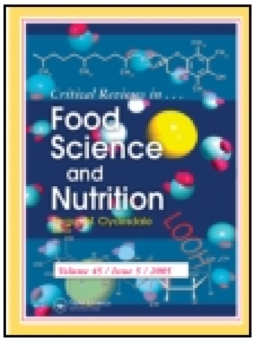

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A Review of Mycotoxins in Food and Feed Products in Portugal and Estimation of Probable Daily Intakes

LUÍS ABRUNHOSA, HÉCTOR MORALES, CÉLIA SOARES, THALITA CALADO, ANA SOFIA VILA-CHÃ, MARTINHA PEREIRA, and ARMANDO VENÂNCIO

CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal

Mycotoxins are toxic secondary metabolites produced by filamentous fungi that occur naturally in agricultural commodities worldwide. Aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone, trichothecenes, and ergot alkaloids are presently the most important for food and feed safety. These compounds are produced by several species that belong to the Aspergillus, Penicillium, Fusarium, and Claviceps genera and can be carcinogenic, mutagenic, teratogenic, cytotoxic, neurotoxic, nephrotoxic, estrogenic, and immunosuppressant. Human and animal exposure to mycotoxins is generally assessed by taking into account data on the occurrence of mycotoxins in food and feed as well as data on the consumption patterns of the concerned population. This evaluation is crucial to support measures to reduce consumer exposure to mycotoxins. This work reviews the occurrence and levels of mycotoxins in Portuguese food and feed to provide a global overview of this issue in Portugal. With the information collected, the exposure of the Portuguese population to those mycotoxins is assessed, and the estimated dietary intakes are presented.

Keywords Mycotoxins, food, feed, daily intakes, Portugal

INTRODUCTION

In 1960, after a severe episode of intoxication accompanied by symptoms of internal bleeding and liver necrosis, more than 100,000 turkeys died in England. Subsequent investigations (Goldblatt, 1969) revealed that those deaths were connected to the ingestion of peanut meal heavily contaminated with toxic compounds (aflatoxins, AFs) produced by the mould *Aspergillus flavus*. Since then, many other toxic compounds produced by filamentous fungi were been discovered, and the generic term mycotoxins came to be used to define them.

Mycotoxins are, therefore, secondary metabolites produced by filamentous fungi that are toxic to animals in small quantities when ingested or inhaled. These compounds pose an important risk to public health because they are potent toxins and ubiquitous in food and feed products. There are currently more than 300 known mycotoxins; nevertheless, only some of these mycotoxins pose a real threat to food safety. The most relevant are the AFs, ochratoxin A (OTA), patulin (PAT), fumonisins (FBs), zearalenone (ZEN), trichothecenes, and ergot alkaloids, which are produced by species from the genera *Aspergillus*, *Penicillium*, *Fusarium,* and *Claviceps*.

Mycotoxins may be carcinogenic, mutagenic, teratogenic, cytotoxic, neurotoxic, nephrotoxic, immunosuppressive, and estrogenic. The severity of the effects depends largely on the ingested amounts and duration of exposure and on toxic synergies that may result from the simultaneous ingestion of different mycotoxins. Namely, the exposure to mycotoxins can cause: (i) acute toxicity resulting in the death of individuals (mycotoxicosis) when high levels are ingested; (ii) losses in weight and in production of milk and eggs in farm animals when levels ingested are below the lethal levels, (iii) suppression of immune functions and decrease resistance to infections when low levels are ingested, and (iv) the development of tumors and of chronic diseases in vital organs when low levels are ingested for long periods of time (Bullerman, 2000). Animal sensitivity to mycotoxins is also dependent of age, gender,

Address correspondence to Luís Abrunhosa, CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar 4710-057, Braga, Portugal. E-mail: luisjap@deb.uminho.pt

and of the physiological state of individuals. In developing countries, where populations are extremely dependent on local crops (e.g., corn in Africa) and where control measures are less restrictive, mycotoxins may cause high morbidity and premature death among humans (FAO/IAEA, 2001). In contrast, in developed countries, where control measures are more restrictive, the main human-health concerns are presently related to mycotoxin carcinogenicity and to the ability of mycotoxins to suppress the immune systems of individuals (FAO/IAEA, 2001).

In addition to the public health risks, mycotoxin contamination causes major economic losses at all levels of the food-production chain. For example, in crops, approximately 5–10% of the total global production appears to be irretrievably lost due to mycotoxins. In the United States, it is estimated that the presence of mycotoxins in corn, wheat and peanuts can cause direct losses of approximately 932 million dollars per year and indirect losses (e.g., costs due to regulation and enforcement, analysis, and implementation of other control measures) of more than 466 million (CAST, 2003). Major losses of livestock production may also occur because contaminated feed can cause feed refusal, resulting in poor feed conversion and thus in a decrease in animals' weight gain, but also immunosuppression, decreasing the resistance of animals to infections, and impaired reproductive capabilities. In addition, mycotoxin residues can carry over into meat, milk, or eggs and be consumed by humans.

To minimize the public health risks associated with mycotoxins, it is generally recommended that the mycotoxin levels in food and feed be reduced as low as technologically possible (Bennett and Klich, 2003). Preventative measures applied in agriculture and food industries are considered the most important. However, contamination of commodities with moulds and the subsequent occurrence of mycotoxins is inevitable under certain environmental conditions (Griessler et al., 2010). Therefore, decontamination or detoxification procedures are also applied in some cases. A great diversity of physical, chemical and biological methods are presently available for that purpose (Karlovsky, 1999; Huwig et al., 2001). To protect consumers' health, many countries have also implemented legislation that imposes limits on the presence of major mycotoxins in commodities. The limits can change according to countries as a result of their stage of development, different perceptions about the levels considered safe for health, or economic interests related to local cultures (van Egmond and Jonker, 2008). However, the limits guarantee with more or less efficiency that highly contaminated products are not traded and introduced into the human food chain. In the particular case of Portugal, as a member of the European Union (EU), the maximum limits of mycotoxins permitted comply with the European legislation. Regarding food, the last consolidated version of the Directive 1881/2006 sets the maximum levels for several mycotoxins in food (EC, 2011). With respect to feed, the only mycotoxin regulated to date is aflatoxin B_1, and its maximum values can be found in the Directive 2002/32/EC (EC, 2006b). For the presence of other mycotoxins in animal feed, there is the Commission recommendation 2006/576/EC, which establishes recommended limits for deoxynivalenol, ZEN, OTA, T-2 toxin, HT-2 toxin and FBs (EC, 2006a).

Aflatoxins

AFs are difuranocoumarins, a group of approximately 20 structurally closely related compounds that are produced by several *Aspergillus* species. These compounds were isolated and characterized for the first time from *A. flavus* after the episode mentioned above of the turkey deaths in England. The most important AFs are from series B and G, namely, aflatoxins B_1 and B_2 (AFB_1 and AFB_2) and aflatoxin G_1 and G_2 (AFG_1 and AFG_2). Aflatoxin M_1 (AFM_1), an AFB_1 hydroxylate that is excreted into milk when cows and other ruminants eat contaminated feed, is also among the most important AFs. The most prevalent aflatoxin in food and feed is AFB_1 (Figure 1). AFs are carcinogenic to humans and classified by the International Agency for Research on Cancer (IARC) as Group 1; AFM_1 is classified as Group 2B. These compounds particularly affect the liver, with several studies linking liver cancer with the presence of AFs in food. AFs also have immunosuppressive properties and can interfere with some nutritional factors (Williams et al., 2004). AFs are mainly found in peanuts and corn but may also occur in soybeans, sorghum, pistachio nuts, dried fruit, beer, milk (AFM_1), and spices. The presence of AFs in feed for farm animals is also very common.

Ochratoxin A

OTA was isolated and characterized for the first time from *Aspergillus ochraceus* (van der Merwe et al., 1965a) after it was found that corn infected with this fungus caused the death of experimental animals (van der Merwe et al., 1965b). This mycotoxin is a di-hydroisocoumarin, consisting of a molecule of 7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3-*R*-methylisocumarin (ochratoxin α) and one molecule of L-β-phenylalanine, which are linked together by an amide bond (Figure 1). OTA is mainly known for its nephrotoxic properties. OTA is considered to be the possible etiological cause of some kidney diseases, such as the porcine nephropathy in Scandinavia, the human Balkan endemic nephropathy (BEN) and associated urothelial tumors and chronic interstitial nephropathy (CIN) in North Africa (Krogh, 1992; Pfohl-Leszkowicz et al., 2002; Abid et al., 2003). OTA is also mutagenic, teratogenic, neurotoxic, hepatotoxic, and immunotoxic (Pfohl-Leszkowicz and Manderville, 2007). OTA is classified by the IARC as Group 2B because there is evidence for experimental animals but not for humans (IARC, 1993). OTA is often found in wheat, rye, coffee beans, nuts, raisins, wine, and pig meat or their processed products (Jørgensen, 2005). Due to its widespread

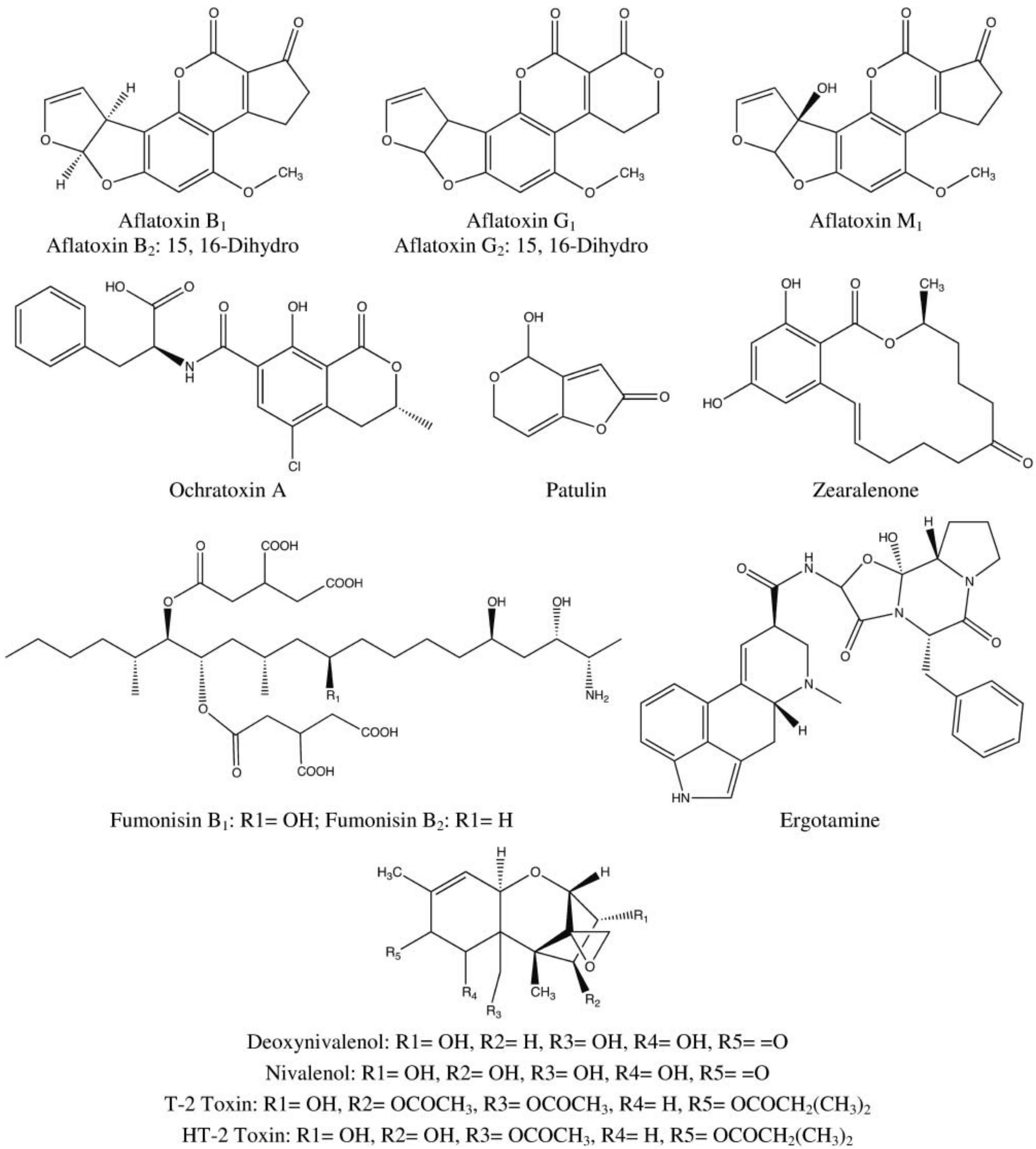

Figure 1 Chemical structure of the main mycotoxins.

occurrence in many products and because it is not readily eliminated from the body (with a half-life of 35.5 days for humans), OTA has been found in human blood (Thuvander et al., 2001) and breast milk (Skaug et al., 1998). In Portugal, OTA was also detected in the blood (Lino et al., 2008) and urine (Pena et al., 2006) of human individuals.

Patulin

PAT is a lactone from the polyketide group (Figure 1) that was discovered during the 1940s. PAT was studied for use as an antibiotic, but the discovery of its toxic effects to animals led to the abandonment of that possibility. This compound is produced by some species from the genera *Aspergillus*, *Penicillium,* and *Byssochlamys*, which are mainly associated with both fresh fruits, such as apples, pears, apricots, peaches, and grapes, and fresh vegetables. Apples and their derived food products, such as juices and purees, are the main dietary source of PAT. *Penicillium expansum* is the main deterioration agent of apples and is responsible for PAT contamination. The ingestion of PAT can cause acute symptoms, such as seizures, shaking, bowel bleeding, edema, and vomiting. Chronic symptoms include neurotoxic, immunotoxic, immunosuppressive, genotoxic, teratogenic, and carcinogenic effects (Moake et al., 2005). PAT is currently classified by the IARC as Group 3, that is, not classifiable with regard to its carcinogenicity to humans.

Fumonisins

FUM are polar secondary metabolites that have a long hydroxylated hydrocarbon chain (20 carbon atoms) containing methyl and amino groups and in which the hydroxyl groups on C_{14} and C_{15} are esterified with tricarboxylic acid. At least 28 FUM have been identified and grouped into four categories based on structural similarities (series A, B, C, and P). For food safety concerns, the most important are fumonisins from the series B (FBs) because they are the most frequently found on naturally contaminated corn. Among them, fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) (Figure 1) are the most relevant. The most important FBs producers are *Fusarium verticillioides*, *Fusarium proliferatum,* and other *Fusarium* species. However, it was recently discovered that *Aspergillus niger* also produced FBs, in particular, FB_2 (Frisvad et al., 2007). The toxic effects of FBs are mainly due to their capacity to inhibit ceramide synthase and, by consequence, to disrupt sphingolipid biosynthesis, resulting in disturbances of cellular processes, such as cell growth, differentiation, morphology, permeability, and apoptosis (Voss et al., 2007). In addition, FB_1 proved to promote the development of cancer in animals and appears to be related to an increased incidence of esophageal and liver cancer in humans (Michael, 1996). FB_1 and FB_2 are classified by the IARC as Group 2B. FBs are also experimentally associated with leukoencephalomalacia in horses and pulmonary edema syndrome in pigs.

Zearalenone

ZEN is a 6-[10-hydroxy-6-oxo-*trans*-1-undecenyl]-B-resorcyclic acid lactone (Figure 1) mainly produced by *Fusarium* species that occurs in several kinds of grains and mainly in corn. ZEN has a relatively low acute toxicity, but it interferes strongly with estrogen receptors and, consequently, with the reproductive tract of individuals. Among other effects, ZEN leads to decreased fertility, precocious puberty, changes in weight of the thyroid, adrenal and pituitary glands, changes of progesterone and estradiol levels in serum, fibrosis in the uterus, breast cancer, endometrial carcinoma, and hyperplasia of uterus (Zinedine et al., 2007). In experimental animals, ZEN also caused liver damages that evolved into liver cancer. According to the available toxicological data, ZEN is classified by the IARC as Group 3.

Trichothecenes

Trichothecenes are a large group of structurally related compounds that are produced by a great diversity of filamentous fungi. These compounds are grouped into type A, B, C, and D trichothecenes according to their structural similarities. The most relevant for food safety are type A and B trichothecenes, which are produced by species from the *Fusarium* genus that are common pathogens of cereals. The major type A trichothecenes are T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (DAS), and the major type B trichothecenes include deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (ADON) and nivalenol (NIV) (Figure 1) (Foroud and Eudes, 2009). Trichothecenes are mainly found in grains, such as wheat, rye, oat, or corn. Trichothecenes are extremely cytotoxic to eukaryotic cells because they inhibit the synthesis of nucleic acids and proteins, cell division and mitochondrial function as well as destabilize cell membranes (Foroud and Eudes, 2009). The epoxide ring present in the chemical structure of trichothecenes is considered the main characteristic responsible for their toxic effects. Trichothecenes have been responsible for some acute toxic episodes, such as alimentary toxic aleukia (ATA), a condition involving gastrointestinal tract irritation, vomiting, diarrhea and, in the more severe cases, leukemia, anemia, and even death of the individual (Rotter, 1996). Throughout history, there have been several episodes of ATA in humans. Trichothecenes also have immunosuppressive and immunostimulant effects, which result in decreased resistance to infections and neoplasms, and in the development of autoimmune diseases. DON, NIV, and T-2 toxin are classified by the IARC as Group 3.

Ergot Alkaloids

The ergot alkaloids are secondary metabolites produced by the genus *Claviceps*, the members of which are common pathogens of cereals and other pasture grasses. Oat, wheat, barley, and sorghum can be infected by these fungi, although rye is the most susceptible crop. *Claviceps* species produce macroscopic structures called sclerotia that grow in contaminated seeds, where they accumulate a mix of toxic alkaloids, including ergotamine (Figure 1). Presently, those sclerotia can be removed by modern technologies of cereal grain cleaning, which largely eliminates the incidence of ergot alkaloids in food for human consumption, but its presence in products intended for animal feed continues to raise concerns (Bennett and Klich, 2003). From the toxicological point of view, when ingested, these mycotoxins induce abdominal pain, vomiting, a burning sensation of the skin, insomnia, and hallucinations. In addition, violent seizures can occur due to its potent effect on the individual's central nervous system, and gangrene of the extremities may result from the toxins' vasoconstricting properties (Krska and Crews, 2008). Throughout history, there have been several deadly episodes of poisoning by ergot alkaloids. These occurrences are known as ergotism or St. Anthony's fire and are now infrequent.

PRESENCE OF MYCOTOXINS IN THE PORTUGUESE MARKET

The presence of mycotoxins in Portugal depends on the quality of food produced locally but also largely on the quality of food traded internationally. In Portugal, approximately 52% of all of the food consumed is imported. This dependence is significantly higher in some food categories; e.g., Portugal imports almost 80% of cereals, 86% of pulses, 25% of fresh fruits, 28% of meat products, 84% of vegetal oils, and 55% of the dried fruits consumed (INE, 2012a). Portugal is self-sustaining only with regard to dairy products, importing only 4% of goods consumed.

In the present work, we review the levels of mycotoxins found in food and feed products commercialized in Portugal, independently of the product origin. This extended overview aims to organize the information available from Portugal to be easily searchable and comparable with similar international data. With the information collected, the Portuguese population's exposure to the main mycotoxins is also assessed by calculating the probable daily intakes (PDI).

Mycotoxins in Food

Regarding food for human consumption, foods for babies and children are among the most protected food categories based on regulations. In the particular case of the European legislation, the maximum levels set for baby food are 0.10 μg/kg for AFB_1, 0.025 μg/kg for AFM_1, 0.50 μg/kg for OTA, 10.0 μg/kg for PAT, 200 μg/kg for DON and FBs, and 20 μg/kg for ZEN (EC, 2011). Only three works report the presence of mycotoxins in this particular kind of food in Portugal. Alvito et al. (2010) evaluated the presence of AFB_1, AFM_1, and OTA in instant flour and infant formulae based on milk powder, while Barriera et al. (2010) and Cunha et al. (2009) evaluated the presence of PAT in apple purees and juices for children. Table 1 summarizes the levels and incidences found.

From 152 samples analyzed, 38 were contaminated with at least one of these mycotoxins, representing an overall incidence of 25%. The most frequently found mycotoxins were AFM_1 in powdered milk and OTA in instant flour, with incidences of 86% and 65%, respectively. However, the concentrations detected are relatively low, as can be seen from the averages presented in Table 1. The higher concentration of AFM_1 found in powdered milk was 0.041 μg/kg in a dry basis. However, the respective European limit refers to products after reconstitution as instructed by the manufacturer. So, a direct comparison of the levels cannot be done. The greatest concern is related to the detection of more than one mycotoxin in five samples of the instant flours because this contamination may constitute an additional health risk for infants due to eventual synergistic toxic effects.

AFs are one of the most targeted mycotoxins in food because of their high toxicity but also because they are the most regulated worldwide. In the EU, the legal maximum limits vary according to the nature of food products from 2 to 12 μg/kg for AFB_1 and from 4 to 15 μg/kg for the sum of all AFs. For AFM_1, there is a limit to 0.05 μg/kg in milk. Table 2 summarizes the works that report the presence of AFM_1 in dairy products in Portugal. A total of 52% of all of the samples were contaminated with this mycotoxin. In the particular case of milk, the incidence is slightly greater, with 68% of the samples with AFM_1 levels ranging from <0.005 to 0.08 μg/kg. However, only 80 samples (9%) had values above the legal limit. The presence of AFM_1 was also detected in samples of yoghurt and cheese, with 6% of the samples with values above the legal limit set for milk. This comparison was done because there is no limit established for these products. Instead the EU

Table 1 Mycotoxins found in foods for infants and children

Mycotoxin/product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
AFB_1/instant flour	20/6 (30%)	0.002–0.009	0.013	0	(Alvito et al., 2010)
AFM_1/instant flour	20/4 (20%)	0.008–0.023	0.017	0	(Alvito et al., 2010)
AFM_1/powdered milk	7/6 (86%)	0.005–0.041	0.014	0	(Alvito et al., 2010)
OTA/instant flour	20/13 (65%)	0.01–0.212	0.065	0	(Alvito et al., 2010)
OTA/powdered milk	7/3 (43%)	0.011–0.136	0.094	0	(Alvito et al., 2010)
PAT/apple puree	2/1 (50%)	9.1	9.1	0	(Cunha et al., 2009)
PAT/apple puree	76/5 (7%)	0.82–5.7	n.a.	0	(Barreira et al., 2010)
Total	152/38 (25%)			0	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

Table 2 Aflatoxin M_1 found in dairy products

Product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit UE[d]	References
Milk	74/29 (39%)	0.060–0.065	0.062	29	(Ouakinin and Martins, 1982)
Milk	101/85 (84%)	0.005–0.061	n.a.	2	(Martins and Martins, 2000)
Milk	68/60 (88%)	0.010–0.024	n.a.	0	(Peito and Venâncio, 2004)
Milk	598/394 (66%)	<0.005–0.08	n.a.	49	(Martins et al., 2005)
Powdered milk	25/0 (0%)	0	0	0	(Martins et al., 2005)
Yoghurt	96/18 (19%)	0.019–0.098	0.048	6	(Martins and Martins, 2004)
Fresh cheese	42/0 (0%)	0	0	0	(Martins et al., 2005)
Cheese	128/8 (6%)	0.005–0.050	0.01	8	(Martins et al., 2007b)
Total	1,132/594 (52%)			94 (8%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

regulation refers only to milk for the manufacture of milk-based products. It should be noted that in the case of yoghurt, nearly all of the contaminated samples were from yoghurt with fruit pieces.

Regarding the other AFs, data for a wide variety of products can be found in the literature, although in some assays a proper amount of analyses was not performed. Table 3 presents a summary of the available data. Peanuts, corn, dried figs, and spices are among the most commonly analyzed. The highest incidence of contamination is found on dried figs, with 82% of samples positive for AFs. The number of samples that exceeded the legal limit is not available, but the maximum concentration detected was 159.4 μg/kg, a value well above that allowed in Europe for dried fruits (4 μg/kg). Spices and pistachios have the second highest incidence (64%). For spices, the concentration detected ranged from 0.4 to 58.0 μg/kg, with 10 samples exceeding the respective EU limit (5 μg/kg). The AF levels detected in pistachios reached a concentration of 360.7 μg/kg, a value that exceeds 36 times the legal limit for this product. In contrast, the incidence of AFs in peanuts does not exceed 18%, despite the fact that peanuts are presumably one of the products more susceptible to these mycotoxins. Nevertheless, the maximum concentration detected (902.4 μg/kg) is very high and exceeds 225 times the EU permitted limit. It should be noted that data from Peito and Venâncio (2004) was obtained from the Portuguese authorities responsible for the control of mycotoxins in imported products and that products with concentration above the EU limits are refused entry. For corn, the incidence and concentrations detected are very low and do not exceed the legal limits of 10 μg/kg. In products, such as honey, pine nuts, raisins, and pig liver, the presence of AFs was not detected.

Regarding OTA, this mycotoxin is mainly found in cereals and cereal-based products, although it is also common in coffee, spices, raisins, and red wine. The legal limits imposed by the EU also differ according to the type of food (EC, 2011). For example, unprocessed cereals have a limit of 5 μg/kg, and cereal-based processed foods have a limit of 3 μg/kg. Products

Table 3 Aflatoxins found in food

Product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
Spices	79/34 (43%)	1.25–58.0	8.8	9	(Martins et al., 2001a)
Spices	83/70 (84%)	0.4–20.1	n.a.	1	(Peito and Venâncio, 2004)
Honey	80/0 (0%)	0	0	0	(Martins et al., 2003a)
Almonds	56/8 (14%)	11.8–167.8	n.a.	n.a.	(Peito and Venâncio, 2004)
Peanuts	745/134 (18%)	0.7–902.4	n.a.	n.a.	(Peito and Venâncio, 2004)
Peanuts	12/6 (50%)	0.199–1.506	0.781	0	(Alves, 2009)
Hazelnuts	22/3 (14%)	0.6–0.9	n.a.	0	(Peito and Venâncio, 2004)
Cashew	23/5 (22%)	0.4–1.1	n.a.	0	(Peito and Venâncio, 2004)
Dry figs	303/247 (82%)	0.9–159.4	n.a.	n.a.	(Peito and Venâncio, 2004)
Nuts	15/1 (7%)	2.5	2.5	0	(Peito and Venâncio, 2004)
Pine nuts	3/0 (0%)	0	0	0	(Peito and Venâncio, 2004)
Pistachio nuts	58/37 (64%)	0.9–360.7	n.a.	n.a.	(Peito and Venâncio, 2004)
Raisins	17/0 (0%)	0	0	0	(Peito and Venâncio, 2004)
Pig liver	37/0 (0%)	0	0	0	(Martins and Magalhães, 2007)
Corn	95/8 (8%)	0.10–0.50	n.a.	0	(Soares and Venâncio, 2011)
Total	1,628/553 (34%)			10 (0.6%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

Table 4 Ochratoxin A found in food

Product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
Wheat	34/2 (6%)	<0.5	0.19	0	(Miraglia and Brera, 2002)
Rice	42/6 (14%)	0.09–3.52	n.a.	0	(Pena et al., 2005)
Cereals	38/6 (16%)	0.27–7.97	0.64	2	(Juan et al., 2008a)
Breakfast cereals	15/9 (60%)	<0.3–0.7	n.a.	0	(Peito and Venâncio, 2004)
Wheat flour	8/2 (25%)	<0.5	0.25	0	(Miraglia and Brera, 2002)
Corn bread	15/9 (60%)	n.a.–2.65	0.43	0	(Juan et al., 2007)
Corn bread	66/55 (83%)	0.10–3.848	0.383	1	(Duarte et al., 2010)
Corn bread	17/15 (88%)	0.06–1.09	0.32	0	(Paíga et al., 2012)
Wheat bread	61/25 (41%)	0.033–5.86	0.230	1	(Juan et al., 2008b)
Wheat bread	50/28 (56%)	0.02–0.490	0.250	0	(Bento et al., 2009)
Wheat bread	208/160 (77%)	0.10–1.510	0.209	0	(Duarte et al., 2010)
Wheat bread	25/18 (72%)	0.03–0.27	0.15	0	(Paíga et al., 2012)
Corn/rye bread	22/21 (95%)	0.03–2.09	0.58	0	(Paíga et al., 2012)
Coffee	38/6 (16%)	0.3–2.7	1.54	0	(Miraglia and Brera, 2002)
Coffee	60/20 (33%)	0.2–7.3	2.38	4	(Martins et al., 2003c)
Coffee	66/51 (77%)	1.0–12.1	n.a.	n.a.	(Peito and Venâncio, 2004)
Green coffee beans	257/229 (89%)	0.3–30.1	n.a.	n.a.	(Peito and Venâncio, 2004)
Spices	9/6 (67%)	0.2–8.5	4.0	0	(Miraglia and Brera, 2002)
Spices	25/22 (88%)	<0.3–52.8	n.a.	1	(Peito and Venâncio, 2004)
Coriander	10/0 (0%)	0	0	0	(Lino et al., 2006a)
Meat	38/5 (13%)	0.01–0.12	0.07	n.a.	(Guillamont et al., 2005)
Peanuts	12/1 (8%)	1.072	1.072	n.a.	(Alves, 2009)
Raw materials for beer	10/10 (100%)	0.121–0.204	n.a.	0	(Vicente et al., 2001)
Beer	2/2 (100%)	0.006–0.0069	0.0065	n.a.	(Nakajima et al., 1999)
Beer	17/2 (12%)	0.043–0.064	n.a.	0	(Vicente et al., 2001)
Beer	7/3 (43%)	0.002–0.006	0.004	0	(Miraglia and Brera, 2002)
Beer	28/10 (36%)	0.002–0.064	n.a.	0	(Peito and Venâncio, 2004)
Raisins	9/8 (89%)	<0.3–13.9	n.a.	n.a.	(Peito and Venâncio, 2004)
Total	1,189/731 (61%)			9 (0.8%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

such as raisins and coffee have a limit of 10 μg/kg, and spices cannot contain more than 15 μg/kg. Table 4 summarizes the occurrence of OTA in products from the Portuguese market.

In the case of cereals and cereal-based products, 59% of the 601 samples were contaminated with OTA. The detected concentrations ranged between 0.02 and 7.97 μg/kg, and only four samples had values above their respective legal limits. In coffee, there is a higher incidence, as 73% of the 421 samples had OTA. In this case, the concentrations found ranged from 0.2 to 30.1 μg/kg, and only four samples exceed the established EU legal limit (Martins et al., 2003c). In Portugal, the highest incidence of OTA is observed in spices, with 82% of samples being positive. In this case, the concentrations found ranged from 0.2 to 52.8 μg/kg, and only one sample exceeded the legal limit. In the case of beer, the incidence of OTA is approximately 31%. However, the concentrations found are very low (from 0.002 to 0.064 μg/kg).

It is well known that OTA occurs frequently in wines, particularly red wines. This issue is of particular importance to Portugal because wine is one of the agro-food products with the greatest economic impact. Table 5 presents a summary of OTA levels found in Portuguese wines and grapes for winemaking. Of 612 analyzed wines, 124 (20%) were contaminated with OTA. However, the detected concentrations were relatively low (from 0.002 to 2.4 μg/kg), and only two wines (0.3% of the samples) showed OTA levels above the legal limit of 2 μg/kg imposed by the EU.

Recently, the possible presence of FB_2 in wines was indicated by Frisvad et al. (2007), who reported that this mycotoxin could also be produced by *A. niger*, a species often found in grapes. To date, the only work available on the levels of FB_2 in Portuguese wines was published by Mogensen et al. (2010). These authors analyzed 7 Portuguese wines from different regions, and FB_2 was found in one only sample at a concentration of 2.8 μg/kg. Currently, there is no legal limit regarding this mycotoxin in wines. In Portugal, recent data showed that only 29% of the *A. niger* strains isolated from wine grapes are FB_2 producers and that only 6% of those can produce FB_2 at concentrations above 1 μg/kg (Abrunhosa et al., 2011). These incidences and production levels were found to be comparable to the production of OTA by *A. carbonarius* strains also isolated from Portuguese grapes, indicating that the concentration foreseen in wine may be similar for both of those mycotoxins. However, an eventual FB_2 legal limit for wine will most likely be far higher than the one set for OTA (2 μg/kg) because the tolerable daily intake (TDI)

Table 5 Ochratoxin A found in wine and wine grapes

Product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
Wine	6/6 (100%)	<0.003–0.017	0.011	0	(Zimmerli and Dick, 1996)
Wine	2/2 (100%)	0.30–0.34	0.32	0	(Majerus and Otteneder, 1996)
Wine	12/8 (67%)	0.003–0.017	n.a.	n.a.	(Burdaspal and Legarda, 1999)
Wine	66/3 (5%)	0.02–0.08	n.a.	0	(Festas et al., 2000)
Wine	61/0 (0%)	<0.02	0.01	0	(Miraglia and Brera, 2002)
Wine	37/5 (14%)	>0.05–n.a.	n.a.	0	(Soleas et al., 2001)
Wine	340/69 (20%)	0.084–2.1	n.a.	1	(Ratola et al., 2004)
Wine	5/5 (100%)	0.03–0.25	n.a.	0	(Shundo et al., 2006)
Wine	9/8 (89%)	0.010–0.139	0.060	0	(Burdaspal and Legarda, 2007)
Wine	12/4 (33%)	0.028–0.057	0.037	0	(Rosa et al., 2004)
Wine	60/12 (20%)	0–2.4	n.a.	1	(Pena et al., 2010)
Wine	2/2 (100%)	0.002–0.014	0.008	0	(Mikulíková et al, 2012)
Grapes	11/3 (27%)	0.035–0.061	0.051	n.a.	(Serra et al., 2004)
Grapes	4/3 (75%)	0.01–0.116	0.073	n.a.	(Serra et al., 2006a)
Grapes	60/26 (43%)	0.008–1.64	0.149	n.a.	(Serra et al., 2006b)
Total	687/156 (23%)			2 (0.3%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

recommended for FB_2 (2 μg/kg bw/day) is 118 times above the OTA limit (17 ng/kg bw/day), which was recently revised (EFSA, 2006).

PAT, as mentioned above, is a mycotoxin mainly associated with apples and apple-based products but also with other fresh fruits. The EU legal PAT limit for juice and cider is 50 μg/kg and for jams and purees is 25 μg/kg. Table 6 presents a summary of the PAT levels found by some researchers in Portugal. In apples, 68% of the samples were contaminated with this mycotoxin at concentrations from 3.0 to 1500.0 μg/kg, and only two samples exceeded the EU legal limit. The most heavily contaminated apples were also observed to contain more visible signs of rot. Regarding juices, 41% of the 135 analyzed samples had PAT at levels from 2.1 to 42.0 μg/kg, but none exceeded the EU legal limit. PAT was also found in quince and quince jam. In quince fruits, the PAT concentrations were between 4.9 and 118.3 μg/kg. In quince jams, PAT was found in 40% of the samples at concentrations between 9.7 and 28.7 μg/kg, with two samples exceeding the EU legal limit for apple-based products. Quince products are not regulated because EU legislation makes an exclusive reference to apple-based products. However, they are a proven source of PAT.

Fumonisins from series B (FBs) occur mainly in corn and corn-based food products. Therefore, the EU only provides legislation for those products. The legal limits vary between 800 μg/kg for breakfast cereals and 4000 μg/kg for unprocessed corn. According to the data available in the literature (Table 7), 65% of the corn samples have FBs at concentrations ranging from 0.10 to 1162.0 μg/kg. Only one sample exceeded the imposed EU limit. In processed corn products, the incidence of positive samples is 60%, and the concentrations found ranged between 50 and 2026.0 μg/kg. In this case, only four samples exceeded the EU legal limit currently enforced. Apart from corn products, FBs were found in black tea and medicinal plants (Martins et al., 2001b). An incidence of 82% was found in these products at levels that ranged from 20 to 700 μg/kg. These products are not currently regulated by the European legislation. However, there seems to be a need for some kind of regulation because other studies have

Table 6 Patulin found in foods

Product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
Apples	3/1 (33%)	740.0	740.0	1	(Nunes et al., 2001)
Apples	351/241 (69%)	3.0–80.5	20.5	n.a.	(Martins, 2002)
Apples	4/3 (75%)	3.2–1,500.0	505.0	1	(Cunha et al., 2009)
Fruit juices	38/9 (4%)	5.0–25.2	13.9	0	(Majerus and Kapp, 2002)
Apple juices	29/18 (62%)	2.1–12.6	5.6	0	(Cunha et al., 2009)
Apple juices	68/28 (41%)	3.9–42.0	n.a.	0	(Barreira et al., 2010)
Fruit puree	5/0 (0%)	0	0	0	(Majerus and Kapp, 2002)
Quinces	4/3 (75%)	4.9–118.3	56.9	2	(Cunha et al., 2009)
Quince jam	10/4 (40%)	9.7–28.7	21.4	2	(Cunha et al., 2009)
Total	512/307 (60%)			6 (1%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

Table 7 Fumonisins found in foods

Product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
Black tea	18/16 (89%)	80.0–280.0	n.a.	n.a.	(Martins et al., 2001b)
Infusion plants	69/55 (80%)	20.0–700.0	n.a.	n.a.	(Martins et al., 2001b)
Corn	11/8 (73%)	113.0–1,162.0	460.0	1	(Lino et al., 2006b)
Corn	95/61(64%)	0.10–100.0	n.a.	0	(Soares and Venâncio, 2011)
Corn-based products	20/6 (30%)	183.0–2,026.0	392.3	1	(Lino et al., 2006b)
Corn-based products	96/77 (80%)	50.0–1,300.0	314.1	3	(Martins et al., 2008b)
Breakfast cereals	20/0 (0%)	0	0	0	(Silva et al., 2007)
Corn snacks	16/1 (6%)	260.0	260.0	0	(Silva et al., 2007)
Corn bread	30/25 (83%)	142.0–550.0	274.0	0	(Lino et al., 2007)
Total	375/249 (66%)			5 (1%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

also reported the presence of mycotoxins in these products (Sewram et al., 2006; Santos et al., 2009). The simultaneous occurrence of several mycotoxins that may result in synergetic toxic effects for consumers should be particularly highlighted (Santos et al., 2009).

Corn is also very susceptible to ZEN contamination, which may also be found in other types of grains. EU regulation established limits for ZEN that vary between 50 μg/kg for bread, bakery products and breakfast cereals and 400 μg/kg for refined corn oil. The data available for Portugal are presented in Table 8. Cereals and cereal-based products are the most analyzed, although in some cases, the number of samples tested is low. The most comprehensive work analyzed 307 samples of corn, corn flour, wheat and wheat-based products, barley, sunflower, soybean, and alfalfa (Marques et al., 2008). In this work, ZEN was detected in 56% of the samples at levels between 5.0 and 930.0 μg/kg, and 13 of those samples exceeded the respective EU legal limits. In breakfast cereal, the observed incidence was slightly higher (66%). In those products, the concentrations ranged from 2.5 to 69.0 μg/kg. Cunha and Fernandes (2010) reported two samples that exceeded the EU legal limit of 50 μg/kg.

Trichothecenes, as mentioned above, are grouped into type A and type B. Among the most important compounds of type A are T-2 and HT-2 toxin. Among the most important of type B are DON, ADON, and NIV. Currently, only DON is regulated in the EU. The existing limits range from 500.0 μg/kg for bread, other bakery products and breakfast cereals to 1750.0 μg/kg for wheat, oats and unprocessed corn. Table 9 summarizes the levels found in Portugal. In breakfast cereals, 72% of the samples had DON at concentrations between 25.0 and 6040.0 μg/kg, and 16 samples exceeded the EU limit of 500 μg/kg. In the other samples of cereals and cereal-based products, 22% of the 445 samples analyzed were contaminated with DON. Of those, only six samples showed a concentration above the EU legal limit. The concentrations found were between 8.0 and 1821.0 μg/kg. Data on the presence of ADON and NIV are not currently available for Portugal. Regarding type A trichothecenes, the only reference found refers to T-2 toxin, which was not found in any of the wheat and corn samples analyzed (Peito and Venâncio, 2004).

Mycotoxins in Feed

In animal nutrition, the only mycotoxin regulated so far in the EU is AFB_1 (EC, 2006b). The limits set range from 5 μg/kg for finish feed for dairy animals to 20 μg/kg for raw materials and other finish feed. Regarding other mycotoxins, the Commission Recommendation 2006/576/EC presents a compilation of the recommended limits for DON, ZEN, OTA, FBs, T-2 toxin, and HT-2 toxin. The lowest concentrations are

Table 8 Zearalenone found in foods

Product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
Wheat-based products	4/2 (50%)	11.0–15.0	13.0	0	(Peito and Venâncio, 2004)
Cereals and corn	307/171 (56%)	5.0–930.0	70.0	13	(Marques et al., 2008)
Breakfast cereals	11/7 (64%)	2.5–11.0	5.1	0	(Peito and Venâncio, 2004)
Breakfast cereals	18/12 (67%)	28.0–69.0	42.7	2	(Cunha and Fernandes, 2010)
Wheat flour	7/1 (14%)	27.0	27.0	0	(Cunha and Fernandes, 2010)
Corn flour	5/0 (0%)	0	0	0	(Cunha and Fernandes, 2010)
Cassava flour	1/1 (100%)	14.0	14.0	0	(Cunha and Fernandes, 2010)
Total	353/194 (55%)			15 (4%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

Table 9 Trichothecenes found in food

Mycotoxin/product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
DON/					
Cereals and corn	307/83 (27%)	96.0–1,790.0	170.0	1	(Marques et al., 2008)
Wheat-based products	10/4 (40%)	333.0–1,821.0	378.7	4	(Peito and Venâncio, 2004)
Corn-based products	105/0 (0%)	0	0	0	(Martins et al., 2008b)
Wheat flour	10/8 (80%)	20.0–77.0	n.a.	1	(Moura et al., 1998)
Wheat flour	7/3 (43%)	205.0–434.0	322.0	0	(Cunha and Fernandes, 2010)
Corn flour	5/1 (20%)	>8.0–<25.0	n.a.	0	(Cunha and Fernandes, 2010)
Cassava flour	1/1 (100%)	48.0	48.0	0	(Cunha and Fernandes, 2010)
Breakfast cereals	10/10 (100%)	25.0–426.0	161.0	n.a.	(Peito and Venâncio, 2004)
Breakfast cereals	88/64 (73%)	103.0–6,040.0	754.0	16	(Martins and Martins, 2001a)
Breakfast cereals	18/10 (56%)	46.0–525.0	194.2	0	(Cunha and Fernandes, 2010)
Total	561/184 (33%)			22 (4%)	
T-2 toxin/					
Wheat	9/0 (0%)	0	0	0	(Peito and Venâncio, 2004)
Corn	10/0 (0%)	0	0	0	(Peito and Venâncio, 2004)

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

100 μg/kg for ZEN in feed for pigs and for OTA in poultry feed. The highest recommended concentrations are for FB_S, which can reach an acceptable concentration of 60 mg/kg in corn used as raw materials for feed. In Portugal, data on mycotoxins in such products is more abundant (Table 10). The only exception relates to T-2 and HT-2 toxins. Regarding AFs, a total of 3535 samples were analyzed between 1999 and 2011. On average, 22% of the samples were contaminated with AFs, and only 3% of those showed a concentration above the EU legal limit established for the products in question. The highest

Table 10 Aflatoxins and OTA found in animal feed and raw materials

Mycotoxin/product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit EU[d]	References
AFs/					
Feed	80/36 (45%)	1.0–16.0	n.a.	0	(Martins and Martins, 1999)
Pig feed	106/19 (18%)	1.0–7,470.0	n.a.	2	(Martins and Martins, 2001b)
Cattle feed	189/92 (49%)	1.0–740.0	n.a.	29	(Martins and Martins, 2001b)
Poultry feed	117/23 (20%)	1.0–68.0	n.a.	1	(Martins and Martins, 2001b)
Raw materials	104/34 (33%)	1.0–166.0	15.0	n.a.	(Novo et al., 2001)
Cattle feed	57/26 (46%)	1.0–16.0	4.0	n.a.	(Novo et al., 2001)
Pet feed	60/0 (0%)	0	0	0	(Martins et al., 2003b)
Cattle feed	399/34 (9%)	5.0–15.0	n.a.	n.a.	(Peito and Venâncio, 2004)
Poultry feed	85/16 (19%)	1.0–20.0	n.a.	n.a.	(Peito and Venâncio, 2004)
Pig feed	74/7 (9%)	1.0–2.0	n.a.	n.a.	(Peito and Venâncio, 2004)
Cattle feed	1,001/374 (37%)	1.0–74.0	18.1	62	(Martins et al., 2007a)
Pet feed	31/0 (0%)	0	0	0	(Guerra et al., 2007)
Raw materials	513/63 (12%)	1.0–45.0	n.a.	n.a.	(Martins et al., 2008a)
Feed	583/62 (11%)	1.0–21.0	n.a.	4	(Martins et al., 2008a)
Oats	45/0 (0%)	0	0	0	(Almeida et al., 2008)
Raw materials	4/1 (25%)	2.0	2.0	0	(Griessler et al., 2010)
Fish feed	87/0 (0%)	0	0	0	(Almeida et al., 2011b)
Total	3,535/787 (22%)			98 (3%)	
OTA/					
Pet feed	60/5 (8%)	2.0–3.6	2.8	0	(Martins et al., 2003b)
Horse feed	50/30 (60%)	2.0–3.2	n.a.	0	(Guerra et al., 2005)
Oats	45/9 (20%)	1.0–1.61	n.a.	0	(Almeida et al., 2008)
Laboratory mice feed	31/0 (0%)	0	0	0	(Almeida et al., 2010)
Raw materials	4/2 (50%)	3.0–4.0	3.5	0	(Griessler et al., 2010)
Pig feed	478/31 (6.5%)	2.0–130.0	11.0	1	(Martins et al., 2012a)
Poultry feed	186/12 (6.5%)	2.0–10.9	5.7	0	(Martins et al., 2012a)
Pig feed	277/21 (7.6%)	2.0–6.8	3.9	0	(Almeida et al., 2011a)
Total	1,131/110 (10%)			1 (0.1%)	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; n.a., not available.

level found was 7470.0 μg/kg in finish feed for pigs. It should be noted that the highest concentrations were recorded in analyses performed between 1999 and 2001. For OTA (Table 10), only 10% of the samples contained this mycotoxin, and only one had a value above that recommended by the EU. The concentrations found varied between 1.0 and 130.0 μg/kg. The highest incidences were found in raw materials and feed for horses.

The occurrence of *Fusarium* mycotoxins in feed and feedstuffs in Portugal are summarized in Table 11. FBs are among the most analyzed. Concentrations between 10.0 and 32,200.0 μg/kg were found in a great variety of products, with corn-based products presenting the highest levels (on average, 6721.3 μg/kg). Nevertheless, the overall incidence of FBs is only 12%. Although some of the FBs levels are quite high when compared with other mycotoxins, no sample exceeded the EU recommended limits. In contrast, ZEN was detected in 25% of the samples. For this mycotoxin, the concentrations found were significantly lower (between 5.0 and 356.0 μg/kg). However, the recommended limits are also lower (between 100.0 and 2000.0 μg/kg). Thus, in this case, only one of the samples exceeded the EU limits. The highest levels of ZEN were found in feed for pigs and in corn. DON, has an incidence of 14%. The highest concentrations were found in raw materials, mainly in corn (between 110.0 and 3793.0 μg/kg); only one sample of feed for pigs exceeded the EU recommended limit of 900.0 μg/kg. As previously mentioned, information on T-2 and HT-2 toxins in feed from Portugal is not

Table 11 Fusarium mycotoxins found in animal feed and raw materials

Mycotoxin/product	No. of samples[a]	Concentration[b] (μg/kg)	Mean[c] (μg/kg)	>Limit UE[d]	References
FBs/					
Poultry feed	12/3 (25%)	24.0–253.0	103.7	0	(Martins and Martins, 2001b)
Pet feed	60/3 (5%)	12.0–24.0	17.3	0	(Martins et al., 2003b)
Corn	12/8 (67%)	25.0–32,200.0	11,900.0	0	(Peito and Venâncio, 2004)
Oats	5/2 (40%)	132.0–421.0	277.0	0	(Peito and Venâncio, 2004)
Poultry feed	22/20 (91%)	31.0–7,437.0	1,177.0	0	(Peito and Venâncio, 2004)
Horse feed	7/6 (86%)	60.0–500.0	307.0	0	(Peito and Venâncio, 2004)
Poultry feed	52/10 (19%)	50.0–109.0	73.6	0	(Martins et al., 2006)
Feed	357/6 (2%)	12.0–34.0	n.a.	0	(Martins et al., 2008a)
Raw materials	208/19 (9%)	10.0–40.0	n.a.	0	(Martins et al., 2008a)
Oats	45/0 (0%)	0	0	0	(Almeida et al., 2008)
Corn	11/11 (100%)	225.0–4,607.0	1,542.6	0	(Monbaliu et al., 2010)
Raw materials	11/7 (64%)	99.0–3,093.0	631.0	0	(Griessler et al., 2010)
Pig feed	127/11 (9%)	50.0–390.0	163.7	0	(Almeida et al., 2011a)
Pig feed	358/51 (14%)	53.7–3,815.5	247.9	0	(Martins et al., 2012b)
Horse feed	31/2 (6%)	79.6–138.8	109.2	0	(Martins et al., 2012b)
Total	1,318/159 (12%)			0	
ZEN/					
Poultry feed	52/16 (31%)	5.1–61.3	25.3	0	(Martins et al., 2006)
Pig feed	30/4 (13%)	104.0–356.0	n.a.	1	(Martins et al., 2008a)
Horse feed	50/0 (0%)	0	0	0	(Martins et al., 2008a)
Corn	11/5 (45%)	73.0–281.0	127.2	0	(Monbaliu et al., 2010)
Raw materials	26/10 (38%)	11.0–57.0	33.5	0	(Griessler et al., 2010)
Pig feed	404/107 (26%)	5.0–73.0	19.2	0	(Almeida et al., 2011a)
Total	573/142 (25%)			1(0.2%)	
DON/					
Pet feed	60/3 (5%)	100.0–130.0	116.0	0	(Martins et al., 2003b)
Poultry feed	52/7 (13%)	100.0–226.5	118.1	0	(Martins et al., 2006)
Raw materials	224/24 (11%)	100.0–500.0	n.a.	0	(Martins et al., 2008a)
Pig feed	291/9 (3%)	100.0–1,649.0	n.a.	1	(Martins et al., 2008a)
Horse feed	50/15 (30%)	100.0–320.0	n.a.	0	(Martins et al., 2008a)
Pet feed	20/3 (15%)	100.0–130.0	n.a.	0	(Martins et al., 2008a)
Oats	45/2 (4%)	309.5–715.4	512.5	0	(Almeida et al., 2008)
Corn [e]	11/10 (91%)	110.0–3,793.0	874.1	0	(Monbaliu et al., 2010)
Raw materials [f]	35/24 (69%)	59.0–1,010.0	399.5	0	(Griessler et al., 2010)
Pig feed	277/47 (17%)	100.0–864.0	223.2	0	(Almeida et al., 2011a)
Total	1,065/144 (14%)			1 (0.1%)	
T-2, HT-2/					
Raw materials	9/3 (33%)	35.0–40.0	14.0	n.a.	(Griessler et al., 2010)
Corn	11/0 (0%)	0	0	0	(Monbaliu et al., 2010)
Total	20/3 (15%)			0	

[a]No. of samples/no. of positive samples (% of contaminated samples); [b]minimum–maximum concentration detected; [c]average of positive samples; [d]no. of samples with concentrations above the European Union legal limit; [e]sum of DON, NIV, and ADON; [f]sum of DON and ADON; n.a., not available.

abundant. The few data available are from Griessler et al. (2010) and Monbaliu et al. (2010), who report the levels of T-2 and HT-2 toxins in Portuguese raw materials. In the first case, three of nine samples were contaminated, with values between 35.0 and 40.0 μg/kg. In the second study, none of the corn samples analyzed had these mycotoxins.

PROBABLE DAILY INTAKES

Using the assembled data of mycotoxin levels in Portuguese food and Portuguese food-consumption data, the PDI was estimated for each mycotoxin. The Portuguese food-consumption data used for calculation were mainly obtained from studies conducted by Rodrigues et al. (2007), which are available at the Dafne-AnemosSoft application tool (http://www.hhf-greece.gr/dafnesoftweb). This application assembles the data from the Data Food Networking project (DAFNE), which is based on information collected in Europe in the context of household budget surveys (HBS). The available information refers to national representative samples of the overall population. For the present work, data from Rodrigues et al. (2007) was used. These researchers have demonstrated that the HBS-derived data are better to evaluate Portuguese dietary habits, since they are closer to data collected from a food-frequency questionnaire conducted in the city of Porto (Lopes et al., 2006) than to data from food balance sheets generated by FAO for the Portuguese population. However, in particular cases when the necessary data were not available from DAFNE, we used data from the Porto consumption survey (Lopes et al., 2006) or the daily edible supply of food products per person available from the Portuguese official statistics database (INE, 2012b).

PDI were calculated according to the methodology of the SCOOP Task Reports that evaluated the dietary intake of some mycotoxins in Europe (Majerus and Kapp, 2002; Miraglia and Brera, 2002; Gareis and Zimmermann, 2003). We used the average level of each mycotoxin in each food commodity by accounting for both positive and negative results and using LOD/2 for results lower than LOD. When the averages were unavailable, we calculated weighed averages using the data available in each publication. In the worst scenario, when data was very scarce, the means were calculated using the 25th percentile of the lowest and highest concentrations reported. The PDI was then calculated using the following equation:

$$PDI\left(\frac{\frac{\text{ng}}{\text{kg}}\text{bw}}{\text{day}}\right) = \frac{\text{mean level in food}\left(\frac{ng}{g}\right) * \text{mean in take of food}\left(\frac{\frac{g}{\text{person}}}{\text{day}}\right)}{\text{body weight (65kg)}}$$

The final PDI for each mycotoxin was calculated by summing the intakes obtained for each food commodity.

The calculated PDI for the Portuguese population are presented in Table 12, where they can be compared with other PDI of the European and French populations available in the literature. All of the Portuguese PDI appears to be quite below the maximum value reported for European countries. However, the Portuguese PDI for AFs, PAT, and deoxynivalenol exceeded the PDI of the same mycotoxins for the French population.

The calculated PDI were also compared with the respective temporary total dietary intake (t-TDI) by expressing them as a percentage of t-TDI. In this case, OTA accounts for 7%, PAT for 8.8%, FBs for 5.4%, ZEN for 17%, and DON for 9.5% of the respective t-TDI. Because the percentages are very low, it can be said that the mycotoxin intakes of Portuguese population are presently at a safe level.

For AFs, a t-TDI was not found for comparative purposes. In this case, because these compounds are carcinogenic, a numerical TDI is not specified by the international expert committees. Instead, it is recommended that AF levels should be as low as reasonably achievable. Nevertheless, the tumor potency of 1 ng/kg bw/day for human liver cancer due to aflatoxin B_1 exposure (which corresponds to approximately one extra

Table 12 Probable daily intake (PDI) of mycotoxins in Portugal, reported European and French PDI, t-TDI enforced and expression of Portuguese PDI as a percentage of t-TDI

	(ng/kg bw/day)						
Mycotoxin	PDI[a]	% of the t-TDI	European PDI (ref.)		French PDI[b]	t-TDI[c] (ref.)	
AFM_1	0.073	—	0.11	(FAO/WHO, 2002)	0.09	—[d]	
AFs	0.501	—	0.03–1.3	(EFSA, 2007)	0.12	—[d]	
OTA	1.196	7.0	0.13–3.14	(Miraglia and Brera, 2002)	2.2	17	(EFSA, 2006)
PAT	35.2	8.8	0.07–140.4	(Majerus and Kapp, 2002)	18.0	400	(EC, 2011)
FBs	108.7	5.4	0.24–264.7	(Gareis and Zimmerman, 2003)	281	2,000	(EFSA, 2005)
ZEN	33.9	17.0	1–420	(EFSA, 2004b)	33.0	200	(EFSA, 2004b)
DON	95.4	9.5	78–480	(Gareis and Zimmerman, 2003)	14.0	1,000	(EFSA, 2004a)

[a]PDI–calculated using the mean of all of the samples analysed by using LOD/2 for results lower than LOD; [b](Leblanc et al., 2005); [c]t-TDI–temporary tolerable daily intake; [d]no t-TDI is set for aflatoxins because they are carcinogenic, and it is recommended that their level should be as low as reasonably achievable (ALARA).

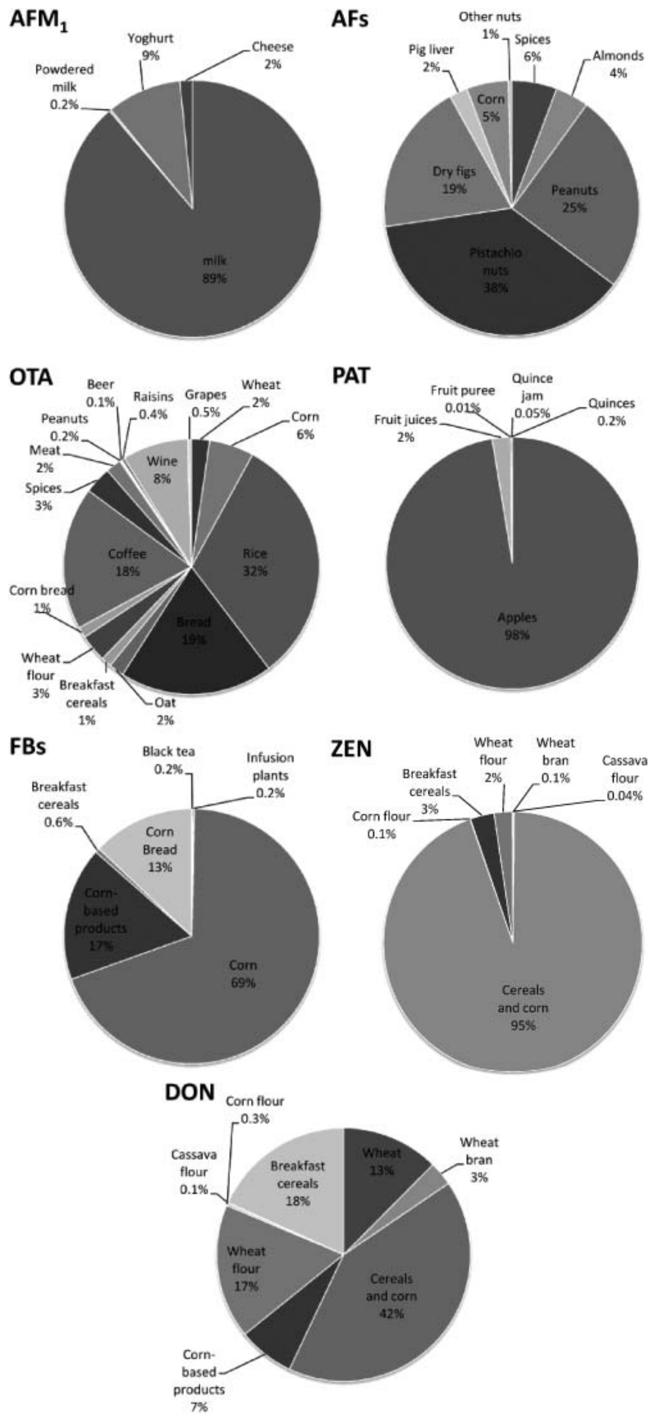

Figure 2 Contribution of each food commodity to the probable daily intake (PDI) of mycotoxins in Portugal.

cancer case for 10^5 individuals) is used as a reference TDI by some authors. The Portuguese PDI for AFM_1 and AFLs are below this level of 1 ng/kg bw/day, and it can, therefore, be considered that the AFM_1 and AFLs daily intakes do not contribute to major health problems for the Portuguese population.

Figure 2 depicts the contribution of each food commodity to the calculated Portuguese PDI. For AFM_1, milk was the main contributor (89%). For AFs, pistachio nuts, peanuts, and dry figs were the main contributors, with 38, 26 and 19%, respectively. For OTA, rice, bread, coffee, and wine were the main contributors, with 32%, 19%, 18%, and 8%, respectively. All cereals and cereal-derived products grouped account for 67% of the OTA intakes, thus becoming to the most important contributor. Miraglia and Brera (2002) also reported similar data. In their evaluation of OTA dietary intakes by the population of EU Member States, they found that cereals (50%), wine (13%), coffee (10%), and spices (8%) were the main contributors for OTA intakes. Compared with the present study, OTA intakes from cereals and coffee are higher in Portugal. However, intakes from wine are lower likely because the OTA contents in Portuguese wines are low (on average, 0.1 μg/L) when compared with other countries. In the particular case of PAT, apples were the main contributor (98%). However, it should be emphasized that the mean PAT levels in the apples used to perform the PDI calculations are probably over-estimated because, according to authors, some apple samples had high visible signs of moulds. For FBs, the main contributors were corn, corn-based products and corn bread, with 69%, 17%, and 13%, respectively. For ZEN, the main contributors were cereals and corn, accounting for 95%, but the data available was insufficient to obtain a more representative and discriminate distribution of the main food contributors. For DON, the main contributors were cereals and corn, breakfast cereals, wheat flour, and wheat, with 42%, 18%, 17%, and 13%, respectively. In the work of Gareis and Zimmermann (2003), the main contributors to DON intakes by the population of EU Member States were wheat, bread and wheat flour (percentages are not available).

CONCLUSIONS

In conclusion, this review on the occurrence of mycotoxins in Portugal shows that mycotoxins occur in a wide variety of products, similar to the case in other European countries (Logrieco and Visconti, 2004) and elsewhere in the world (Murphy et al., 2006). Furthermore, this review allows to draw some conclusions on the overall incidence of major mycotoxins and on the average levels found in different products. Namely, the highest incidences of positive samples are found in food for human consumption and not in feed. In fact, the global incidences of FBs, PAT, OTA, ZEN, and AFM_1 in food were 66%, 60%, 59%, 55%, and 52%, respectively, while in feed, the highest incidence did not exceed 25% (for ZEN). Regarding the average levels, the opposite tendency was observed, as average levels are generally three times higher in feed than in food. Major differences were observed for OTA and FBs, with concentrations sevenfold and fourfold higher in feed, respectively. It may also be observed from the global data that in food for humans, 3% of the samples exceeded the respective EU legal limit, whereas in animal feed, only 1% of the samples exceeded the EU recommended limits. However, it should be noted that the latter limits are substantially higher than the limits set for human food.

The continuous control of mycotoxin levels in food and feed is an important tool to support measures to reduce consumer exposure to mycotoxins and, therefore, to strengthen food safety. Additionally, such control may be also important as a source of useful data to estimate, for example, the dietary intakes of mycotoxins for particular populations. Unfortunately, some of the data reported are not always comprehensive because some important numbers are often not included in the studies. For this purpose, it is advisable that researchers follow the recommendations of the European Mycotoxins Awareness Network (http://www.mycotoxins.org) when they publish information on mycotoxin surveys. Nonetheless, using the available data for Portugal, we could obtain reliable PDI for the most important mycotoxins, which are in accordance with those that can be found in the literature for other European countries.

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