CHAPTER 5

MYCOTOXINS. OCHRATOXIN A

5.1. MYCOTOXINS

The term mycotoxin is derived from the Greek word 'mycos' meaning mould, and the Latin word 'toxicum', which means poison. Mycotoxins are relatively low-molecular weight secondary metabolites of fungal origin that are harmful to animals and humans. They have always been a hazard to men and domestic animals, but until the decade following 1970, their effects have not been largely studied.

Mycotoxins are considered secondary metabolic products because they are not necessary for fungal growth and are simply a product of the primary metabolic processes. Secondary metabolism usually occurs after a phase of balanced growth and it is often associated with developmental processes. Thus, sometimes mycotoxins are secreted by growing colonies at the approximate time of sporulation (Calvo et al., 2002), but the functions of mycotoxins are still an enigma. They are believed to protect the mould and act as a defence mechanism by excluding or poisoning animals, plants or other competing fungal species in the same environment. The production of particular secondary metabolites such as mycotoxins, phytotoxins or antibiotics, is usually restricted to a small number of species and may be species, or even strain, specific (Smith and Moss, 1985).

The amount of mycotoxins needed to produce adverse health effects varies widely among toxins, as well as for each animal or person's immune system. Two concepts are needed to understand the negative effects of mycotoxins on human health:

- **Acute toxicity**, defined as the rapid onset of an adverse effect from a single exposure.

- **Chronic toxicity**, the slow or delayed onset of an adverse effect, usually from multiple, long-term exposures.

Mycotoxins can be acutely or chronically toxic, or both, depending on the kind of toxin and the dose. In terms of acute toxicity, the mycotoxins most commonly encountered in food are about a factor of a million times less toxic than the most virulent of the botulism toxins (Moss, 1995). It is the long term toxicity which is of special concern because certain mycotoxins ingested in minor quantities with the daily diet for an extended period are known to be carcinogenic and to influence the immune response of a number of animal species, being also a risk to human health (Table 1).

Table 1. Rating health risks from foods (Kuiper-Goodman, 1998).

Acute		Chronic
HIGH		
Microbiological		**Mycotoxins**
Phycotoxins		Anthropogenic contaminants
Mycotoxins		Unbalanced diet
Anthropogenic contaminants		Phycotoxins
Pesticide residues		Food additives
Food additives		Pesticide residues
		Microbiological
LOW		

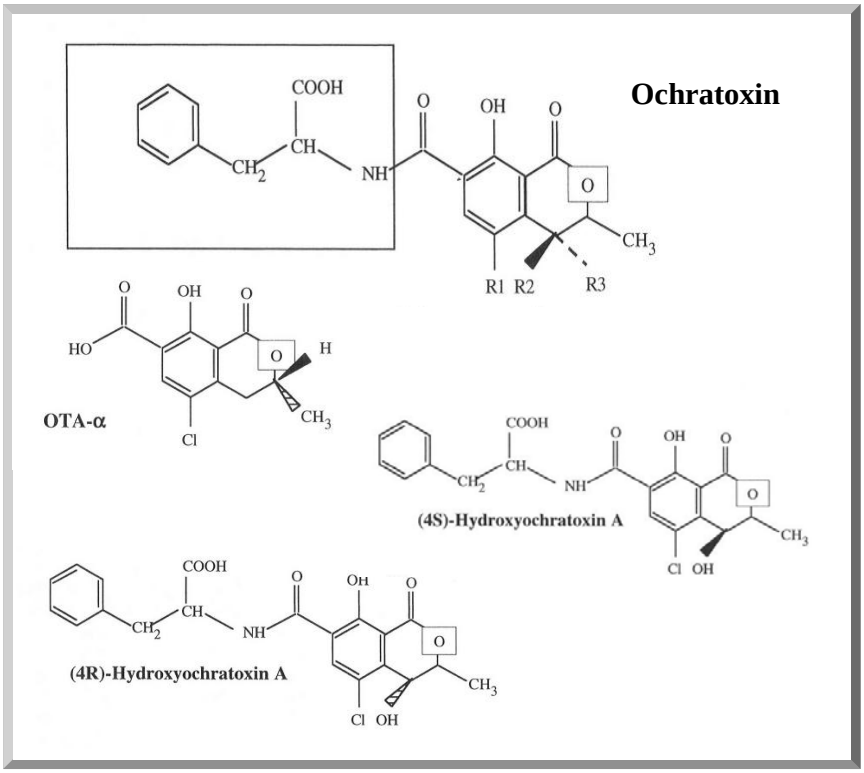
Over 300 mycotoxins have already been identified, produced by approximately 350 species of fungi (Betina, 1989). Nowadays, this number has increased – Bennet and Klich (2003) have recently made an estimation of near 400 mycotoxins -, but the exact number has never been accurately determined. However, not all fungi produce mycotoxins and among the toxigenic species, some only produce one type of mycotoxin, while others are able to produce several. Also, a specific type of mycotoxin can be produced by different fungal species (Boutrif and Bessy, 2001). The suspicion is that nearly all fungal metabolites, if tested, would show some sort of toxicity, and that all foods and feeds susceptible to mould growth may be potentially contaminated under the appropriate environmental conditions (Pohland, 1993). Therefore, when the pathogen is a mycotoxigenic fungus, information has to be acquired not only by monitoring host, pathogen, environment and disease, but also the toxins which may accumulate (Battilani et al., 2003).

The main mycotoxins that have been related to human intoxication include aflatoxins, cyclopiazonic acid, citreoviridin, fumonisins, 3-nitropropionic acid, ochratoxins, certain trichothecenes and zearalenone (Peraica and Dominjan, 2001).

5.2. OCHRATOXINS

Ochratoxins are mycotoxins produced by two main genera of fungi, *Aspergillus* and *Penicillium*. Chemically, ochratoxins are described as weak organic acids consisting of a dihydroisocumarin moiety joined by a peptide bond to 1-phenylalanine (O'Brien and Dietrich, 2005). There are three generally recognized ochratoxins, designated A, B and C (Figure 4). Structurally, these three toxins differ only very slightly from each other;

however, ochratoxin A (OTA) is chlorinated and is the most toxic, followed by OTB (substitution of chloride for a hydrogen atom in the isocumarin moiety), which is at least an order of magnitude less toxic, and OTC, or ethyl OTA, with little or no toxic potential (van der Merwe et al., 1965; Li et al., 1997). OTA, and occasionally OTB, occur naturally in mouldy products. However, a wide range of related compounds like ochratoxin α -the isocoumarin nucleus of OTA-, its dechlorinated analogue known as ochratoxin β, methyl and ethyl esters, and several amino acid analogues, are synthesized in laboratory cultures (Moss, 1996; Xiao et al., 1995). Ochratoxin α and β, are hydrolysis products of OTA and OTB respectively, and as consequence of the lack of the phenylalanine molecule, they are not toxics.



	R1	R2	R3	Box
Ochratoxin A	-Cl	-H	-H	Phenylalanine
Ochratoxin B	-H	-H	-H	Phenylalanine
Ochratoxin C	-Cl	-H	-H	Phenylalanine ethyl ester

Figure 4. Chemical structure of the main ochratoxins (O'Brien and Dietrich, 2005).

5.3. OCHRATOXIN A (OTA)

Ochratoxin A is the most toxic of the ochratoxins. It derives its name from *Aspergillus ochraceus*, the first mould from which it was isolated (van der Merwe et al., 1965), although later, other genera were reported to be capable of producing this toxin. In some countries, OTA is found in food and beverages often enough and at high enough levels to cause concern for human safety.

5.3.1. Chemical and physical properties

OTA, $C_{20}H_{18}ClNO_6$ (molecular weight: 403.82 daltons), is a phenylalanyl derivative of a substituted isocoumarin. It is listed in Chemical Abstracts' index as L-phenylalanine N-[5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H2-benzopyran-7-yl]carbonyl-(R)- (C.A. No. 303-47-9). OTA is structurally similar to the amino acid phenylalanine (Phe). For this reason, it has an inhibitory effect on a number of enzymes that use Phe as a substrate, in particular, Phe-tRNA synthetase, which can result in inhibition of protein synthesis. For the same reason, OTA may also stimulate lipid peroxidation (see 5.3.3).

OTA is a colourless crystalline compound soluble in organic solvents and in alkaline water. It crystallises from benzene to give a product melting at 90 ºC containing one molecule of benzene. This can be removed under vacuum at 120 ºC to give a substance melting at 168 ºC. It crystallises in a pure form from xylene. OTA is optically active and exhibits blue fluorescence under UV light, but the ultraviolet spectrum varies with pH and with the solvent polarity. Fluorescence emission is maximum at 467 nm in 96 % ethanol and 428 nm in absolute ethanol (Scott, 1994).

5.3.2. Stability of OTA

OTA is a very stable mycotoxin in different solvents. It can be stored in ethanol for at least one year under refrigeration and protected from light, as photolysis may occur on exposure to fluorescent light (Neeley and West, 1972). It has been reported that OTA solutions in methanol stored at -20 ºC are stable over a period of some years (Valenta, 1998).

5.3.3. Toxicology of OTA

Often, a single mycotoxin can cause more than one type of toxic effect. The target organ of OTA toxicity in all mammalian species tested is the kidney, in which lesions can be produced by both acute and chronic exposure (Harwig et al., 1983). Animals can demonstrate variable susceptibilities to OTA depending on genetic factors (species, breed

and strain), physiological factors (age, sex, nutrition, other diseases) and environmental factors (climatic conditions, management, etc.). The LD_{50} is one way to measure the short-term poisoning potential (acute toxicity) of a compound. LD stands for '*Lethal Dose*', and LD_{50} is the amount of a material, given all at once, which causes the death of 50 % (one half) of a group of test animals. Therefore, in acute toxicity studies, LD_{50} values of OTA, vary greatly among species, ranging from an oral LD_{50} of 0.20 mg kg^{-1} in dogs and 1 mg kg^{-1} in pigs, to more than 30 mg kg^{-1} in rats (Table 2). LD_{50} values are also strongly influenced by the administration routes (oral feeding, intubation, intravenous or intraperitoneal injection), the solvents of toxins, the presence of other mycotoxins and the composition of the diet. Thus, data obtained in toxicological studies will be relative and not conclusive for the evaluation of the toxicological features of individual mycotoxins.

Table 2. LD_{50} values of OTA of different animal species (several sources).

Animal	LD_{50} (mg kg^{-1})	Administration way
Mice (female)	22	intraperitoneal
Rat (male)	30.5	oral
Rat (female)	21.4	oral
Rat (male)	12.6	intraperitoneal
Rat (female)	14.3	intraperitoneal
Chicken	3.3	oral
Turkey	5.9	oral
Quail	16.5	oral
Rainbow trout	4.7	intraperitoneal
Dog	0.2	oral
Pig (female)	1	oral

OTA is nephrotoxic, mutagenic, carcinogenic, teratogenic and immunosuppressive in a variety of animal species. It is a mitochondrial poison causing mitochondrial damage, oxidative burst, lipid peroxidation and interferes with oxidative phosphorylation. In addition, OTA increases apoptosis in several cell types. Much has been written about the possible role of OTA in the etiology of these phenomena and detailed reviews on OTA toxicology have been published (Kuiper-Goodman and Scott, 1989; Dirheimer, 1996; Creppy, 1999; Petzinger and Ziegler, 2000; Mantle, 2002; O'Brien and Dietrich, 2005). Although a complete review of the toxicology of OTA is beyond the scope and intention of this text, the most important points are outlined afterwards.

5.3.3.1. Carcinogenesis

Oral administration of OTA produced renal tumours in rats and mice (Boorman, 1989). Moreover, in mice OTA give rise to liver tumours in both sexes (Kuiper-Goodman and Scott, 1989). Nephrotoxic effects have also been demonstrated in other mammalian species. In the early 1970s, observers in Denmark noted a high incidence of nephritis in pigs (Krogh, 1972), a disease known nowadays as **Danish porcine nephropaty**, which was associated with the use of mouldy rye, and particularly, with the presence of OTA in feed samples. Given that OTA is a kidney toxin in all mammals tested, it would appear prudent to assume it is also a kidney toxin in humans. Particularly, kidney failure rates in rural Scandinavian populations were proved high, and a possible cause was the ingestion of those pig tissues containing excessive levels of OTA (Krogh et al., 1976; 1977).

Observational studies have associated OTA with two human disease states:

- **Balkan endemic nephropathy** (BEN).

- **Urothelial tumours** (UT).

The first was initially described in the 1950s as a human kidney disease, in a series of publications from different Easter Europe countries, where OTA is relatively high in the diet. Subsequent studies have also shown a high incidence of kidney cancer and cancer of the urinary tract in some BEN afflicted populations. The connection between human urinary tract tumours and OTA was postulated by a Danish study, based on regional coincidence of tumours of the urinary tract in humans, human chronic kidney disease and, as an indication of regional OTA contamination of grain, the occurrence of nephropathy in pigs (Olsen et al., 1993). Studies carried out in several countries including Tunisia, Egypt and France, have also indicated a link between dietary intake of OTA and the development of renal and urothelial tumours (Abdelhamid, 1990; Maaroufi et al., 1995; Fillastre, 1997; Godin et al., 1998; Wafa et al., 1998).

To sum up, it is not possible to conduct studies in humans under controlled conditions but, the parallels between the pathological changes and functional deficits observed in pigs and those noted in human BEN/UT cases, suggest that OTA may play a role in human kidney and urothelial cancer. Recently, it has also been suggested that OTA can cause testicular cancer in humans, as positive associations have been found between the incidence of testicular cancer and the consumption of foods typically associated with OTA contamination (Schwartz, 2002).

To adequately assess the human cancer risk of OTA, a variety of factors must be considered, such as specific exposure information, ample follow-up time, large sample sizes including adequate numbers of both males and females, control for confounding factors that may also affect cancer risk, etc. (FAO/WHO, 2003). The major difficulty with

epidemiological studies on mycotoxins is obtaining data on historical exposure, since many of the effects observed are of a chronic nature. Even when using biomarkers, the estimate of exposure usually reflects only the recent past (van der Brandt et al., 2002). Furthermore, without thorough studies that take all these factors into account, it is not possible to conclude whether or not exposure to OTA increases cancer risk in humans.

In 1993, the International Agency for Research on Cancer (IARC) classified OTA as a possible human carcinogen (Group 2B) (Table 3), based on sufficient evidence of carcinogenicity in experimental animal studies and inadequate evidence in humans (IARC, 1993). In the subsequent years since the IARC classification, studies have shown a tendency in the direction of group 2A toxicity (Kuiper-Goodman, 1996), as well as indicating the occurrence of synergistic multiple actions of diverse mycotoxins.

5.3.3.2. Mutagenesis/ Genotoxicity

Mutagenic or genotoxic chemicals are those capable of causing damage to DNA. For a long time, OTA was not considered to be genotoxic. However, in 1985, Creppy et al. showed that OTA caused DNA single-stranded breaks in mice-spleen cells (*in vitro*) and in mouse spleen, kidney and liver, after injection of high OTA doses. Moreover, in 1991, Pfohl-Leszkowicz et al. found several DNA adducts after oral application of OTA to mice. This discussion received considerable stimulus when it became known that cells from target organs of animals and also human ureter cells, react much more sensitively to changes in DNA (Föllmann et al., 1995; Dörrenhaus and Föllman, 1997). However, there is still some disagreement about whether OTA reacts directly with nucleic acids or acts via an indirect mechanism to disrupt DNA.

5.3.3.3. Teratogenesis

OTA is a potent teratogen in rodents (Hayes et al., 1974; Brown et al., 1976), chickens (Gilani et al., 1978) and pig (Shreeve et al., 1977). Both teratogenic and reproductive effects have been demonstrated. OTA causes birth defects in rodents. It is seen that OTA crosses the placenta and is also transferred to newborn rats and mice via lactation (Hallen et al., 1998). In the foetus, the major target is the developing central nervous system, thus OTA is also considered a neurotoxic compound. In addition, OTA-DNA adducts are formed in liver, kidney and other tissues of the progeny (Pfohl-Leszkowicz et al., 1993; Petkova-Bocharova et al., 1998). The mechanism of induced teratogenesis by OTA is still not clear, but it seems to affect both the progenitor and the embryo, in a direct way (Hood et al., 1976). Thus, sufficient experimental evidence exists in the scientific literature to classify OTA as a teratogen, affecting the nervous system, skeletal structures and immune system of research animals.

Table 3. Summary of the IARC evaluations and classification of mycotoxins on the basis of the carcinogenic risk to humans (IARC 1993, 1998).

Mycotoxin	Risk carcinogenic to[a]		IARC[b] classification
	humans	animals	
Penicillic acid	AD	L	3
Aflatoxins	S	S	1
Aflatoxin B_1	S	S	
Aflatoxin B_2		L	
Aflatoxin G_1		S	
Aflatoxin G_2		I	
Aflatoxin M_1	I	S	2B
Citrinin	AD	L	3
Cyclochlorotin	AD	I	3
Griseofulvin	AD	S	2B
Luteoskyrin	AD	L	3
OTA	I	S	2B
Patuline	AD	I	3
Rugulosine	AD	I	3
Sterigmatocytin	AD	S	2B
F. graminearum toxins	I		3
F. culmorum toxins	AD		
F. crookwellense toxins	AD		
Zearalenone		L	
Vomitoxin		I	
Nivalenol		I	
Fusarenone X		I	
F. sporotrichioides toxins	AD		3
T-2 toxin		L	
F. moniliforme toxins	I	S	2B
Fumonisin B_1		L	
Fumonisin B_2		I	
Fusarin C		L	

[a] Evidence of carcinogenicity: (S) sufficient, (L) limited, (I) inadequate, (AD) absence of data;
[b] Classification criteria: Group 1: carcinogenic to humans; Group 2B: carcinogenic to animals and possible carcinogenic to humans; Group 3: non-classifiable for carcinogenicity to humans.

5.3.3.4. Immunosuppression

OTA is known to affect the immune system in a number of mammalian species. The type of immune suppression experienced appears to be dependant a number of factors, including the species involved, the route of administration, the doses tested, and the methods used to detect the effects (O'Brien and Dietrich, 2005). OTA causes immunosuppression following prenatal, postnatal and adult-life exposures. These effects include reduced phagocytosis and lymphocyte markers (Muller et al., 1999), and increased susceptibility to bacterial infections and delayed response to immunization in piglets (Stoev et al., 2000). Purified human lymphocyte populations and subpopulations are adversely affected by OTA *in vitro* (Lea et al., 1989).

5.3.3.5. Action on different enzymes

Because of its structure, OTA was first shown to inhibit protein synthesis both *in vitro* and *in vivo*, by competition with phenylalanine. OTA might act on other enzymes that use phenylalanine as a substrate, such as phenylalanine hydroxylase (Dirheimer, 1996), and lower the levels of phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis (Meissner and Meissner, 1981). Inhibition of protein and RNA synthesis is also considered another toxic effect of OTA.

5.3.3.6. Lipid peroxidation and mitochondrial damage

OTA enhance lipid peroxidation both *in vitro* and *in vivo* (Rahimtula et al., 1988; Omar et al., 1990). This action might have an important effect on cell or mitochondrial membranes. Several lines of experimental observations demonstrate that OTA effects mitochondrial function and causes mitochondrial damage (Wei et al., 1985; Wallace, 1997).

5.3.3.7. Apoptosis

OTA also induces apoptosis (programmed cell death) in a variety of cell types *in vivo* and *in vitro* (Seegers et al., 1994). The apoptosis is also mediated through cellular processes involved in the degradation of DNA.

5.3.4. Synergistic effects with other mycotoxins

Many toxicological studies have used pure OTA, free from the complex matrix of the biosynthesising fungus. In nature there are other microorganisms and their metabolites

that increase the complexity of the matrix, which could protect from or enhance the effects of OTA. It appears logical to assume that exposure to several nephrotoxic substances could have more severe consequences than exposure to a single substance. But certain combinations of mycotoxins could be more toxic than the sum of their individual actions (O'Brien and Dietrich, 2005). Accordingly, a hypothesis about synergistic effects between OTA and penicillic acid and possibly other fungal metabolites such as citrinin has emerged, and all together are suspected to be the responsible for the BEN (Stoev et al. 2001). The authors described differences in the renal pathologies resulting from OTA exposure alone and those observed following a combination of two or more other mycotoxins. One year later, Speijers and Speijers (2004) confirmed the synergistic effect of combine both nephrotoxic compounds: OTA and citrinin.

5.3.5. Half-life

Protein binding is probably the decisive factor in determining the half-life of OTA in any given species. Several studies have determined OTA to have an extremely high affinity for serum albumin and other macromolecules in the blood (Galtier et al., 1981; Hult and Fuchs, 1986). This bond with serum albumin has been suggested to result in the generation of a mobile reservoir of ochratoxin, which can be slowly released and hence rendered bioavailable over extended periods of time and furthermore, retard the elimination of OTA from the body (O'Brien and Dietrich, 2005).

OTA is absorbed passively throughout the gastrointestinal tract and actively in the kidneys (Marquardt and Frohlich, 1992). Highest amounts of OTA could be found in the blood and it is distributed in kidney, liver, muscle and adipose tissue in a decreasing order (Gareis and Scheuer, 2000). The toxin is excreted primarily in the urine, and to a lesser degree in the faeces, as ochratoxin α or OTA, in bile and also in milk.

The half-life of experimentally orally ingested OTA is shorter than intravenously injected OTA, as part of the toxin is subjected to a hepatic first-pass elimination and is removed by the bile before it can enter the systemic blood circulation. Following intravenous administration OTA is eliminated with a half-life from body in rats in 3 days, in 3-5 days in pigs (Galtier et al. 1981) and in vervet monkeys in 19-21 days (Hagelberg et al., 1989; Stander et al., 2001). Studer-Rohr (1995) showed human serum half-life of OTA to be 35 days after oral ingestion. Assuming that it takes eight-times the half-life to reach a zero value, a detectable serum level would still be found in humans 280 days after a single uptake.

5.3.6. OTA presence in food

OTA is found in a variety of foods and beverages, including both plant-based products and animal products (Table 4). Among the first ones, its presence in cereal grains (corn, wheat, barley, flour, oats, rye, rice, etc.), beans (coffee, cocoa, soy, etc.), spices, and beverages like coffee and wine must be highlighted. In 1983, OTA was reported in olive oil (Letutour et al. 1983) and recently it was detected again in this product (Papachristou and Markaki, 2004). OTA can be absorbed from contaminated feed by monogastric animals such as pigs, where it is accumulated in the blood and kidneys, and therefore it can be found in products made from them, such as black pudding, sausages, etc. Moreover, OTA has been detected in milk, cheese and other animal products. The presence of OTA in grape and its derivatives such as dried vines, grape juice, musts, wine, vinegar, etc. will be reviewed in chapter 6 (see 6.3).

Table 4. Occurrence of OTA in several food and feed. Note that the studies are a representative sample of the whole range.

Food/feed	Reference
Animal feed	van Egmond and Speijers (1994); Höhler (1998); Dalcero et al. (2002); Accensi et al. (2004)
Bee pollen	Medina et al. (2004)
Beer	Scott and Kanhere (1995); Zimmerli and Dick (1995); Jørgensen (1998); Legarda and Burdaspal (1998); Ueno (1998); Degelmann et al. (1999); Bresch et al. (2000a); Tangni et al. (2002)
Cereals (Rye, wheat, barley, oat, maize, etc.) and cereal products (bread, muesli, breakfast cereals)	Speijers and van Egmond (1993); Wood et al. (1996); Trucksess et al. (1999); Engel (2000); Wolff (2000); Legarda and Burdaspal (2001); Blesa et al. (2004)
Cheese	Sinha and Ranjan (1991); Elsawi et al. (1994); Engel (2000)
Chocolate and cocoa	van Egmond and Speijers (1994); MAFF (1999); Engel (2000); Serra-Bonvehí (2004)
Coffee	Levi et al. (1974); Zimmerli and Dick (1995); Nakajima et al. (1997); Bucheli et al. (1998); Burdaspal and Legarda (1998a); Jørgensen (1998); Ueno (1998); Trucksess et al. (1999); Bresch et al (2000a); Joosten et al. (2001); Otteneder and Majerus (2001); Varga et al. (2001a); Pardo et al. (2004)
	(…/…)

Cow milk	Engel (2000); Breitholtz-Emanuelsoon et al. (1993)
Dried fig, dried prunes	Majerus et al. (1993); Zohri and Abdelgawad (1993); Doster et al. (1996); Engel (2000); Bayman et al. (2002)
Fruit and vegetal fruits	Majerus et al. (2000); OTA in grapes (see 6.3.1.)
Grape juices	(see 6.3.1.)
Liquorice	Bresch et al. (2000b)
Meat and meat products (pork, beef, sausages, etc.)	Gareis (1996); Jørgensen (1998); Gareis and Scheuer (2000)
Nuts (hazelnuts, peanuts)	Engel (2000)
Olive oil	Letutour et al. (1983); Papachristou and Markaki (2004)
Pulses	Scott et al. (1972); Jørgensen (1998); MAFF (1999)
Raisins	(see 6.3.2.)
Sauces (ketchup, moustard, barbacue)	Majerus et al. (2000)
Seeds (sunflower seed, sesame, linseed)	Engel (2000)
Spices	Patel et al. (1996); Hübner et al. (1998); Thirumala-Devi et al. (2001); Abdulkadar et al. (2004)
Tea	Bresch et al (2000a)
Yoghurt	Engel (2000)
Vinegar	(see 6.3.3.)
Wine	(see 6.3.4. and 6.4.)

To sum up, OTA can be found in a wide range of raw commodities and also in processed foods made from contaminated resources, thus, it is difficult to avoid this substance.

5.3.7. Human exposure

Mycotoxins can affect human and animal health, as mentioned before. In general, animals are directly exposed to mycotoxins through the consumption of mouldy feedstuff. Human exposure can be via one of two routes; direct exposure due to the consumption of mouldy plant products, or indirect exposure through the consumption of contaminated animal products, containing residual amounts of the mycotoxin ingested by the food producing animals (Boutrif and Bessy, 2001) (Figure 5). However, animal derived food products contribute to a lesser extent to human OTA exposure, with the exception of babies and

infants, due to their high consumption of milk and milk products, and their specific metabolism (Kuiper-Goodman, 1998; Gilbert et al., 2001).

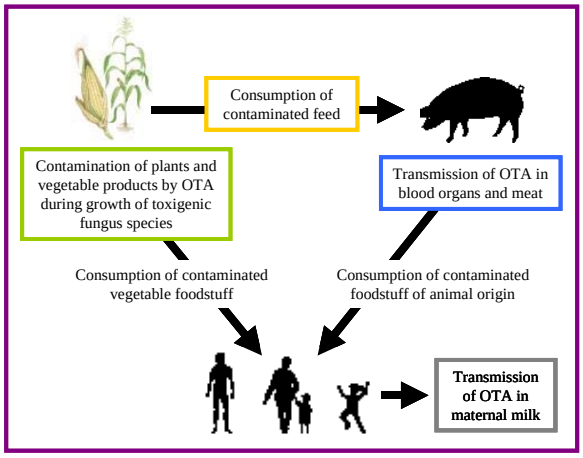


Figure 5. OTA in the food chain. Possible routes for contamination of humans by OTA (Bauer and Gareis, 1987).

5.3.7.1. OTA levels in human fluids and tissues

It is possible to verify exposure to OTA by directly measuring OTA levels in human blood, breast milk and some tissues. This is the most direct type of exposure measurement. OTA is metabolised slowly in the human body so it tends to remain present for several months or more allowing for measurement for a length of time after exposure. Human exposure to OTA has been clearly demonstrated by its detection in blood (Breitholtz et al., 1991; Hald, 1991; Breitholtz-Emanuelsson et al., 1993; Peraica et al., 1999), serum (Rosner et al., 2000), plasma (Ueno, 1998; Burdaspal and Legarda, 1998b) and breast milk (Gareis et al., 1988; Breitholtz-Emanuelsson et al., 1993; Jonsyn et al., 1995; Micco et al., 1995; Miraglia et al., 1996). The wide dispersal of food made possible by modern transportation and trade makes exposure more likely. Numerous studies performed worldwide have detected OTA in biological samples from healthy people living outside BEN-endemic areas, suggesting that the general population may be exposed to low levels of OTA. However, no cases of acute intoxication in humans have been reported (JECFA, 2002).

The toxicology and human health risks of OTA have been assessed at both European and International levels by the European Commission Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), respectively, who have established tolerable intakes of OTA from food (European Commission, 1998;

JECFA, 2001) (Table 5). These are levels of OTA that experts believe a person may ingest on a daily or weekly basis without harm over a lifetime. But humans are not continuously exposed throughout lifetime to a certain high level of the mycotoxin and on the contrary, the ingestions might be exceeded during certain periods. In principle, the evaluations are based on the determination of a No-Observed-Adverse-Effect-Level (NOAEL) in toxicological studies and the application of an uncertainty factor. The uncertainty factor means that the lowest NOAEL in animal studies is divided by 100, 10 for extrapolation from animals to humans and 10 for variation between individuals, to arrive at a tolerable intake level. In cases where the data are inadequate, JECFA uses a higher safety factor.

Table 5. Provisional tolerable daily intake (PTDI) values of OTA.

PTDI (ng/kg body weight/day)	Reference
1.2-5.7	Kuiper-Goodman (1990)
5-5.7	NNT (1991)
5.0	Kuiper-Goodman (1996)
1.2-14	European Commission (1998)
14	JEFCA (2001)

Although the similarities among these estimated values, there is still no worldwide consensus on what levels of OTA are considered tolerable for people to ingest. These guidelines are primarily meant to be used by scientists and regulatory agencies in their efforts in food safety protection and are not intended to be used by consumers for calculating their personal intake levels.

This hazard assessment approach does not apply for toxins where carcinogenicity is the basis for concern as is, for example, the case of aflatoxins. Assuming that a no-effect concentration limit cannot be established for genotoxic compounds, any small dose will have a proportionally small probability of inducing an effect. Imposing the absence of any amount of genotoxic mycotoxins would then be appropriate, but as natural contaminants that they are, they will never be completely eliminated without outlawing the contaminated food or feed. In these cases, JECFA does not allocate a PTWI or PTDI. Instead it recommends that the level of the contaminant in food should be reduced so as to be As Low As Reasonably Achievable, known as the ALARA approach.

5.3.7.2. OTA levels in contaminated food

Apart from measuring OTA in human fluids and tissues, exposure can also be estimated by measuring OTA levels in contaminated food that may have been consumed. Studies on some foods show that there are differences between the contamination level of different batches of food, and even within the batches, the mycotoxin might not be homogeneously distributed but be restricted to a small part of the batches (Speijers, 2001). Furthermore, the occurrence of mycotoxins can fluctuate considerably in time. Sometimes the mycotoxin concentration can be high for a certain episode, whereas for another it might be negligible low.

It is difficult to compare OTA levels between countries or between types of food, as data on the occurrence of OTA in food and beverages are not available for many commodities in many countries, and the data that are available are often out of date and/or incomplete. The consumption data used were mainly based on intake in Europe (Table 6). The European Commission (2000) calculated and summarised intake figures for OTA. The total mean intake of OTA for Europe was estimated to be 3.7 ng/kg body weight per day, assuming a body weight of 60 kg.

Table 6. The relative contribution of different food categories to human OTA exposure (JECFA, 2001).

Food category	OTA contamination (μg/kg)	Intake (g)	Daily intake of OTA (ng/kg body weight[a]/ day)	% of total intake
Cereals	0.94	230	3.58	57.8
Wine	0.32	240	1.23	20.8
Grape juice	0.39	69	0.44	7.3
Coffee	0.76	24	0.30	5.1
Pork meat	0.17	76	0.21	3.5
Beer	0.023	260	0.09	1.6
Dry fruits	2.2	2.3	0.08	1.1
Pulses	0.19	25	0.08	1.1
Cocoa	0.55	6.3	0.06	0.8
Poultry	0.041	53	0.06	0.8

[a] Body weight 60 kg

Exposure assessments indicate that cereals and cereal products are the main contributors to the dietary intake of OTA (50-70 %), as almost all cereals seem to have the possibility

to contain OTA and their consumption is generally high (JECFA, 2001). Grape juice and wines, were considered in a first approach to be the second most prominent source of OTA intake for humans, with 7-20 %, respectively. Otteneder and Majerus (2000) reduced this figure for wine to 2 % after new calculations, and more recently, Miraglia and Brera (2002) estimated it to be 10 %. Other products contribute less to the dietary intake, but the incidence of contamination can be high in coffee, beer, raisins and spices. Therefore, if intakes are not greatly above what seems tolerable, why bother?. One reason is that average intake means that some individuals exceed this value and so some people may be at risk. Also, individuals may differ in their sensitivity to OTA. Further, OTA may be additive to, or synergistic with, other chemicals in food and the environment. Thus, the importance of human ochratoxicosis could be under-estimated because of the presence in our diet of substances such as phenylalanine, aspartame, vitamins, etc., which are capable of alleviating some of the effects of OTA, and could also change its profile of distribution and metabolism (Creppy, 1999). Indeed, the prevention of human ochratoxicosis could be achieved by using the sweetener aspartame, a structural analogue of OTA, which prevents the distribution of the toxin and accumulation in the organism by avoiding the binding to blood proteins (Creppy et al., 1995, 1996; Baudrimont et al., 1997). It also greatly reduces the cytotoxic and nephrotoxic effects of OTA in the normal food contamination ranges.

5.3.7.3. OTA levels in air

Finally, exposure to OTA has also been estimated by sampling air and dust in households or workplaces, such as farms or food processing facilities, where airborne exposure to OTA can occur, adding to the daily intake of the mycotoxin via the respiratory tract. Thus, OTA has been demonstrated in dust and fungal conidia in samples taken from cowsheds. A very high level (1500 $\mu g\ kg^{-1}$) of OTA was found in dust collected from inside the ducts of the heating system in a household (Richard et al., 1999). Furthermore, OTA was detected in dust samples from the heating ducts of a house where animals showed signs of ochratoxicosis (Skaug et al., 2000). Exposure to mycotoxins from inhalation is receiving increasing attention nowadays, as farm workers are often exposed to high concentrations of airborne organic dust and fungal conidia, especially when working with plant materials, constituting a potential health hazard for them.

5.4. DECONTAMINATION OF MYCOTOXINS

Most research effort has concentrated on the means for prevention of mycotoxin formation, and this must remain the best defence for protecting the consumer. However, prevention is not always possible, especially for those mycotoxins formed under field conditions. However, it is possible to recuperate infected products by decontaminating them.

Detoxification consists in removing, destroying or reducing the toxic effects of mycotoxins. Traditionally, detoxification strategies are classified based on whether they use chemical, physical or microbiological processes. However, treatments have their own limitations, since the treated products should be health safe from the chemicals used and their essential nutritive value should not be deteriorated. Decontamination of mycotoxins has been frequently investigated for cereals, and much attention has been paid on aflatoxins.

The ideal decontamination procedure should:

- Completely inactivate, destroy, or remove the toxin, or reduce its concentration to acceptable levels.
- Not produce or leave toxic residues in the food.
- Preserve the nutritive value of the food.
- Not alter the acceptability or the technological properties of the product.
- Destroy fungal spores and mycelia so as to prevent revival and toxin production.
- Be integrated, if possible, into the regular food-processing and preparation steps.
- Be cost-effective.
- Be easy to use.
- Not destroy or damage equipment or pose a health hazard to workers.
- Be approved by regulatory agencies.

Physically, fungi-contaminated solid food can be removed by hand picking or photoelectric detecting machines. The method would consume time and labour or expensive. Heating, dry and oil roasting, cooking under pressure, etc. can destroy different percentages of mycotoxins. Some mycotoxins resist higher temperatures, so special attention should be paid in long-time cooking and overheating as they would destruct essential vitamins and amino acids in treated foods.

Chemical treatment has been used as the most effective means for the removal of mycotoxins from contaminated commodities. Ionizing radiation such as gamma-rays can stop growth of food spoilage organisms, including bacteria, moulds and yeasts. It also inactivates pathogenic organisms including parasitic worms and insect pests. It has been

reported that gamma-irradiation (5-10 Mrad) caused reduction of aflatoxin (Sommer and Fortlage, 1969). The irradiation, however, could not completely destroy the toxin and its mutagenicity. The treatment combination of gamma irradiation and ammoniation should be therefore attempted for more aflatoxin decontamination.

Organic solvents (chloroform, acetone, hexane and methanol) have frequently been used to extract toxins from agricultural products. Methods should be sure that the detoxification system is capable of converting the toxin to a nontoxic derivative without deleterious change in the raw product. Mutagenicity of the treated products should be assessed. Many common chemicals have been brought to test the effectiveness in detoxification of aflatoxin. Other mycotoxins which are like aflatoxin and have a lactone grouping in the molecule, can be similarly destroyed by alkaline condition using ammonia, sodium hydroxide and sodium bicarbonate. These toxins are patulin, penicillin acid, citreoviridin, citrinin, cyclochlorotin, OTA, rubratoxin, trichothecenes and zearalenone.

Certain conditions such as moisture content, heat, ultraviolet or gamma irradiation, sunlight and pressure at different treatment-periods have been simultaneously combined with the chemicals for the enhancement of detoxification. Inactivation methods can be achieved by mixing, packing, fumigation and immersion with the chemical used.

Microorganisms and their enzymes can also be applied for mycotoxin detoxification, and a brief review for OTA appeared in Varga et al. (2001b).

5.4.1. Decontamination of OTA

Once OTA has been formed in a food it would be difficult to remove by most forms of food processing (Moss, 1996). A number of these processes have been examined in detail although much remains to be done. Hypochlorite (Castegnaro et al., 1991), ammoniation (Chelkowski et al., 1982), ozone (McKenzie et al., 1997), alkaline hydrogen peroxid (Fouler et al., 1994) and gamma irradiation (Refai et al., 1996) treatments, have shown different degrees of success for detoxify OTA in animal feed. Boudra et al. (1995) showed that even at as high temperature as 250 ºC, complete destruction of OTA in wheat was not achieved. However, none of these physical and chemical processes was recommended for practical detoxification of OTA-contaminated grains and feeds (Scott, 1996). Scudamore et al. (2004) found a significant reduction on the OTA content of wheat wholemeal by extrusion cooking at the highest temperature and initial moisture content of the samples. The effect of this procedure on the reduction of other mycotoxins content in cereals, has been reviewed (Castells et al., 2005a). A recent study about OTA reduction in artificially contaminated barley meal showed up to 86 % of reduction after extrusion cooking the samples (Castells et al., 2005b). In general, the degree to which OTA is destroyed will further depend on other parameters such as pH, temperature, contamination levels, measurement methods used, etc. Scientists are working to better

understand the conditions under which OTA degrades or remains intact throughout food processing.

Several reports of OTA biodegradation have been published. *Streptococcus salivarius, Bifidobacterium bifidum, Lactobacillus delbrueckii* and yogurt bacteria have completely reduced OTA levels in milk samples (Skrinjar et al, 1996). Cell cultures of several vegetal plants have been reported to completely transform OTA into a number of other products (Karlovski, 1999).

Varga et al. (2000) examined more than 70 *Aspergillus* species for their ability to degrade OTA in ochratoxin α, which still has limited toxicity. Only *A. fumigatus* and black aspergilli strains were able to do it. The kinetics of the degradation of OTA of an atoxigenic *A. niger* strain was further studied. OTA degradation was faster in solid media than in liquid cultures. *A. niger* could also degrade ochratoxin α to an unknown compound within some days (Varga et al., 2000). This is a promising result because it might allow the biological elimination of this mycotoxin and may provide a source of enzymes which could be used for detoxification of OTA in contaminated agricultural products.

Abrunhosa et al. (2002) isolated 51 strains (67 % of the strains tested) of filamentous fungi from grapes, with ability to degrade more than 80 % of OTA added to a culture medium, being black aspergilli, *A. clavatus*, *A. ochraceus*, *A. versicolor* and *A. wentii*, the most effective species.

Furthermore, several reports have describe the OTA degrading activities of the microbial flora of the mammalian gastrointestinal tract, including rumen microbes of the cow and sheep (Galtier and Alvinerie, 1976; Hult et al., 1976; Pettersson et al., 1982; Kiessling et al., 1984; Xiao et al., 1991; Özpinar et al., 1999). The velocity of the degradation of OTA increased with concentration of starch in the animal diet and the resulting higher number of protozoa, while an influence of the pH-value was not apparent (Özpinar et al., 1999). It is reported that the human intestinal microflora can also partially degrade OTA (Akiyama et al., 1997).

Detoxification of OTA in wines is detailed in section 6.5.

5.5. REFERENCES

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