) and the clearance of ochratoxin A from blood is slower than from kidney, liver and other tissues (Hult *et al.,* 1979). This indicates that at a constant level of intake from the diet, steady-state blood concentrations will be reached after about 3 weeks of exposure. Elimination occurs via the kidneys and with bile fluid. In the kidneys (only the non-protein-bound fraction will pass glomerular filtration) re-absorption can occur in the proximal tubules, contributing to the slow elimination and tissue accumulation of ochratoxin A.

Diaplacental transfer has been demonstrated in rats (WHO/FAO, 2001), but there are conflicting reports concerning placental transfer in the pig. Previous data had suggested that

ochratoxin A administered to pregnant sows at a dose of 380 µg/kg b.w on days 21 - 28 of gestation did not cross the placenta (Patterson *et al.,* 1976) and no residues were found in the piglets of sows fed diets containing ochratoxin A throughout gestation at 7 - 16 µg/kg b.w. (Mortensen *et al.,* 1983b). Conversely, ochratoxin A was reported to be transmitted to piglets *in utero* when the sow was fed naturally contaminated feed; the blood levels in newborn piglets were 0.075 - 0.12 ng/ml compared to maternal levels of 0.20 ng/ml (Barnikol and Thalmann, 1988).

Ochratoxin A may be displaced from serum albumin by other xenobiotics, including other mycotoxins and veterinary drugs, some of which have a high binding affinity to serum proteins as well (Galtier *et al.,* 1980). For example, in a 90-day feeding trial with pigs, given ochratoxin A at 100 µg/kg; zearalenone at 250 µg/kg; and deoxynivalenol at 1000 µg/kg diet, changes in the metabolism and rate of excretion of ochratoxin A, dependent on the presence of other mycotoxins in the feed, were observed (Lusky, 2001).

7.2 Poultry

The overall rate of absorption of ochratoxin in chickens is approximately 40 % (Galtier *et al.,* 1981). The serum half-life of ochratoxin A is about 4 hours (Galtier *et al.,* 1981) i.e. much shorter than in the pigs and other mammals. Like in other species, the highest residual amounts of ochratoxin A are found in the kidneys and livers.

There are conflicting reports about transfer to eggs. No ochratoxin A was found in eggs from hens fed ochratoxin A (Krogh *et al.,* 1976a); conversely, detectable levels were found in eggs of birds fed 10 mg/kg b.w. (Juszkiewicz *et al.,* 1982) but not 1 mg/kg b.w. (Piskorska-Pliszczynska and Juszkiewicz, 1990). In a study in which laying hens received feed containing 1.3, 2.6 and 5.2 mg ochratoxin A/kg feed, levels in eggs were determined using a validated HPLC method (LOD 0.3 µg/kg egg white, LOD 1.0 µg/kg egg yolk). After 4 weeks exposure, concentrations in the eggs were between 0.1 and 0.2 mg/kg in egg white, independent of dose; levels in yolk increased in a dose-dependent manner from 1.6 µg/kg to 4.0 µg/kg (Scholtyssek *et al.,* 1987; Bauer *et al.,* 1988). In the latter study, mean serum concentrations varied between 4.7 and 11.7 µg/L while residues in liver averaged from 9.1 to 18.0 µg/kg in the three dose groups (see also table 4).

7.3. Ruminants

In young (pre-ruminant) calves, 85 – 90 % of orally administered ochratoxin A is excreted in urine, mainly as ochratoxin α (Sreemannarayana *et al.,* 1988). Ochratoxin α may undergo intestinal re-absorption.

In adult ruminants, ochratoxin A is largely degraded by the ruminal microflora into the less toxic ochratoxin α, as mentioned above. *In vitro* studies with rumen contents indicate that there is effective conversion towards ochratoxin α by ruminant protozoa and it has been calculated that up to 12 mg/kg feed could be converted in this way in cattle (Hult *et al.,* 1976; Pettersson *et al.,* 1982).

Sheep rumen flora seems to have a lower capacity to degrade ochratoxin A before it is absorbed (Kiessling *et al.,* 1984). In a recent study, the metabolism of ochratoxin A was investigated over a period of 29 days, feeding various doses of ochratoxin A (0, 9.5, 19.0, and

28.5 µg/kg b.w.) to sheep (Blank *et al.*, 2003). Animals were fed 70 % concentrates and 30 % grass silage. Significant levels of ochratoxin A were detected in serum of sheep at all levels of ochratoxin A tested. Serum concentrations of ochratoxin A slightly increased with time of exposure, and were linearly dependent on the administered dose of ochratoxin A. The serum values seemed to reach a maximum level at day 23 and the levels ranged from 6.0 to 18.2 µg/L in the highest dose group. In addition to the parent compound, small amounts of ochratoxin α could be detected.

7.4. Fish

The toxicokinetics of ochratoxin A have been studied in carp (*Cyprimus carpio*) given doses of 50 µg/kg b.w. by oral gastric tube, or intravenously. The serum half-life was 8.3 hours following intravenous administration and 0.68 hours after oral dosing. It was suggested that the low bioavailability was due to hydrolysis or conjugation (Hagelberg *et al.,* 1989).

Whole-body radiography studies in rainbow trout have shown that the highest concentrations of ochratoxin A or its metabolites are found in kidney, urine and bile, and not in the blood or muscular tissue (Fuchs *et al.,* 1986).

8. Carry-over and residues

In a number of feeding experiments in Denmark and Germany, residues of ochratoxin A in tissues and blood from pigs have been studied (Krogh *et al.,* 1974, Madsen *et al.,* 1982a,b, Mortensen *et al.,* 1983a,b, Lusky *et al.,* 1993, 1994, 1995). In most cases naturally contaminated feeding materials were used, at toxin levels varying between 25 and 1989 µg/kg feed. Data from these experiments are displayed in table 3.

Reviewing these data no obvious linear relationship between feed levels and tissue concentrations can be established, which is in line with the toxicokinetic characteristics of ochratoxin A showing a saturation kinetic in blood serum (due to high protein binding) and a selective accumulation in renal tissue (due to selective transport mechanisms). Furthermore levels in renal tissue depend on integrity of the kidneys and duration of exposure. As yet, all studies show that residue concentrations in slaughtered pigs can be ranked as follows: serum levels > kidney > liver > muscle tissue and fat (Harwig *et al.,* 1983; Mortensen *et al.,* 1983a; Madsen *et al.,* 1982a,b).

Surveillance data confirm that the highest amount of ochratoxin A occur in blood serum, and that these levels do not normally exceed 15 ng/ml although levels of several hundred ng/ml occur occasionally (Holmberg *et al.*, 1990a,b; Langseth *et al.,* 1993; Ominski *et al.,* 1996; Curtui and Gareis, 2001). Levels in pork edible offal generally are below 10 µg/kg (table 5 and 6), and in muscle tissue and fat < 0.3 µg/kg.

Ochratoxin A was determined in 90 samples of pork ham (derived from hind legs) obtained from 9 different farms in Italy (Spotti, 2002). Sixty percent of the samples were positive and the average concentration was 0.125 µg/kg. Only 3 of the samples showed a contamination exceeding 1 µg/kg.

Residue analyses after feeding chickens various diets have been performed by Krogh *et al.* (1976a), Prior *et al.* (1980), Golinski *et al.* (1983), Niemiec *et al.* (1988) and Micco *et al.* (1987; 1988). Most residue analyses have been performed after feeding with more than 1000 µg ochratoxin A per kg of feeding stuff. In these experiments, the residues in the liver varied between 2 and 11 µg/kg, and in muscles 2 µg/kg were found.

There are five studies on residue formation in hens following exposure to ochratoxin A (Juszkiewicz *et al.,* 1982; Reichmann *et al.,* 1982; Micco *et al.,* 1987; Bauer *et al.,* 1988; Niemiec *et al.,* 1994). The results of these studies are included in table 4 (annex). No direct relation between the concentrations in the feed and the reported residue concentrations could be established. This might be attributed to the fact that all residue levels were rather low, approaching the limit of quantification of the methods of analysis used.

According to the presented estimations eggs may contain 0.11 % of the toxin concentration present in the feed. Following administration of ochratoxin A to laying hens at dietary levels of 1.3, 2.6 and 5.2 mg/kg feed, concentrations in egg white were 0.1 - 0.2 µg/kg and in yolk were 1.6 – 4 µg/kg (Scholtyssek *et al.,* 1987; Bauer *et al.,* 1988).

Transmission of ochratoxin A into milk has been shown in monogastric animals such as rats, pigs and humans (Breitholtz-Emanuelsson *et al.,* 1993a; Mortensen *et al.,* 1983a,b). Ochratoxin A has also been found in bovine milk in low concentrations (Breitholtz-Emanuelsson *et al.,* 1993b), indicating that small amounts may escape rumen metabolism, and are absorbed. Concentrations of ochratoxin A (10 - 58 ng/L) have been found in Swedish and Norwegian milk samples from road tankers (Breitholtz-Emanuelsson, 1993b) and in commercial samples (Skaug, 1999), but not in retail samples collected in Germany (Valenta and Goll, 1996).

Carry over into tissues might be affected by other mycotoxins, and other xenobiotics in general. For example, a 90-day feeding trial with pigs, given ochratoxin A at 100 µg/kg, zearalenone at 250 µg/kg, and deoxynivalenol at 1000 µg/kg diet, indicated that metabolism and excretion of ochratoxin A were influenced by the presence of other mycotoxins in the feed (Lusky, 2001). Residue concentrations were recorded in different tissues, including blood (77.0 µg/kg), muscle tissue (10.4 µg/kg), kidneys (43.6 µg/kg), liver (36.7 µg/kg) and heart (50.1 µg/kg) (Lusky, 2001).

9. Human dietary exposure

In the SCOOP report (EC, 2002) the number of data on ochratoxin A in meat products was 1890. Almost all of them concerned pork-derived products but 41 data, all being negative, were given for poultry meat. The highest daily intake value, 0.2 ng/kg b.w. was observed for girls, 4 - 6 years old. The general intake from meat products was low, representing about 3 % of the total intake. However, data were provided only by 4 member states and Norway (table 5). Furthermore, no or few data was available on products containing pig blood/plasma. Pig blood may contain high levels of ochratoxin A and in some member states pig blood/plasma is used in high amounts in different kind of meat products (sausages, black pudding). More intake estimates are presented in table 6, using additional data provided in the SCOOP report and other published surveys.

JECFA (WHO/FAO, 2001) estimated the intake of ochratoxin A from pork to be 1.5 ng/kg b.w./week and from poultry to be 0.25 ng/kg b.w./week. For this report, most of the data submitted for pig meat and products were for pig liver and kidney, whereas the figures for food consumption were based on pig meat. Hence, the resulting estimate has been considered as a gross overestimation of intake of ochratoxin A from pork meat.

In a report from the Swedish Ministry of Agriculture (Olsen, 1997), written in connection with the Swedish derogation from EC legislation, a theoretical estimation of exposure to ochratoxin A from foods of animal origin was presented. These theoretical levels were based on the assumption that the mean values of ochratoxin A in all feed materials, amount to 50 % of the Swedish maximum permitted levels for feedingstuffs (100 µg/kg feed intended for pigs). Results from these estimations indicate that the intake of ochratoxin A by adult men was larger than 10 % of the TDI (5 ng/kg b.w.). However, children (17 kg b.w.) could even exceed the TDI, merely through high consumption (95 percentile corresponding to 13 g/day) of black pudding (containing 8.3 µg ochratoxin A/kg).

In a German study (Gareis and Scheuer, 2000) a total of 620 samples of meat and meat products available on the market were analysed for the presence of ochratoxin A. Among the meat products, blood sausages and liver-type sausages were most frequently contaminated (77 and 68 %, respectively). Maximum levels in liver and blood sausages were 4.6 and 3.2 µg/kg, respectively. These two studies indicate that the assumptions made in the above-mentioned estimation of the human intake of ochratoxin A from products such as black pudding are not unrealistic.

Measurements of ochratoxin A in human blood have been widely used as an indicator of exposure (Bauer and Gareis, 1987; Breitholtz *et al*., 1991; Hald, 1991; Golinski *et al*, 1991; Creppy *et al*., 1993; Studer-Rohr, 1995, Palli *et al*., 1999; Domijan *et al.,* 1999). The correlation between ochratoxin A levels in human blood and the consumption of different food items was studied among 406 blood donors (Thuvander *et al.*, 2001). Compared to other food products (such as cereal products and wine), roast pork and liver paste were correlated to higher plasma levels of ochratoxin A to only a minor degree.

Ochratoxin A occurrence in mothers milk may also be used as an indicator of exposure (Gareis *et al*., 1988, Micco *et al*., 1991; Breitholt-Emanuelsson *et al*., 1993b). Human milk samples were collected from 80 Norwegian women whose habitual food intake during the previous years was recorded using a quantitative food frequency questionnaire (Skaug *et al.*, 2001). The concentration of ochratoxin A in the human milk was determined by HPLC (detection limit 10 ng/L). Seventeen out of 80 human breast milk samples (21 %) contained ochratoxin A in the range 10 - 182 ng/L. It was found that women with a high dietary intake of liver paste (liverwurst, liver pate) and cakes (cookies, fruitcakes, chocolate cakes, etc.) were more likely to excrete ochratoxin A with breast milk.

Gilbert *et al*. (2001) found a linear correlation between ochratoxin A consumption by humans and the urine concentration of ochratoxin A, expressed as the total amount excreted, indicating the suitability of urine analyses in exposure assessment of human populations.

CONCLUSIONS

- Ochratoxin A is produced by several fungi of the *Aspergillus* and *Penicillium* genera, most notably by *P. verrucosum* with maize, wheat, rye, oats and barley as the principal substrates. Post-harvest formation is the most important source of contamination.

- Because of the difference in diet composition and the very heterogeneous distribution of ochratoxin A in contaminated feed it is difficult to determine exposure of domestic animals. However, experimental data are available on the relationship of exposure to tissue and serum levels, and thus exposure can be estimated from data on occurrence of ochratoxin A in serum and tissues.

- Pigs are generally considered to be the most sensitive farm animal species to the nephrotoxicity of ochratoxin A. Progressive nephropathy is seen in pigs at dietary concentrations of 1 mg/kg (equivalent to 40 µg/kg b.w.). No data are available for establishing a NOEL, but based on effects on renal (diagnostic) enzyme levels and kidney function, the dietary concentration of 0.2 mg/kg (equivalent to 8 µg/kg b.w.) is considered to be the LOEL.

- Chickens are also sensitive species, and it is assumed that ochratoxin A is the most important cause of poultry nephropathy. Quantitative dose response data, however, allowing establishment of a NOEL or LOEL are not available. Immunotoxicity was observed at 0.5 mg/kg complete diet.

- Ruminants are less sensitive than monogastric species. This is consistent with data indicating that prior to absorption, a significant microbial degradation of ochratoxin A to the less toxic ochratoxin α occurs in the rumen.

- Herbivores such as horses, rabbits and related species that rely on caecal rather than ruminal fermentation may absorb intact ochratoxin A in the small intestine. Therefore they are likely to be more sensitive than ruminants, but quantitative data are lacking.

- Other monogastric animal species including dogs, cats and fish are expected to be sensitive to renal toxicity and immunosuppressive effects, as these have been observed in all species tested so far. However, quantitative data are available only for dogs, and these data indicate that renal tubular damage as well as necrotic changes in lymphoid tissues occurs at 0.2 mg ochratoxin A/kg diet.

- Following ingestion, ochratoxin A is retained in blood serum and may accumulate in tissues such as kidney and liver. Subsequently, the highest concentrations in slaughter animals can be found in blood serum, followed by kidney, liver, muscle tissue and fat. In contrast, in chicken the highest levels of ochratoxin A were found in the liver, followed by the kidneys, whereas levels in other edible tissues are substantially lower.

Multiple source exposure assessment indicates that food of animal origin contributes only to a minor extent (generally < 3 %, and in populations with dietary preferences < 10 %) to human dietary exposure to ochratoxin A.

RECOMMENDATIONS

- There is a need to establish measures to reduce the formation of ochratoxin A in feed commodities during transport and storage, including on-farm storage, and to implement adequate control of moisture (water activity) and temperature changes during storage.
- Analytical methods with appropriate limits of quantification for feeding stuff need to be validated by collaborative studies.
- The efficacy of feed control programmes should be assessed by surveys of blood levels of ochratoxin A in pigs at slaughter.
- More data are needed in order to establish a NOEL for pigs and poultry.
- In order to assess the significance of residue levels in animal tissues, both with respect to animal health and to human exposure, more extensive occurrence data on ochratoxin A in animal tissues and products thereof and form other foods, covering all member states, are required.

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ANNEX

Table 1. Regulations on ochratoxin A in various animal feedstuffs (FAO, 2004)

Country	Feed product	Limit (µg/kg)	Remarks
Bangladesh	Maize and mixed feed for poultry	Not given	In preparation
Canada	Feed for swine and poultry	2000	Guideline limit
Estonia	Feedingstuffs of vegetable origin Complete feedingstuffs for cattle, pigs and other farm animals Complete feedingstuffs for young cattle, young pigs and other young farm animals Complementary feedingstuffs for cattle, pigs and other farm animals Complementary feedingstuffs for young cattle, young pigs and other young farm animals	100 100 50 200 50	
Israel	All grains for feed	300	
Lithuania	Feeds for pigs and poultry Feeds for young pigs and young poultry	50 20	
Mozambique	Corn for feed	Unknown	
Slovenia	Feedstuffs for pigs Feedstuffs for poultry	200 1000	
Sweden	Complete feedstuffs for pigs Complete feedstuffs for poultry	100 200	
Yugoslavia (Serbia/ Montnegro)	Feed for pigs Feed for swine Feed for poultry Feed for egg laying hen	100 200 1000 250	

Table 2. Collation of data for ochratoxin A in unprocessed cereals taken from SCOOP Report (EC, 2002).

Country	Sample type	Survey Year	Total no samples	Numbers of samples containing ochratoxin A in range μg/kg			
				< 1	1 – 5	5 – 10	10 - 25
Finland	Wheat	95-99	125	122	3	0	0
	Oats	99	7	7	0	0	0
	Rye Barley	98-99	52	47	4	0	1
	Wheat	97-99	21	21	0	0	0
France	Oats	98-99	22	22	0	0	0
	Barley	98-99	1	1	0	0	0
	Wheat	98-99	7	6	1	0	0
Germany	Oats	95-98	27	27	0	0	0
	Rye Barley	95-98	29	29	0	0	0
	Cereals	95-98	26	26	0	0	0
	Oats	95-98	22	22	0	0	0
Greece	Cereals	99	42	40	2	0	0
	Wheat	98	3	0	3	0	0
Ireland	Maize	99	42	40	2	0	0
Italy	Rye	99	30	30	0	0	0
	Barley	99	40	37	3	0	0
	Wheat	99	42	42	0	0	0
	Oats	99	24	23	1	0	0
Norway	Rye	95-98	193	186	4	2	1
	Wheat	95-98	72	72	0	0	0
	Wheat	95-96	8	7	1	0	0
Portugal	Oats	99	34	34	0	0	0
Sweden	Rye	97-99	132	123	8	1	0
	Wheat	97-99	33	32	1	0	0

Country	Sample type	Survey Year	Total no samples	Numbers of samples containing ochratoxin A in range µg/kg			
				< 1	1 – 5	5 – 10	10 - 25
	Wheat	97-99	47	39	7	0	1
Netherlands	Maize	95	31	30	0	1	0
UK	Oats	97-99	138	131	6	1	0
	Rye Barley	99	139	137	2	0	0
		97	22	21	0	1	0
	Cereals	97	22	21	1	0	0
		97-99	67	64	2	1	0
TOTAL		**95-99**	**1500**	**1439**	**51**	**7**	**3**

Table 3. Residual levels of ochratoxin A in pig's tissue after feeding a diet of naturally ochratoxin A contaminated feedstuff (after Pettersson, 2004)

Feed conc. (µg/kg)	Residual ochratoxin A concentrations (ng/g)						Reference
	Blood	Serum	Kidney	Muscle	Liver	Fat	
25			4	1	2		Madsen *et al.* 1982a
50			5	1	1		Madsen *et al.* 1982a
90	41		20.7	4.23	12.4	5.6	Lusky *et al.* 1995
100			11	5	2		Madsen *et al.* 1982a
100	20		16.2	2.7	7.9		Lusky *et al.* 1994
101			1.5				Madsen *et al.* 1982a
140		88	10	4	1	1	Madsen *et al.* 1982a
140		88	12	4	4	3	Madsen *et al.* 1982a
200		103	11	6	4	2	Madsen *et al.* 1982a
200			5		2		Krogh *et al.* 1974
266			17	8	10	3	Madsen *et al.* 1982a
271			4.8				Madsen *et al.* 1982a
400			43.6	10.3	36.7		Lusky *et al.* 1994
880		429	61	32	25	17	Mortensen *et al.* 1983b
1000			14	4	10	7	Krogh *et al.* 1974
1380			71	38	21	29	Madsen *et al.* 1982a
1380			62	37	22	30	Madsen *et al.* 1982a
1380			49	44	20	59	Madsen *et al.* 1982a
1380			45	33	20	51	Madsen *et al.* 1982a
1400			67	37	30	8	Madsen *et al.* 1982b
1550		838	36	28	15	12	Mortensen *et al.* 1983b
1600		665	38	17	12	8	Madsen *et al.* 1982a
1600		665	50	23	20	10	Madsen *et al.* 1982a
1600		665	39	22	15	10	Madsen *et al.* 1982a
1600		665	54	25	19	16	Madsen *et al.* 1982a
1600		319	50	31	16	18	Madsen *et al.* 1982a
1720			61	31	30	20	Madsen *et al.* 1982a
1989			55.8	6.3			Madsen *et al.* 1982a

Different feeding times were used in the cases with the same feed concentration cited in Madsen (1982a) as follows:

140 µg/kg – 3 and 4 weeks resp.

1380 µg/kg – 2, 4, 6 and 8 weeks resp.

1600 µg/kg – 1, 2, 3 and 4 weeks resp.

Table 4. Residual levels of ochratoxin A in tissues from poultry having been fed diets containing ochratoxin A (after Petterson, 2004)

Feed conc. (µg/kg)	Residual ochratoxin A concentrations (ng/g)						Reference
	Blood/plasma	Kidney	Liver	Muscle	Fat	Egg	
Hens							
500		36.8	26.3	8	0		Prior and Sisodia 1978
1000		3.0-10.0	1.5-2.5				Reichmann *et al.* 1982
1000		77	57.6	12.6	0		Prior and Sisodia 1978
1300	4.7		9.1			1.6	Bauer *et al.* 1988
2100	13.3	8.1	3.3			4.1	Niemiec *et al.* 1994
2500	*0.25*	3.8	3	*0.25*		*0.25*	Juszkiewicz *et al.* 1982
2600	14.1		17.9			2.5	Bauer *et al.* 1988
4000		106.9	72.6	20.8	0		Prior and Sisodia 1978
4100	37	9	11.9			7.9	Niemiec *et al.* 1994
5200	11.7		18			4	Bauer *et al.* 1988
10000	4	5.7	5.2	2.4		1.3	Juszkiewicz *et al.* 1982
Chicken							
50		0.5	5	0.5			Micco *et al.* 1987
50		0.8	11	0.5			Micco *et al.* 1987
324		12	2	2			Krogh *et al* .1976b
1000			32.8	0.8			Golinski *et al.* 1983
1000			22.8	2.8			Golinski *et al.* 1983
1052		19	4	2			Krogh *et al.* 1976b
1052		32	7	4			Krogh *et al.* 1976b
1500	1.24		16.16	7.46			Niemiec *et al.* 1988
1500	4.56		10.98	3.02			Niemiec *et al.* 1988
1500			14.2	3			Golinski *et al.* 1983
1500			39.8	5			Golinski *et al.* 1983
2000		41	24	*1.75*	*1.75*		Prior *et al.* 1980
2000			34.3	4.5			Golinski *et al.* 1983
2000			58.6	8.5			Golinski *et al.* 1983

The same feed concentration for a specific reference represents in the case of Golinski *et al.* 1983 separate groups of males and females, in the other cases groups with different feeding times Micco *et al.* 1987 – 36 and 64 days; Niemic *et al*. 1988 – 7 and 14 days; Krogh *et al*. 1976b - 14 and 176 days.

The italic values in the table represents half detection limit, when reported as not detected.

Table 5. Examples of dietary intake of ochratoxin A from food of animal origin according to the SCOOP report.

Food category	Food intake average/95th g/day	Contamination level (n) µg OTA/kg	OTA intake average/95th ng OTA/kg b.w.	Country
Sausages	46.9/ - (adults)	0.09 (201)	0.06	D
Sausages	36.3/ - (children)	0.09 (201)	0.11	D
Sausages	28.6/ - (girls 4-6 years)	0.09 (201)	0.20	D
Salami	2.5/14.3	0.25 (8)	0.01/0.05	I
Salami	10.6/28.6 (consumers only)	0.25 (8)	0.04/0.10	I
Milk	463/1113 (consumers only)	0.01 (165)	0.07/0.16	N
Pork edible offal	0.05/ - (consumers only)	0.14 (1011)	< 0.01	F

Table 6. Estimates of dietary intake of ochratoxin A from food of animal origin calculated from different surveys.

Food category	Contamination level average/maximum µg OTA/kg (n)	Food intake GEMS/Food[1] g/person and day	OTA intake ng OTA/kg b.w. (60 kg b.w.)	Refer-ence
Pork meat	0.01/0.13 (58)	75.8	0.01/0.16	2
Pork meat	0.11/1.3 (76)	75.8	0.13/1.6	3
Pork edible offal	0.79/9.3 (120)	5	0.07/0.78	2
Black pudding	0.60/1.80 (32)	2.0	0.02/0.06	2
Pork liver pate	0.09/1.8 (38)	5	< 0.01/0.15	4
Chicken meat	0.03/0.18 (65)	53	0.03/0.16	3
Turkey meat	0.02/0.11 (17)	53	0.02/0.10	3
Turkey liver	0.04/0.28 (17)	0.4	< 0.01	3

1) Global Environmental Monitoring System/Food Europe, WHO. Data for Europe, available at http://www.euro.who.int/eprise/main/WHO/Progs/FOS/Chemical/20020905_1

2) EC, 2002

3) Jorgensen, 1998

4) Jiménez *et al*., 2001