



## OCCURENCE OF FUSARIUM MYCOTOXINS IN WHEAT FROM EUROPE – A REVIEW

Oana STANCIU<sup>\*1</sup>, Roxana BANC<sup>\*</sup>, Anamaria COZMA<sup>\*</sup>, Lorena FILIP<sup>\*</sup>,  
Doina MIERE<sup>\*</sup>, Jordi MAÑES<sup>\*\*</sup>, Felicia LOGHIN<sup>\*\*\*</sup>

*\*Department of Bromatology, Hygiene, Nutrition, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Romania*

*\*\*Department of Food Chemistry and Toxicology, Faculty of Pharmacy,  
University of Valencia, Spain*

*\*\*\*Department of Toxicology, Faculty of Pharmacy, Iuliu Hațieganu  
University of Medicine and Pharmacy Cluj-Napoca, Romania*

**Abstract:** The quality of cereals is very important for both human and animal nutrition. Fusarium mycotoxins include a great number of compounds. Trichothecenes, zearalenone (ZEN) and fumonisins are the major Fusarium mycotoxins occurring in cereal grains, animal feeds and forages. Conditions that predispose to mycotoxin production by *Fusarium* species include humidity, temperature, aeration and substrate type. Even if a great number of fungal metabolites have been designated as mycotoxins, a small number are known to have significant animal/human health and economic significance. For this, the world-wide impact of mycotoxins on human and animal health is likely underestimated and the future in this area is to identify additional specific biomarkers and group of biomarkers that can be used to establish the exposition of human and animals to individual mycotoxins.

**Keywords:** *Fusarium* mycotoxins, trichothecenes, fumonisins, zearalenone, wheat, Europe.

### INTRODUCTION

Nowadays, industrialization, globalization and liberalization make it possible to have greater varieties of foods worldwide. But globalization and technological development lead also to increased risks in food chain (Smyth

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<sup>1</sup> Corresponding author. Mailing address: Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Faculty of Pharmacy, Department of Bromatology, Hygiene, Nutrition, 6 Pasteur Street 400349 Cluj-Napoca, Romania. Phone: 0040 724184687. E-mail: [oana.stanciu@umfcluj.ro](mailto:oana.stanciu@umfcluj.ro)

et al., 2014). World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) are involved in ensuring food security and for this in 1963 the Sixteenth World Health Assembly approved the establishment of the Joint FAO/WHO Food Standards Programme with the Codex Alimentarius Commission as its principal organ. Moreover, European Food Safety Authority (EFSA) was established by the Council and European Parliament in 2002 to provide scientific advice and technical support in all areas impacting on food safety: to share information, data and best practices, to identify emerging risks and to develop coherent communications on risks in the food chain (Regulation (EC) no 178/2002; EFSA, 2009). Food security is defined as existing “when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life” (World Food Summit, 1996).

Safe foods are those that ensure food safety and food quality (Grunert, 2005; Babu et al., 2014). Over the past fifty years, more attention has been given on food safety and quality, because of the higher incidence of foodborne diseases, the large-scale outbreaks as well as the incidents and the recalls due to unacceptable levels of chemical hazards in our foods (Motarjemi & Lelieveld, 2014). For this, the prevention and control of these situations are international public health, policy and industry goals (Grunert, 2005; CODEX, 2007; WHO, 2013).

Even if the terms food safety and food quality can sometimes be confusing, each term defines a different area. Food safety refers to all those hazards (biological, chemical, physical), whether chronic or acute, that can make food injurious to the health of the consumer (Bouxin, 2014). Food quality includes all other attributes that influence a product's value to the consumer or all those characteristics of excellence that make it acceptable (Ferree, 1973; Grunert, 2005).

The European Union defines contaminants as substances that have not been intentionally added to food or feed for food producing animals, but may be present in food or feed as a result of the various stages of its production, packaging, transport or holding or which also might result from environmental contamination (EC, 2007; CODEX, 2012). The potential range of food or feed contaminants is very large, but could be interpreted as restricted to synthetic compounds (any industrial chemical or environmental contaminant), metals, mycotoxins, and bacterial toxins (Slorach, 2000). The European Union has established the maximum levels for the following contaminants: nitrate, mycotoxins (aflatoxins, ochratoxin A, patulin, deoxynivalenol, zearalenone, fumonisins and citrinine), metals (lead, cadmium, mercury, inorganic tin), 3-monochloropropano-1,2-diol (3-MCPD), dioxins, dioxin-like and non dioxin-like polychlorinated biphenyls (PCBs),

polycyclic aromatic hydrocarbons (PAHs: benzo(a)pyrene and sum of 4-PAHs), melamine and erucic acid (Regulation (EC) no 1881/2006). Even if the definition of food contaminants excludes agricultural and veterinary chemicals, food additives, and processing aids, certain compounds may also be regarded as contaminants (e.g., some pesticides) (Slorach, 2000).

The European Union is one of the world's biggest cereal producers and an important cereal trader. Cereals account for one-quarter of the EU's crop production value and for one-eighth of the total value of its agricultural products. In terms of quantity and area, wheat is by far the most popular cereal grown in the EU, making up nearly half the total (EC, 2014). In 2013, wheat was grown in Europe on approximately 57600000 hectares (FAOSTAT, 2015a). According to the latest informations of the European Union, the five biggest wheat producers are France, Germany, United Kingdom, Poland and Romania (EC, 2015). Regarding the latest informations from FAOSTAT, in 2011, per capita wheat and wheat products consumption in Europe was 107.8 kg/year (295 gr/capita/day) (FAOSTAT, 2015b). Larger feed consumption of wheat in the EU is behind most of the year-on-year increase. Consumption of wheat as food, which is projected to rise by 1.1 percent (5 million tonnes) in 2014/2015 to 485 million tonnes, still accounts for the bulk of wheat utilization (FAO, 2015).

The aim of this work is to evaluate the occurrence of three types of *Fusarium* mycotoxins, trichothecens, zearalenone and fumonisins, in wheat, which is the most cultivated and consumed cereal in Europe.

## MYCOTOXINS

The word "mycotoxin" is derived from "myco" and "toxin", greek words "mykes" and "toxikon", meaning mold and a poison produced by a living organism. The term "mycotoxins" defines secondary fungal metabolites (metabolites not essential to the normal growth and reproduction of the fungus) that cause biochemical, physiologic, and/or pathologic changes in other species, including vertebrates, other animal groups, plants, and other microbes. Mycotoxins have low molecular weight molecules ( $M_w < 700$ ) and are toxic in low concentrations (Sorriano, 2007; Haschek & Voss, 2013).

Even if hundreds of compounds have been classified as mycotoxins, and have been isolated and chemically characterized, only approximately 50 have been studied in detail (CAST, 2003). The class of mycotoxins constitutes a toxigenically and chemically heterogeneous class. From the chemical viewpoint, mycotoxins are classified into: cyclopeptides, polycetoacids, terpenes, and nitrogenous metabolites, depending on their biological origin and structure (Ferrante et al., 2012). Another classification more common is

according to the type of origin genera. The major mycotoxin-producing fungal genera are *Fusarium*, *Aspergillus*, and *Penicillium*, but mycotoxins can also be produced by *Cladosporium*, *Claviceps*, *Alternaria* and *Helminthosporium* genera (Oancea & Stoia, 2008; Coppock & Dziwenka, 2014).

Humans and animals are exposed to mycotoxins by the oral, inhalation, and dermal routes. Mycotoxins commonly enter the food chain through contaminated food and feed crops, mainly cereals (Figure 1). FAO estimated that approximately 25% of the cereals produced in the world are contaminated by mycotoxins, but perhaps this value is closer to 50%, if one takes into account emerging mycotoxins of which so far have limited data. According to EFSA and FAO there are five mycotoxins, or groups of mycotoxins, that occur quite often in food: deoxynivalenol, nivalenol, HT-2 and T-2 toxins; zearalenone; ochratoxin; fumonisins; and aflatoxins.

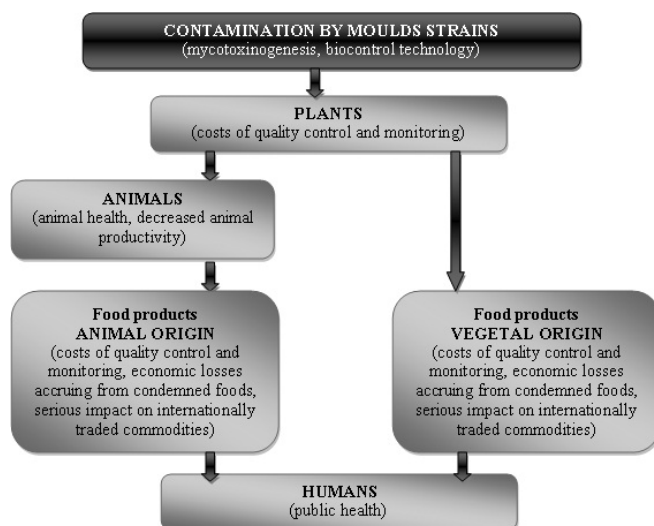


Figure 1. Mycotoxins in the food chain, impact and risks

The accumulation of mycotoxins in foods and feeds represents a major threat to human and animal health as they are responsible for many different toxicities, and also it has a big economic significance. Acute exposure to high levels of mycotoxins is not very common, but the adverse effects in a chronic exposure continue to attract worldwide attention because of their impact on human health. Mycotoxins can produce, alone or in combination (two or more mycotoxins): endocrine dysfunction, immune alterations, induction of cancer, mutagenicity, estrogenic, gastrointestinal, urogenital, vascular, kidney and nervous disorders; some mycotoxins are also immuno-compromising,

and can thus reduce resistance to infectious disease (Sorriano, 2007; Oancea & Stoia, 2008; Ferrante et al., 2012; Haschek & Voss, 2013; Marroquín-Cardona et al., 2014).

## **FUSARIUM MYCOTOXINS**

Fungal species of the *Fusarium* genus are of major importance worldwide. This genus is widespread, both in soil and in organic substrates, it contains over 70 phytopathogenic species, occurring in natural conditions in different regions of the world and affecting in principal cereals, other vegetables and fruits (Sorriano, 2007). *Fusarium* spp are considered some of the most dangerous pathogens for cereals and other plants, not only because of significant economic losses, but also for mycotoxins production (Kotowicz et al., 2014).

The factors influencing the occurrence of diseases produced by *Fusarium* spp are linked to substrat (composition, pH, humidity) and environment conditions respectively. Weather conditions (warmer weather, heat waves, the humidity, greater precipitation and drought) can affect both plant growth and presence of infection (Paterson & Lima, 2011; Tantaoui Elaraki, 2014); for this, it is very important to take into account the climate conditions and also the climate changes when *Fusarium* spp and *Fusarium* mycotoxins are studied and/or analysed (Zain, 2011). The species from *Fusarium* genus are commonly found in vegetables grown in America, Europe and Asia (Escriva et al., 2015).

*Fusarium* spp produce three of the most important classes of mycotoxins with respect to animal and human health and production: fumonisins (FBs), zearalenone (ZEA), and trichothecenes. *Fusarium* genus also produce emerging mycotoxins, such as fusaproliferin (FUS), beauvericin (BEA), enniatins (ENNs) and moniliformin (MON), or fusaric acid, fusarin A-D, gliotoxin, butenolite which are more recently discovered and less studied (Haschek & Voss, 2013; Escriva et al., 2015).

*Fusarium* species are responsible for mycotoxin production before harvest, but cereal products may be contaminated by mycotoxins at any stage, during processing, storage and transport of the end product (Kotowicz et al., 2014). An important aspect for *Fusarium* genus is that the same mycotoxin can be produced by different *Fusarium* species and one species can produce various mycotoxins at once (Table 1), so in the same substrate we can find more than one metabolite. The problem of co-occurrence of *Fusarium* mycotoxins in cereal grains and animal feed is a recurring feature, raising the question of interactions, synergistic or antagonistic actions in the manifestation of toxicity (Placinta et al., 1999; Streit et al., 2012).

Table 1. Important species of *Fusarium* infecting cereals and mycotoxins produced (Kotowicz et al., 2014)

<b>Species</b>	<b>Mycotoxins produced</b>
<i>F. avenaceum</i>	moniliformin, beauvericin
<i>F. cerealis</i>	nivalenol, fusarenone, zearalenone
<i>F. culmorum</i>	deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenone, zearalenone
<i>F. equiseti</i>	fusarochromanone, zearalenone, diacetoxyscirpenol
<i>F. graminearum</i>	deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenone, zearalenone
<i>F. oxysporum</i>	moniliformin, fusaric acid
<i>F. poae</i>	T2-toxin, HT2-toxin, nivalenol, diacetoxyscirpenol, fusarenone
<i>F. proliferatum</i>	fumonisin, moniliformin, fusarin C
<i>F. sporotrichioides</i>	T2-toxin, HT2-toxin, neosolaniol, diacetoxyscirpenol, fusarenone, zearalenone
<i>F. tricinctum</i>	moniliformin
<i>F. verticillioides</i>	fumonisin, moniliformin, fusarin C

## EU REGULATIONS AND GUIDANCE VALUES

Other than the direct health risk, economic losses and implications arising from mycotoxicoses are enormous. To protect human and animal exposure to mycotoxins, but also to reduce financial losses and to confer international trade advantages, a number of measures are needed at different levels: good agricultural practices (early harvesting, proper drying, physical treatment, sanitation, proper storage, insect management), biological and chemical control, decontamination, breeding for resistance and adequate legislation (Zain, 2011). As a result, many countries have adopted regulations to limit mycotoxin exposure, especially for deoxynivalenol and zearalenone (Zinedine & Ruiz, 2014; Bianchini et al., 2015).

According to the annual report of the Rapid Alert System for Food and Feed (RASFF), in 2013 mycotoxins were the third hazard category in border rejection notifications in the European Union, after pathogenic micro-organisms and pesticides residue. Analysis of these notifications reveals which products are more susceptible to be contaminated by these fungal metabolites. In the report, aflatoxins were the primary mycotoxins associated to the notifications, followed by ochratoxin A, deoxynivalenol and fumonisins (Table 2), and nuts, nut products and seeds, fruits and vegetables, herbs and spices, cereals/bakery products, and foodstuffs, were the most affected categories (RASFF Annual Report, 2014).

Table 2. Mycotoxin notifications in EU during 2004–2013 (RASFF Annual Report, 2014)

Hazard	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Aflatoxins	839	946	801	705	902	638	649	585	484	341
Deoxynivalenol				10	4	3	2	11	4	8
Fumonisin	14	2	15	9	2	1	3	4	4	7
Ochratoxin A	27	42	54	30	20	27	34	35	32	54
Patulin		6	7		3					
Zearalenone			1	6	2				4	
Total mycotoxins	880	996	878	760	933	669	688	635	528	410

The European regulations refer to maximum tolerated limits or guidance values for several mycotoxins in food and feed and also to the methods of sampling and analysis of mycotoxins (Regulation (EC) no 1881/2006 consolidated version by Regulation (EC) no 1126/2007, Regulation (EC) no 629/2008, Regulation (EC) no 165/2010; Regulation (EC) no 401/2006; Recommendation (EC) no 165/2013).

The maximum level is designed to prevent the occurrence of each mycotoxin at levels considered harmful to human and/or animal health. Selected examples of maximum values for some *Fusarium* mycotoxins in wheat are given in Table 3.

Table 3. Selected examples of maximum levels for some *Fusarium* mycotoxins in wheat (Regulation (EC) no 1881/2006 consolidated version; Recommendation (EC) no 165/2013)

Mycotoxin	Foodstuffs	Maximum levels [ $\mu\text{g/kg}$ ]
Deoxynivalenol	Unprocessed durum wheat and oats	1750
	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption	750
Zearalenone	Unprocessed cereals other than maize	100
	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption	75
HT-2 and T-2 toxins	Unprocessed wheat	100
	Wheat grains for direct human consumption	50
Fumonisin (B <sub>1</sub> +B <sub>2</sub> )	Not mentioned for wheat	

## **FUSARIUM MYCOTOXINS IN EUROPE**

### **TRICHOTHECENS**

Tricothecene mycotoxins are a family of tetracyclic sesquiterpenoid substances (12,13-epoxytricothec-9-ene skeleton) comprising over 200 compounds of widely varying toxicity. They possess a C9–C10 double bond, an epoxide between carbon atoms C12 and C13 (considered essential for toxicity), plus a variable number of hydroxyl and acetoxy groups in the molecule. Tricothecenes are divided into four types, named A, B, C, D, according to their functional groups present in the molecule, types A and B being the most common (Yazar & Omurtag, 2008; Santini et al., 2009; McCormick et al., 2011; Juan et al., 2012). Tricothecenes can also be divided into two groups, macrocyclic and non-macrocyclic tricothecenes, based on the presence or absence of a macrocyclic ring linking C-4 and C-15 (Mostrom, 2011; Haschek & Voss, 2013). The tricothecenes with major economic importance in agriculture are non-macrocyclic mycotoxins (Haschek & Voss, 2013).

Type A tricothecenes include HT-2 toxin, T-2 toxin, diacetoxyscirpenol (DAS), and neosolaniol (NEO) and differ from type B tricothecenes, such as deoxynivalenol (DON), nivalenol (NIV) and their acetyl derivatives, respectively 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON) and fusarenon-X (FUS-X), by the absence of a carbonyl group at the C-8 position (Yazar & Omurtag, 2008; Santini et al., 2009; Juan et al., 2012).

Tricothecens are small, amphiphilic molecules that can move passively across cell membranes and can be absorbed through the gastrointestinal and respiratory tracts, as well as skin (McCormick et al., 2011; Haschek & Voss, 2013). Understanding the toxicokinetics of tricothecenes is important for understanding potential effects in humans and animals. The tricothecenes can undergo all four basic reactions in xenobiotic metabolism: phase I (hydrolysis, oxidation, reduction) and phase II (glucuronide conjugation). The ability to remove the epoxide oxygen (deepoxidation) is an important step in the detoxification of tricothecenes. Specific metabolic pathways of these mycotoxins differ, and the metabolites produced often differ among species. The majority of these reactions occur in tissues and result in reduced toxicity; however some metabolites may be more toxic than the parent mycotoxin. It should be noted that HT-2 toxin is a major metabolite of T-2 toxin. Excretion is via the biliary system and urine. Enterohepatic recirculation may occur, resulting in delayed excretion and, ultimately, increased toxicity (Mostrom, 2011; Haschek & Voss, 2013).



Trichothecens target the 60S ribosomal subunit, suggesting that the major mechanism of toxicity is translational inhibition. The trichothecens have multiple effects on eukaryotic cells, the most important being the inhibition of protein, RNA and DNA synthesis. The mechanism of DNA synthesis inhibition has not yet been clarified, however, it may be a secondary effect of the inhibition of the protein synthesis or of the apoptotic effect of trichothecens (Yazar & Omurtag, 2008). Furthermore, they can produce: alteration of membrane structure and mitochondrial function, stimulation of lipid peroxidation, induction of programmed cell death or apoptosis, activation of cytokines and chemokines, activation of mitogen activated protein kinases - MAPKs, modulation of immune responses or alteration at neurotransmitter levels (Sorriano, 2007; Mostrom, 2011; Haschek & Voss, 2013; Marin et al., 2013; Marroquín-Cardona et al., 2014; Escriva et al., 2015).

Trichothecens are known to cause neurotoxicity, immunosuppression and renal toxicity. Different studies and reviews have shown that trichothecens can cause adverse effects in humans consuming grain-based foods and in animals ingesting contaminated grain or hay, including in chronic low-level exposure: emesis, nausea, anorexia, growth retardation, neuroendocrine changes, immunosuppression, gastroenteritis and gastrointestinal toxicity, exacerbation of infections (Placinta et al., 1999; CAST, 2003; Sorriano, 2007; McCormick et al., 2011; Mostrom, 2011; Zain, 2011; Streit et al., 2012; Haschek & Voss, 2013; Marin et al., 2013; Marroquín-Cardona et al., 2014). Because of their effects on the immune system, the exposure of trichothecenes could predispose humans and animals to infectious disease, especially for sensitive populations (examples for humans: young children, immuno-depressed people and old people) (Escriva et al., 2015).

DON is probably the most important trichothecene because it is commonly detected in cereal grains throughout the world, followed by T-2 and HT-2 toxins (Mostrom, 2011; Haschek & Voss, 2013); also NIV was usually found associated with DON and has been intensively studied (Santini et al., 2009; Zain, 2010; McCormick et al., 2011; Juan et al., 2012). In the Tables 4-7 are presented the incidence, the occurrence and the ranges for the positive wheat samples for the most important trichothecens, DON, NIV, HT-2 and T-2.

Table 4. Contamination of wheat and wheat derivatives by DON in Europe

Country	Commodity	Incidence (%)	Occurrence (a)	Range <sup>b</sup> (µg/kg)	Reference
<b>Belgium</b>	wheat	66.66	4/6	max. 150	De Boevre et al., 2012
<b>Bulgaria</b>	wheat	67.0	94/140	max. 1800	Vrabcheva et al., 1996
<b>Croatia</b>	wheat	65	33/51	max. 278	Pleadin et al., 2013
<b>Czech Republic</b>	wheat	100	48/48	17 - 2265.2	Hajšlova et al., 2007

	wheat	91.5	43/47	22.8 - 804.9	Hajšlova et al., 2007
	wheat	97.7	44/45	18.6 - 721.9	Hajšlova et al., 2007
	wheat	97.5	40/41	6.8 - 702.0	Hajšlova et al., 2007
<b>Denmark</b>	wheat flour	89	108/120	10 - 2591	Rasmussen et al., 2003
<b>Finland</b>	wheat	29.9	101/338	max. 5865	Van Der Fels-Klerx et al., 2012
<b>Germany</b>	wheat flour	100	28/28	15 - 965	Schollenberger et al., 2002
	wheat flour	92	12/13	38 - 756	Schollenberger et al., 2002
	whole grain wheat flour	100	19/19	15 - 1379	Schollenberger et al., 2002
	wheat flour	11.11	2/18	max. 177	Reinhold & Reinhardt, 2011
<b>Hungary</b>	wheat	78.2	287/367	70 - 1560	Rafai et al., 2000
<b>Italy</b>	wheat	28	16/57	9.6 - 99.6	Juan et al., 2013
	wheat	62.8	27/43	13 - 1230	Alkadri et al., 2014
<b>Lithuania</b>	wheat	98.4	61/62	max. 642	Mankevičienė et al., 2007
	wheat	94.3	83/88	max. 1121	Mankevičienė et al., 2007
	wheat	100	48/48	max. 223	Mankevičienė et al., 2011
	wheat	94.11	32/34	max. 445	Mankevičienė et al., 2011
<b>The Netherlands</b>	wheat	71.4	671/940	max. 10000	Van Der Fels-Klerx et al., 2012
<b>Norway</b>	wheat	14	24/169	max. 350	Langseth & Rundberget, 1999
	wheat	29.4	245/832	max. 890	Van Der Fels-Klerx et al., 2012
<b>Poland</b>	wheat	59.4	19/32	max. 997	Perkowski et al., 2007
	wheat	89	17/19	max. 455	Perkowski et al., 2012
	wheat	80	12/15	max. 341	Perkowski et al., 2012
<b>Portugal</b>	wheat products	40	4/10	333 - 1821	Peito & Venâncio, 2004
	wheat flour	80	8/10	20 - 77	Abrunhosa et al., 2014
	wheat flour	43	3/7	205 - 434	Cunha & Fernandes, 2010
<b>Romania</b>	wheat	100	25/25	max. 5600	Curtui et al., 1998
	wheat	83.3	10/12	6.1 - 154.3	Stroia et al., 2010
	wheat	42.5	17/40	max. 95.7	Banu et al., 2011
	wheat	73.08	19/26	294 - 3390	Alexa et al., 2013
	wheat	19.23	5/26	254 - 1440	Alexa et al., 2013
	wheat	90	38/42	21 - 3395	Macri et al., 2009
<b>Serbia</b>	wheat	85.7	24/28	52 - 3306	Stankovic et al., 2012
	wheat	93.3	70/75	50 - 1090	Stankovic et al., 2012
	wheat	50.0	2/4	0.63 - 1.84	Jajic et al., 2008
	wheat	34.5	19/55	0.057 - 0.42	Jajic et al., 2008
	wheat	27.78	15/54	41 - 309	Škrbić et al., 2011
	wheat flour	86.7	13/15	17.5 - 976	Škrbić et al., 2012
<b>Slovakia</b>	wheat	76.6	229/299	max. 7880	Šliková et al., 2013
	wheat	78	145/186	200 - 2940	Šliková et al., 2014
	wheat	100	20/20	220 - 7880	Šliková et al., 2014
<b>Slovenia</b>	wheat and wheat products	68.8	55/80	max. 3070	Kirinčič et al., 2015
<b>Spain</b>	wheat products	79.8	95/119	max. 83.2	Rodríguez-Carrasco et al., 2013
<b>Sweden</b>	wheat	20.6	114/554	max. 890	Van Der Fels-Klerx et al., 2012

	winter wheat	81	25/31	max. 303	Lindblad et al., 2013
	winter wheat	64	21/33	max. 1394	Lindblad et al., 2013
	spring wheat	93	26/28	max. 1189	Lindblad et al., 2013
	spring wheat	91	30/33	max. 6460	Lindblad et al., 2013
	wheat	90	26/29	max. 3230	Nordkvist & Häggblom, 2014
<b>United Kingdom</b>	wheat	86.0	1396/1624	max. 20333	Edwards, 2009
<b>Austria, Germany, Slovakia</b>	wheat	100	23/23	203 - 4130	Berthiller et al., 2009
<b>Europe</b>	wheat and wheat flour	61.0	3891/6350	max. 3600	SCOOP, 2003
<b>Europe</b>	flour	50.0	51/103	20 - 2270	Biselli & Hummert, 2005
<b>Europe and Mediterranean region</b>	wheat	62	157/254	max. 5510	Binder et al., 2007

<sup>a</sup> number of positive samples/number of total samples

<sup>b</sup> in positive samples

Table 5. Contamination of wheat and wheat derivatives by NIV in Europe

<b>Country</b>	<b>Commodity</b>	<b>Incidence (%)</b>	<b>Occurrence (<sup>a</sup>)</b>	<b>Range<sup>b</sup> (µg/kg)</b>	<b>Reference</b>
<b>Czech Republic</b>	wheat	78.0	32/41	15.4 - 25.9	Hajšlova et al., 2007
<b>Denmark</b>	wheat flour	47.5	57/120	10 - 234	Rasmussen et al., 2003
<b>Germany</b>	wheat flour	4	1/28	max. 25	Schollenberger et al., 2002
	wheat flour	8	1/13	max. 25	Schollenberger et al., 2002
	whole grain wheat flour	26	5/19	max. 25	Schollenberger et al., 2002
<b>Hungary</b>	wheat	9	33/367	50 - 590	Rafai et al., 2000
<b>Italy</b>	wheat	19.3	11/57	12 - 106	Juan et al., 2013
<b>The Netherlands</b>	wheat	0	0/134	-	Van Der Fels-Klerx et al., 2012
<b>Norway</b>	wheat	0	0/169	-	Langseth & Rundberget, 1999
<b>Poland</b>	wheat	44	14/32	max. 80	Perkowski et al., 2007
	wheat	53	8/15	max. 23	Perkowski et al., 2012
	wheat	84	16/19	max. 18	Perkowski et al., 2012
<b>Romania</b>	wheat	2	1/42	max. 30	Macri et al., 2009
<b>Serbia</b>	wheat	0	0/54	-	Škrbić et al., 2011
<b>Spain</b>	wheat products	13.4	16/119	max. 53.6	Rodríguez-Carrasco et al., 2013
<b>Sweden</b>	wheat	0	0/75	-	Van Der Fels-Klerx et al., 2012
	winter wheat	94	29/31	max. 111	Lindblad et al., 2013
	winter wheat	33	11/33	max. 39	Lindblad et al., 2013
	spring wheat	50	14/28	max. 39	Lindblad et al., 2013
	spring wheat	27	9/33	max. 50	Lindblad et al., 2013
<b>United Kingdom</b>	wheat	67.0	1088/1624	max. 430	Edwards, 2009

<sup>a</sup> number of positive samples/number of total samples

<sup>b</sup> in positive samples

Table 6. Contamination of wheat and wheat derivatives by HT-2 in Europe

Country	Commodity	Incidence (%)	Occurrence <sup>a</sup> (°)	Range <sup>b</sup> (µg/kg)	Reference
<b>Belgium</b>	wheat	33.33	2/6	max. 14	De Boevre et al., 2012
<b>Czech Republic</b>	wheat	14.6	6/41	12.7 - 18.3	Hajšlova et al., 2007
<b>Denmark</b>	wheat flour	16.7	6/36	max. 33	Rasmussen et al., 2003
<b>Germany</b>	wheat flour	0	0/28	-	Schollenberger et al., 2002
	wheat flour	8	1/13	max. 12	Schollenberger et al., 2002
	whole grain wheat flour	16	3/19	max. 4	Schollenberger et al., 2002
	wheat products	94	122/130	max. 22	Gottschalk et al., 2009
<b>Italy</b>	wheat	0	0/20	-	EFSA, 2010
	wheat	29	6/20	max. 13.7	EFSA, 2010
	wheat	5.3	3/57	6.78 - 60.1	Juan et al., 2013
<b>The Netherlands</b>	wheat	0	0/134	-	Van Der Fels-Klerx et al., 2012
	wheat	5	4/85	max. 38	EFSA, 2010
<b>Poland</b>	wheat	22	7/32	max. 66	Perkowski et al., 2007
	wheat	7	1/15	max. 9	Perkowski et al., 2012
	wheat	26	5/19	max. 2	Perkowski et al., 2012
<b>Romania</b>	wheat	50	21/42	3 - 18	Macri et al., 2009
<b>Serbia</b>	wheat	5.56	3/54	128 - 129	Škrbić et al., 2011
	wheat flour	0	0/15	-	Škrbić et al., 2012
<b>Spain</b>	wheat products	16.8	20/119	max. 28.2	Rodríguez-Carrasco et al., 2013
<b>Sweden</b>	wheat	10	3/29	max. 13	Nordkvist & Häggblom, 2014
<b>Norway</b>	wheat	1.2	2/169	max. 20	Langseth & Rundberget, 1999
<b>United Kingdom</b>	wheat	31.0	503/1624	max. 193	Edwards, 2009
	wheat	20	12/60	max. 49	Scudamore et al., 2009

<sup>a</sup> number of positive samples/number of total samples<sup>b</sup> in positive samples

Table 7. Contamination of wheat and wheat derivatives by T-2 in Europe

Country	Commodity	Incidence (%)	Occurrence <sup>a</sup> (°)	Range <sup>b</sup> (µg/kg)	Reference
<b>Belgium</b>	wheat	0	0/6	-	De Boevre et al., 2012
<b>Bulgaria</b>	wheat	0.7	1/140	55	Vrabcheva et al., 1996
<b>Croatia</b>	wheat	25	13/51	max. 18	Pleadin et al., 2013
<b>Czech Republic</b>	wheat	39.0	16/41	5.7 - 8.2	Hajšlova et al., 2007
<b>Denmark</b>	wheat flour	29	11/38	max. 153	Rasmussen et al., 2003
<b>Finland</b>	wheat	0	0/338	-	Van Der Fels-Klerx et al., 2012
<b>Germany</b>	wheat flour	0	0/28	-	Schollenberger et al., 2002
	wheat flour	0	0/13	-	Schollenberger et al., 2002
	whole grain wheat flour	16	1/19	max. 4	Schollenberger et al., 2002
	wheat products	85	110/130	max. 1.9	Gottschalk et al., 2009
<b>Hungary</b>	wheat	6.5	24/367	80 - 370	Rafai et al., 2000
<b>Italy</b>	wheat	8	2/20	max. 1.4	EFSA, 2010
	wheat	29	6/20	max. 4.9	EFSA, 2010

	wheat	3.5	2/57	7.14 - 17.8	Juan et al., 2013
<b>Lithuania</b>	wheat	69	33/48	max. 18.8	Mankevičienė et al., 2011
	wheat	100	34/34	max. 23.0	Mankevičienė et al., 2011
<b>The Netherlands</b>	wheat	0	0/159	-	Van Der Fels-Klerx et al., 2012
	wheat	13	11/85	max. 7	EFSA, 2010
<b>Norway</b>	wheat	0.6	1/169	max. 20	Langseth & Rundberget, 1999
<b>Poland</b>	wheat	0	0/32	-	Perkowski et al., 2007
	wheat	0	0/15	-	Perkowski et al., 2012
	wheat	16	3/19	max. 4	Perkowski et al., 2012
<b>Romania</b>	wheat	24	6/25	max. 63	Curtui et al., 1998
	wheat	100	2/2	0.8 - 1.0	Galbenu-Morvay et al., 2011
	wheat	2	1/42	max. 7	Macri et al., 2009
<b>Serbia</b>	wheat	75.0	21/28	60 - 495	Stankovic et al., 2012
	wheat	60.0	45/75	86 - 200	Stankovic et al., 2012
	wheat	0	0/54	-	Škrbić et al., 2011
	wheat flour	26.7	4/15	9.8 - 26.9	Škrbić et al., 2012
<b>Spain</b>	wheat products	0.8	1/119	max. 13.7	Rodríguez-Carrasco et al., 2013
<b>Sweden</b>	wheat	7	2/29	max. 12	Nordkvist & Häggblom, 2014
<b>United Kingdom</b>	wheat	16.0	260/1624	max. 52	Edwards, 2009
	wheat	5.3	3/57	max. 13	Scudamore et al., 2009

<sup>a</sup> number of positive samples/number of total samples

<sup>b</sup> in positive samples

## FUMONISINS

Fifteen different fumonisins have been described and grouped by chemical properties into four categories A, B, C, and P. Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is the most abundant compound from class B, followed by fumonisin B<sub>2</sub> (FB<sub>2</sub>) and fumonisin B<sub>3</sub> (FB<sub>3</sub>) (Voss et al., 2011; Marin et al., 2013; Coppock & Dziwenka, 2014; Marroquín-Cardona et al., 2014). Fumonisin B<sub>1</sub> is a diester of propane-1,2,3-tricarboxylic acid and 2S-amino-12S,16R-dimethyl-3S,5R,10R,14S,15R-pentahydroxyeicosane in which the C-14 and C-15 hydroxy groups are esterified (Coppock & Dziwenka, 2014).

The bioavailability, distribution and toxicokinetics of fumonisins have been studied in multiple species. Absorption is low and only small amounts accumulate in tissues. Gastrointestinal absorption is poor and absorbed FB<sub>1</sub> is quickly eliminated from the blood. Minor amounts accumulated in liver and kidneys can be found while only negligible amounts can be found in other tissues (Voss et al., 2011).

Fumonisin are a group of mycotoxins with a strong structural similarity to sphingosine and sphinganine, the backbone precursor of sphingolipids (Haschek & Voss, 2013; Marin et al., 2013). The toxicity of fumonisins is a result of competition with sphingosine in sphingolipids metabolism

(Kotowicz et al., 2014). Fumonisin bind to and are potent inhibitors of sphinganine N-acetyltransferases (ceramide synthases) (Coppock & Dziwenka, 2014). The inhibition of these enzymes disrupts overall sphingolipid metabolism, resulting in accumulation of sphingolipid bases, with implications in the development of disease symptoms (Voss et al., 2011; Kotowicz et al., 2014).

FB<sub>1</sub>, the most predominant and well-studied isoform, is nephrotoxic and hepatotoxic in several species and has been classified as a Group 2B, possible human carcinogen (IARC, 2002). The ingestion of fumonisin-contaminated cereals in animals has been associated with: appetite loss, reduced litter weight, low bone development in fetus, fetal mortality, respiratory problems, porcine pulmonary edema, hepatic lesions, fibrosis, neurotoxicity, hepatocellular carcinoma, hypercholesterolemia, lethargy, hydrothorax, equine leukoencephalomalacia, immunosuppression (Sorriano, 2007; Oancea & Stoia, 2008; Voss et al., 2011; Zain, 2011; Streit et al., 2012; Haschek & Voss, 2013; Coppock & Dziwenka, 2014; Marroquín-Cardona et al., 2014; Escriva et al., 2015). The risks to humans posed by fumonisins are undetermined at present; however, a link between fumonisins and esophageal cancer was detected (Prieto-Simon et al., 2007; Silva et al., 2014).

A wide variety of commodities in the world have been analysed for fumonisins contamination (Stankovic et al., 2012); the studies have shown that fumonisins occur naturally at biologically significant levels in maize and a variety of maize-based human foodstuffs and animal feeds, but also can be found in low concentrations in other cereals like wheat (Haschek & Voss, 2013; Kotowicz et al., 2014). Some concentrations for fumonisins in wheat samples are presented in Table 8.

Table 8. Contamination of wheat and wheat derivatives by FB<sub>1</sub> in Europe

Country	Commodity	Incidence (%)	Occurrence (°)	Range <sup>b</sup> (µg/kg)	Reference
Germany	wheat bran	0	0/32	-	Rubert et al., 2013
	wheat flakes	13.7	6/44	20.2 - 59.8	Rubert et al., 2013
France	wheat flakes	14.3	9/63	75.8 - 125.8	Rubert et al., 2013
Romania	wheat	0	0/25	-	Curtui et al., 1998
	wheat	0	0/12	-	Stroia et al., 2010
	wheat	0	0/1	-	Galbenu et al., 2011b
Serbia	wheat	82.1	23/28	750 - 5400	Stankovic et al., 2012
	wheat	92.0	69/75	750 - 4900	Stankovic et al., 2012
	wheat flour	0	0/15	-	Škrbić et al., 2012
Europe	wheat	79.0	87/110	max. 736	SCOOP, 2003
	wheat flour	16.0	42/256	max. 4343	SCOOP, 2003

<sup>a</sup> number of positive samples/number of total samples

<sup>b</sup> in positive samples

FB<sub>1</sub> concentrations usually exceed those of FB<sub>2</sub> and FB<sub>3</sub> of about 3 or more times, although higher concentrations of FB<sub>2</sub> or FB<sub>3</sub> can also be observed (Voss et al., 2011). Even so, most studies analyze fumonisins as sum of FB<sub>1</sub>, FB<sub>2</sub> or FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>.

## **ZEARELENONE**

Zearalenone (ZEA) is a non-steroidal oestrogenic mycotoxin, which is prevalent in temperate and warm countries. Chemically, ZEA, is 6-[10-hydroxy-6-oxo-trans-1-undecenyl]-beta-resorcylic acid lactone (Marin et al., 2013).

ZEA is readily and rapidly absorbed from gastrointestinal tract (Haschek & Voss, 2013) and undergoes both phase I and phase II metabolism with involvement of different enzymes catalyzing the first biotransformation step (Mostrom, 2011). ZEA is reduced to  $\alpha$ - and  $\beta$ -isomers in mammalian tissues, and these metabolites can also be produced at low concentrations by the fungi. ZEA and the metabolite zearalenol (as a combination of free and conjugated forms) are excreted relatively rapidly in feces, urine, and to a small extent in milk (Zinedine et al., 2007).

ZEA and its derivatives are the only known mycotoxins with primarily estrogenic effects, thus they are considered mycoestrogens, a subset of naturally occurring estrogenic compounds or xenoestrogens, and are classified as endocrine disruptor chemicals. Also, ZEA can act on the hypothalamic-hypophyseal axis with release of prolactin and luteinizing hormone, can activate the pregnane X receptor, the constitutive androstane receptor, the aryl hydrocarbon receptor mRNA levels, as well as a number of CYP enzymes in human hepatocyte cultures (Haschek & Voss, 2013).

The predominant adverse effects are related to the estrogenic activity of ZEA and its metabolites: alterations in the reproductive tract, uterus enlargement, decreased fertility, increased embryo/lethal resorptions, reduced litter size, and changes in the serum levels of progesterone and estradiol. ZEA has been shown also to be an enhancer of lipid peroxidation, immunotoxic, hepatotoxic, nephrotoxic, genotoxic and with a high potential of carcinogenicity (Zinedine et al., 2007; Mostrom, 2011). According to the available toxicological data, ZEN is classified by the IARC as Group 3 (IARC, 2002).

ZEA occurs in many agricultural products, including cereals, mixed feeds, rice, and corn silage. The most frequently contaminated crop is corn, followed by wheat. A synthesis of results for wheat contamination in Europe is related in Table 9.

Table 9. Contamination of wheat and wheat derivatives by ZEA in Europe

Country	Commodity	Incidence (%)	Occurrence (a)	Range <sup>b</sup> (µg/kg)	Reference
<b>Bulgaria</b>	wheat	69.0	97/140	max. 120	Vrabcheva et al., 1996
	wheat	1.9	1/54	max. 10	Manova & Mladenova, 2009
<b>Croatia</b>	wheat	66.7	4/6	13 - 50	Klarić et al., 2010
	wheat	69	35/51	max. 107	Pleadin et al., 2013
<b>Denmark</b>	wheat flour	33.3	10/30	max. 2	Rasmussen et al., 2003
<b>Germany</b>	wheat	92	22/24	11 - 860	Schneweis et al., 2002
	wheat flour	11	3/28	1 - 2	Schollenberger et al., 2002
	wheat flour	31	4/13	1 - 8	Schollenberger et al., 2002
	whole grain wheat flour	79	15/19	2 - 24	Schollenberger et al., 2002
<b>Hungary</b>	wheat	58.6	215/367	50 - 890	Rafai et al., 2000
<b>Lithuania</b>	wheat	31.4	16/51	max. 95.6	Mankevičienė et al., 2007
	wheat	32.6	16/49	max. 33.4	Mankevičienė et al., 2007
	wheat	69	33/48	max. 28.1	Mankevičienė et al., 2011
	wheat	97	33/34	max. 45.8	Mankevičienė et al., 2011
<b>Italy</b>	wheat	8.8	5/57	2.35 - 27.15	Juan et al., 2013
<b>The Netherlands</b>	wheat	8.7	27/312	max. 310	Van Der Fels-Klerx et al., 2012
	wheat flour	100	2/2	12.4 - 13.7	Aldana et al., 2014
	mixed flour with mainly wheat flour	50	2/4	19.8 - 37.2	Aldana et al., 2014
<b>Portugal</b>	wheat based products	50	2/4	11.0 - 15.0	Peito & Venâncio, 2004
	wheat flour	14	1/7	max. 27	Cunha & Fernandes, 2010
	wheat flour	23.5	4/17	7.4 - 15.3	Aldana et al., 2014
	mixed flour with mainly wheat flour	30.8	4/13	5.4 - 39.4	Aldana et al., 2014
<b>Romania</b>	wheat	100	25/25	max. 170	Curtui et al., 1998
	wheat	10	2/20	0.88 - 3.57	Galbenu et al., 2011a
	wheat flour	31.25	5/16	0.41 - 41.8	Galbenu et al., 2011a
	wheat bran	100	1/1	max. 0.42	Galbenu et al., 2011a
	wheat	50	6/12	36.7 - 67.3	Stroia et al., 2010
	wheat	10	4/40	max. 5.52	Banu et al., 2011
	wheat	69.23	18/26	37.6 - 1000	Alexa et al., 2013
	wheat	76.92	20/26	28 - 105.6	Alexa et al., 2013
	wheat	5	17/336	max. 80	Misca et al., 2014
<b>Serbia</b>	wheat	88.6	22/28	10 - 143	Stankovic et al., 2012
	wheat	94.6	71/75	16 - 201	Stankovic et al., 2012
	wheat	0	0/54	-	Škrbić et al., 2011
	wheat flour	33.33	5/15	1.9 - 21.1	Škrbić et al., 2012
<b>Slovenia</b>	wheat and wheat products	23.8	19/80	max. 113	Kirinčič et al., 2015
<b>Spain</b>	wheat products	0	0/119	-	Rodríguez-Carrasco et al., 2013
<b>Sweden</b>	wheat	0	0/51	-	Van Der Fels-Klerx et al., 2012
	winter wheat	100	31/31	max. 86	Lindblad et al., 2013
	winter wheat	9	3/33	max. 25	Lindblad et al., 2013
	spring wheat	36	10/28	max. 32	Lindblad et al., 2013



	spring wheat	39	13/33	max. 678	Lindblad et al., 2013
	wheat	34.5	10/29	max. 116	Nordkvist & Häggblom, 2014
<b>United Kingdom</b>	wheat	19.0	309/1624	max. 1292	Edwards, 2009
<b>Europe</b>	wheat milling products	14.0	432/3088	max. 507	Marin et al., 2013
<b>Europe and Mediterranean region</b>	wheat	92	44/48	max. 921	Binder et al., 2007

<sup>a</sup> number of positive samples/number of total samples

<sup>b</sup> in positive samples

## CONCLUSIONS AND FUTURE PERSPECTIVES

Food security and food quality are important issues in the context of globalization. The European Union is one of the world's biggest cereal producers, but it is also a big cereals consumer, wheat being the most important in this category. *Fusarium* genus produce an extraordinary diversity of biologically active secondary metabolites, some of which are harmful to animals and humans, like mycotoxins. Health risks associated with the consumption of cereal products, contaminated with *Fusarium* mycotoxins are worldwide recognized. Trichothecenes, zearalenone and fumonisins are distributed widely in cereals, including wheat. The problem of co-occurrence of *Fusarium* mycotoxins in wheat is a recurring feature, raising the question of interactions, synergistic or antagonistic actions in the manifestation of toxicity, which can be the future in this field of research. The relationship between climate change in Europe and mycotoxin development is an accepted idea by most scientists. Global warming will possibly increase the fungi development, but will definitely also produce the growth of new fungi species, and consequently new mycotoxins, in crops. Following this, an increase of analytical methods for food control and measures that influence food security, such as Good Practice, HACCP and others are required. To avoid negative impacts on humans and animal health, compliance with EU regulations and the development of programs of risk assessment based on hazard and exposure evaluation are necessary .

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