In The Name Of Allah,
The Most Beneficent, The Most Merciful

مایکوتوکسین ها و آلودگی مواد غذایی
Mycotoxins and Food Contamination

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بیماریهای ناشی از غذا
Food-borne diseases

- pathogenic microorganisms: acute effects on human health.
- presence of various chemical substances(chronic, and in some cases acute) including:
  - Residues of pesticides
  - Veterinary drugs,
  - Unlawful food additives
  - **Mycotoxins**,
  - Biotoxins
  - Radionuclides

Epidemiology of FBD :

- WHO estimates that one in three people worldwide suffer from a food-borne disease every year,
- 1.8 million die from severe food and waterborne diarrhea ,related illness
- Most of these illnesses are due to microorganisms and chemical contaminants, which may occur naturally or be introduced at some point along the food chain.
- Campylobacter and Salmonella species account for over 90% of all reported cases of bacteria related to food poisoning worldwide.
- As most cases of food borne disease are not reported, **the true dimension of the problem is unknown.**
- **Today food safety is one of WHO's top eleven priorities**

Food safety and security :

- The World Food Summit, organized by FAO in 1996, recognized that access to safe food is in itself an element of food security,
- The World Health Assembly Resolution on Food safety from May 2000 stated that :

1. Everyone should have the right to an adequate supply of safe, nutritious food ,
2. Encourages WHO member states "to implement and keep national and, when appropriate, regional mechanisms for food borne disease surveillance"
3. Governments should take the necessary measures to ensure the availability of safe food for all in order to sustain the health and economic development of their people.

Discovery of mycotoxins

- Serious worldwide concern began in the early 1960s after "Turkey X disease" was discovered in UK.

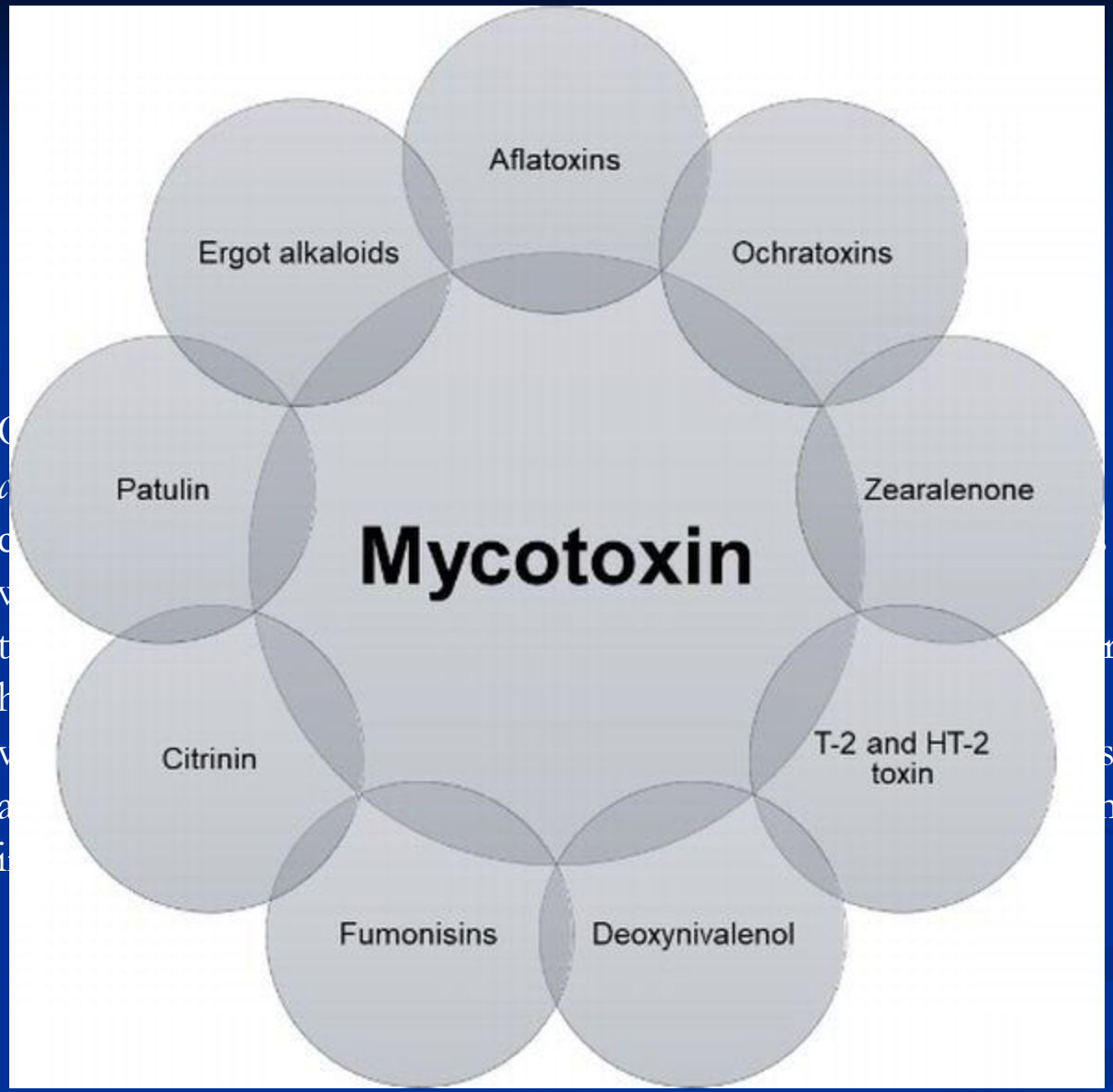
- More than 100,000 young turkeys on poultry farms died in the course of a few months.

- Investigation of the early outbreaks showed that they were all associated with feeds, namely Brazilian peanut meal .

Impact of Mycotoxins

- Significant economic losses are associated with their impact on human health, animal productivity, and both domestic and international trade.
- It is estimated that 25% of the world's food crops, including many basic foods, are affected by mycotoxin producing fungi.
- According to FAO estimates global losses of foodstuffs due to mycotoxins are in the range of 1000 million tonnes per year [1].
- Over $100 billion of exported commodities all over the world are susceptible to mycotoxin contamination [2].

[1] FAO wesbite (http://www.fao.org/food/food-safety-quality/a-z-index/mycotoxins/en/)
[2] Cardwell K. F. (2001), Food and nutrition Bulletin, 21:488–492


Aflatoxins
Ergot alkaloids
Ochratoxins
Patulin
Mycotoxin
Zearalenone
Citrinin
T-2 and HT-2 toxin
Fumonisins
Deoxynivalenol

Mycotoxin may cause a harmful effect to animals as well as humans such as carcinogenic,nephrotoxicity, mutagenic, immunosuppressive, estrogenic neurotoxicity, reproductive and developmental toxicity, hepatotoxicity and indigestion

- Most of the countries agreed to set the limits of mycotoxins present in food because of the effects of the mycotoxins to human health.
- The permitted level is slightly different, which depends on the type of food products.
- The minimum limits for mycotoxins in single ppb (part per billion) and even below (0.05 ppb for infant foods) are established in EU, with similar standards in China and Japan

Methods to test

Conventional techniques:

- thin-layer chromatography (TLC),
- high-performance liquid chromatography (HPLC)
- mass spectrometry
- enzyme-linked immunosorbent assay (ELISA)

(it has slight defects of cross-reactivity and possible false-positive or false-negative outcomes Also, those techniques usually costly and available in a specialized research laboratory needs highly personnel trained and laborious).

- Recently, advanced methods used to detect the presence of mycotoxins in food samples, which show high sensitivity, low cost, simple operation, and portable on-field use
- Besides, portable and easy-to-use biosensor devices suitable for express, in-field detection of mycotoxins.
- The development of biosensors for mycotoxins has risen sharply in the last decade with a large number of different bio-sensing technologies application

- Aflatoxins B1 and M1 (AFT B1 & M1) [15] produced by Aspergillus flavusand A. parasiticusspecies grown on grains and cereals, spices, tree nuts.

- Aflatoxin B1(AFB1) is one of the most carcinogenic substances produced by fungi and results in inevitable contamination of food and feed at deficient concentrations.

- Four main types of aflatoxin naturally contaminate foods which are aflatoxin B1 (AFB1), G1 (AFG1) and their dihydroderivatives B2 (AFB2) and G2 (AFG2).
- Others without additional metabolites known as Aflatoxin M1 and Aflatoxin M2.

- AFT M1 being a 4-hydroxylated metabolite of AFT B1, is found in cow and sheep milk and milk products.

What are Mycotoxins?

Low molecular weight (small molecules) produced as fungal Secondary metabolites that exert toxic effects on animals and human beings.

- 300-400 compounds are recognized as mycotoxins, many received attention as human threats
- Mold (fungi) produce mycotoxins to destroy their microbial competitors.
- Most literature attributes mycotoxicosis to ingestion of contaminated foods but airborne contamination is a problem as well

Mycotoxins are associated with human disease and cause acute and chronic effects

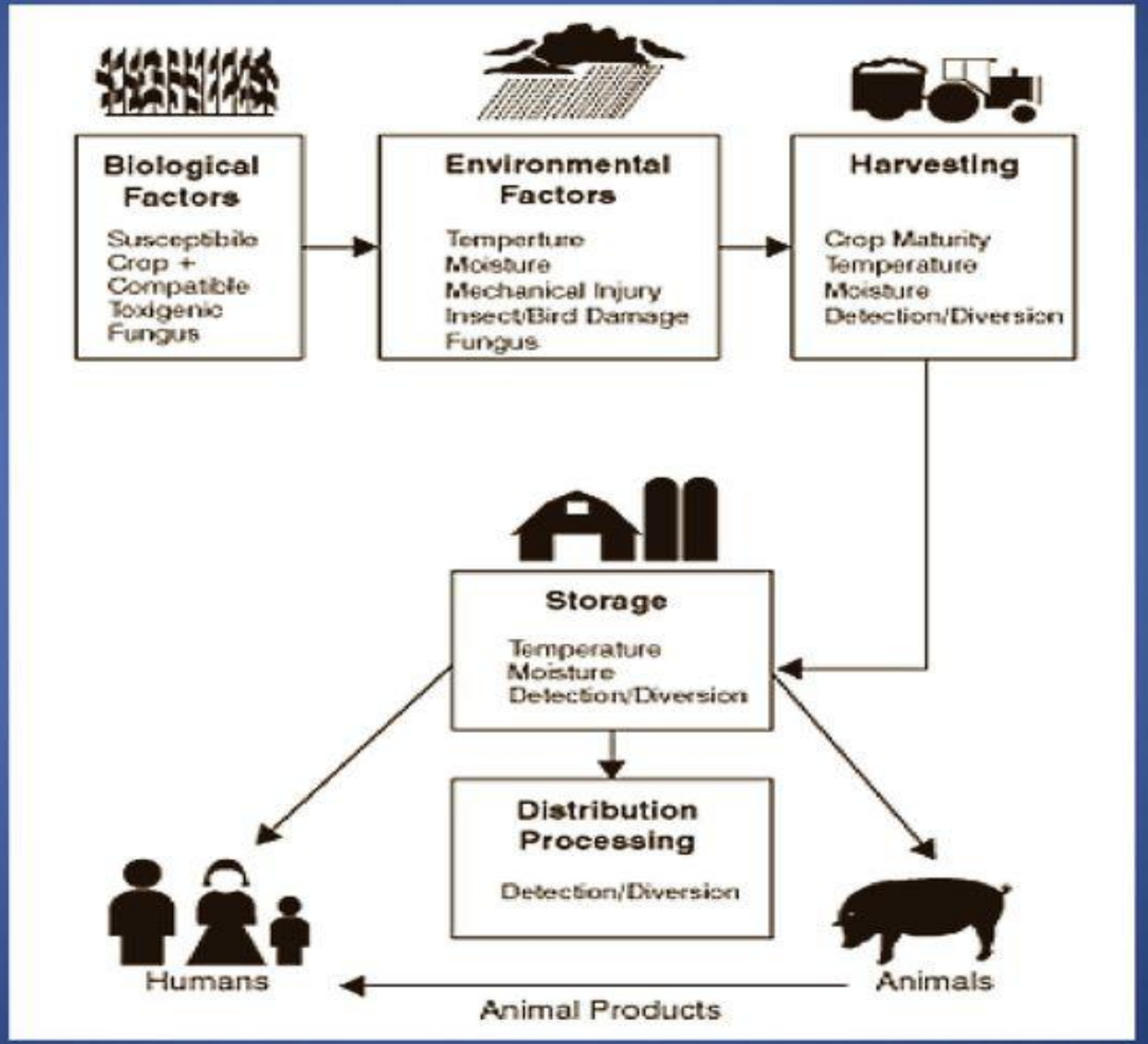
Fungal Infection

- Can occur at any stage in crop production.
- While in the field.
- During harvesting.
- While in silage and storage.
- Spores can lay dormant for months to years, waiting for positive conditions for germination.

Conditions to Encourage Fungal Growth

- Relative humidity over 70%.
- Temperatures over 30 degrees Celsius for a period of a few days to a week.
- Stress to the affected plant, such as drought, flood, or insect infestation.
- High moisture content of crop (20% or higher).

Mycotoxin Chain of Events



Mycotoxin occurrence

Field Crops

Field mycotoxins contamination

Harvesting

Field mycotoxins contamination

Drying/Silo storage

Storage mycotoxins contamination

Biological factors, Agronomic practices, Environmental factors(Temperature, Humidity, Time)

Feed mills

Storage mycotoxins contamination

Local farms

Storage mycotoxins contamination

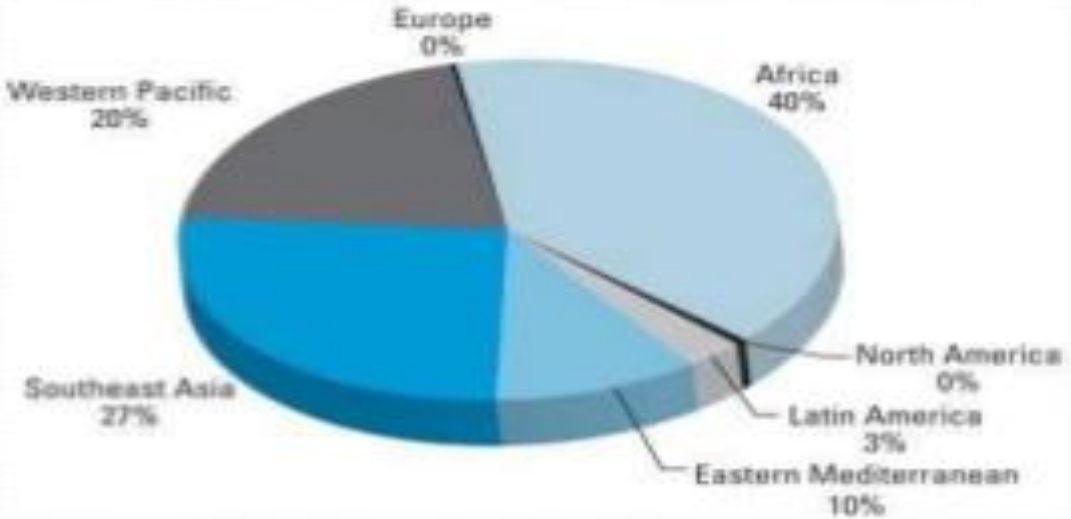
Processing

Storage mycotoxins contamination

Mycotoxin contamination of meat/milk supply

Mycotoxins in Food

- Today, 300-400 mycotoxins are known[1].
- Aflatoxin B1 has been classified by the IARC to be a Class 1 human carcinogen.
- A 2010 study by Liu, Y. Wu in collaboration with WHO estimated that Aflatoxin causes between 5-30% of all liver cancer cases in the world[2].



[1] Berthiller F., Sulyok M., Krska R., Schuhmacher R., Int. J. Food Microbiol. 2007; 119:33–37.
[2] Environ Health Perspect. 2010 Jun;118(6):818-24. doi: 10.1289/ehp.0901388. Epub 2010 Feb 19

Mycotoxins in Food

- There are six agriculturally important mycotoxins : aflatoxins, trichothecenes, fumonisins, zearalenone and ochratoxin.

Mycotoxins	Crops
Aflatoxins	Groundnut, Maize, Almond, Fig, Pistachios, Hazenut, Sunflower seed, Sorghum, Cashew, Chestnut, Nutmeg, Rice, Chilli, Pepper, Turmeric, Milk
Ochratoxins	Coffee, Grape, Paprika, Fig, Pepper, Barley, Nutmeg, Corn
Fumonisins	Maize, Wheat, Rice
Trichothecenes	Wheat, Oats, Corn
Zearalenone	Corn, Wheat, Soyabean, Rice, Barley
Ergot alkaloids	Rye, Barley, Wheat, Oats

References : RSAFF, Int. J. Mol. Sci., 2008, 9, pp2062-2090

Mycotoxins	Organisms	Foods
Aflatoxins	*A. flavus, A. paraciticus*	Corn,peanuts,cottonseed,etc.
Ochratoxin A	*A. ochraceus, R. veridicatum, R. cyolopium*	Corn,barley,wheat, peanuts
Zearalenone	*Fusarium roseum, E. moniliforme, F. nivale, E. oxysporum*	Corn, sorghum, wheat
Fumonisin	*Fusarium*	Corn
Patulin	*A. clavatus, R. patuluns*	Silage, apples
Trichothecene	Fusarium graminearum	Cereal grains,coffee

Toxicity of Mycotoxins in Food

Mycotoxin	Major Foods	Species	Health effects	LD50 (mg/kg)
flatoxins	Maize, groundnuts, figs, tree nuts (Aflatoxin M1 (secreted by cow after metabolism of aflatoxin B1), milk, milk products	*Aspergillus flavus* *Aspergillus parasiticus*	Hepatotoxic, carcinogenic	0.5 (dog) 9.0 (mouse)
Fumonisin	Maize	*Fusarium moniliforme*	Esophageal cancer	?
Ochratoxin	Maize, cereals, coffee beans	*Penicillium verrucosum* *Aspergillus ochraceus*	Nephrotoxic	20-30 (rat)
T-2 toxin	Cereals	*Fusarium sporotrichioides*	Alimentary toxic, aleukia	4 (rat)

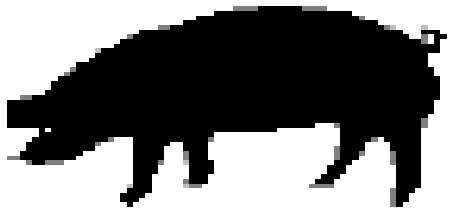
Occurrence data (EFSA chemical occurrence database)

Distribution of total Aflatoxins by sampling year in cereals and milling products

		Year of sampling												
		2007		2008		2009		2010		2011		TOTAL (2007-2011)		
		N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	P95
Barley grain	Lower bound [a]	1	0.00	5	0.03	1	0.00	4	0.00	5	0.00	16	0.01	.
	Upper bound [b]	1	0.40	5	0.69	1	0.64	4	0.58	5	1.00	16	0.74	.
Buckwheat grain	Lower bound	4	0.00	9	0.00	12	0.00	3	0.00	28	1.12	56	0.56	.
	Upper bound	4	0.12	9	0.64	12	0.64	3	0.40	28	2.43	56	1.48	.
Buckwheat milling products	Lower bound	.	.	.	.	1	0.00	10	0.00	2	0.00	13	0.00	.
	Upper bound	.	.	.	.	1	0.40	10	2.56	2	0.50	13	2.08	.
Corn grain	Lower bound	.	.	1	0.00	3	0.00	8	0.00	38	0.07	50	0.06	.
	Upper bound	.	.	1	0.80	3	0.35	8	0.39	38	1.48	50	1.21	.
Corn milling products	Lower bound	3	0.00	35	0.16	18	0.00	34	0.56	29	0.01	119	0.21	0.00
	Upper bound	3	0.09	35	0.52	18	0.34	34	1.21	29	0.59	119	0.69	0.99
Millet grain	Lower bound	.	.	.	.	.	.	1	0.00	2	0.00	3	0.00	.
	Upper bound	.	.	.	.	.	.	1	0.08	2	0.70	3	0.49	.
Oats, grain	Lower bound	1	0.00	2	0.00	.	.	4	0.00	.	.	7	0.00	.
	Upper bound	1	0.40	2	0.70	.	.	4	0.52	.	.	7	0.55	.
Oat milling products	Lower bound	.	.	1	0.00	.	.	2	0.00	3	0.00	6	0.00	.
	Upper bound	.	.	1	0.64	.	.	2	0.80	3	2.60	6	0.89	.
Rice	Lower bound	57	0.16	118	0.15	88	0.39	167	0.40	200	0.53	630	0.37	1.74
	Upper bound	57	0.38	118	0.43	88	0.65	167	0.84	200	1.38	630	0.87	2.60
Rice milling products	Lower bound	.	.	.	.	2	0.41	4	0.00	4	0.23	10	0.17	.
	Upper bound	.	.	.	.	2	0.66	4	0.40	4	0.65	10	0.55	.

❑۱۱ ماده غذایی با بالاترین ریسک آلودگی به مایکوتوکسین

Consumption of contaminated feed



Contamination of plants and vegetable products by OTA during growth of toxigenic fungus species

Transmission of OTA in blood organs and meat

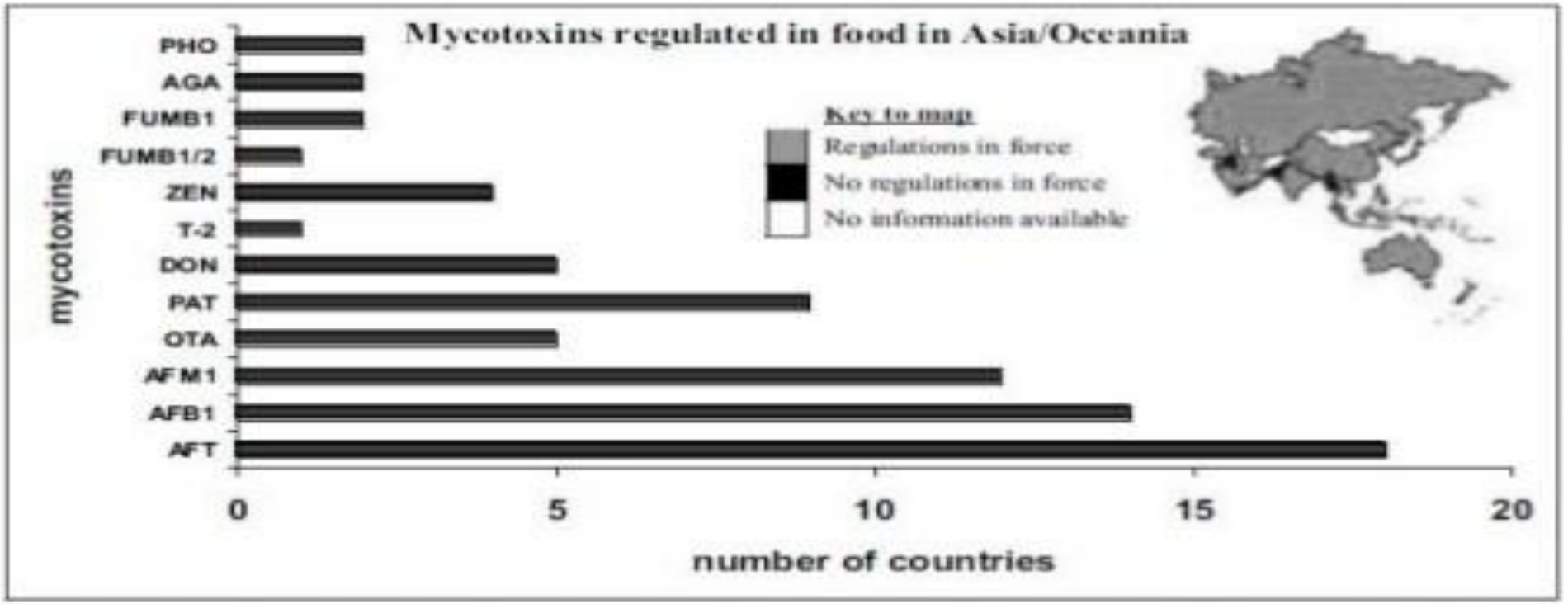
Consumption of contaminated vegetable foodstuff

Consumption of contaminated foodstuff of animal origin

Transmission of OTA in maternal milk

Mycotoxin control - regulations

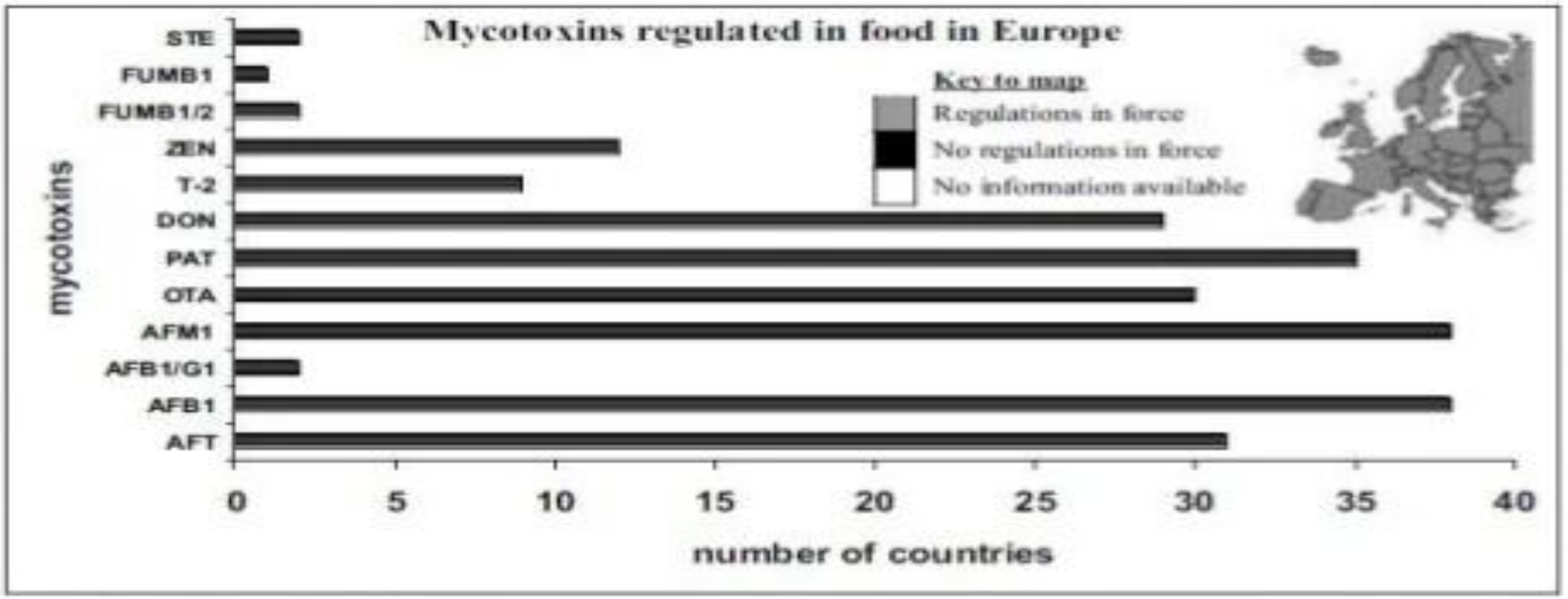
- Legislation is established in many countries worldwide.
- In Asia/Oceania, 26 countries have specific mycotoxin regulations.



Reference : Worldwide regulations for mycotoxins in food and feed in 2003

Mycotoxin regulations

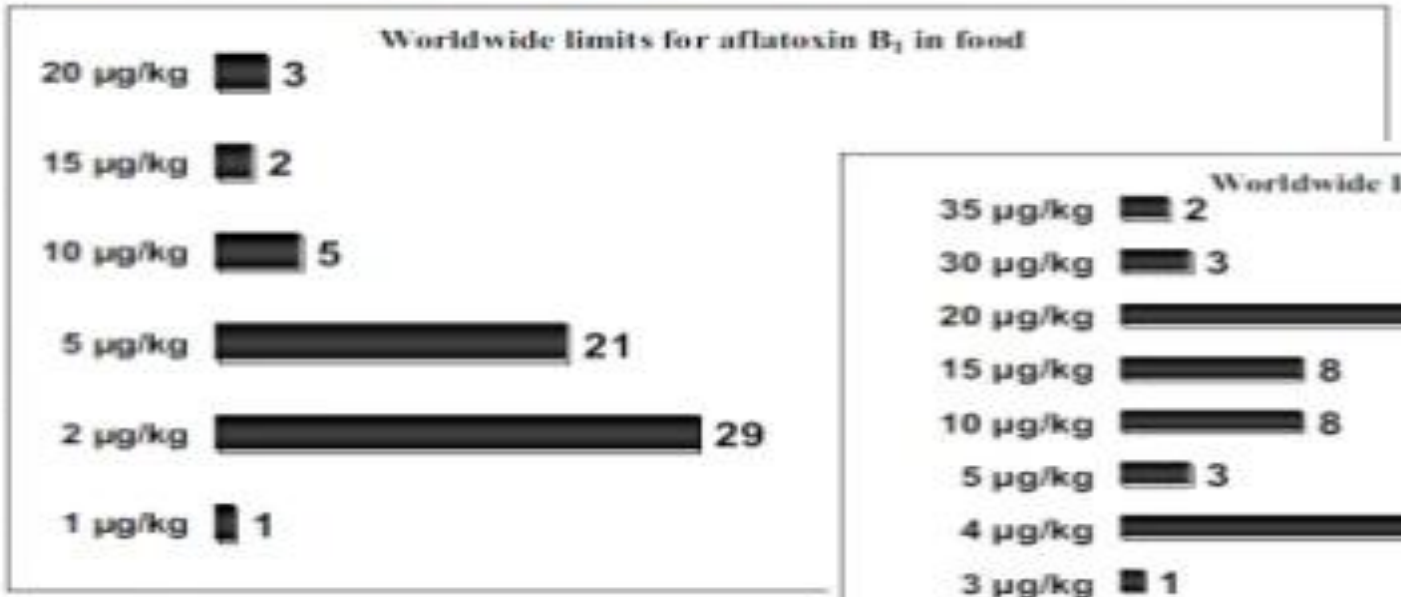
In Europe, ~ 99% of the 39 countries have specific mycotoxin regulations.

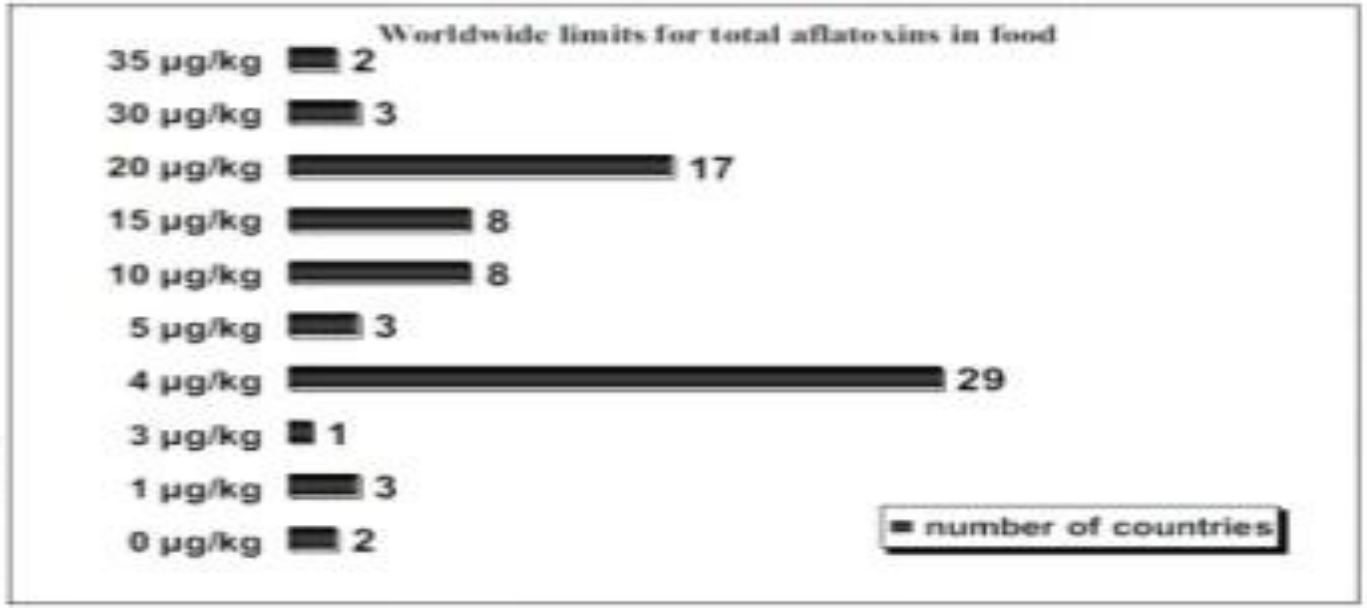


Reference : Worldwide regulations for mycotoxins in food and feed in 2003

Mycotoxin regulations

Regulated levels of mycotoxins differ from nation to nation.





Reference : Worldwide regulations for mycotoxins in food and feed in 2003

Mycotoxin regulations

- Codex Standard 193-1995

  Maximum limits exists for Total Aflatoxins, Aflatoxin M1, Ochratoxin A and Patulin.

  Codex standards (Maximum limits) on 14 types of foodstuffs

- EU Legislations on mycotoxins in foodstuffs

  Regulatory standards (Maximum limits) on 62 types of foodstuffs

Reference : JRC Technical Notes, Mycotoxin Factsheet, 2011

Commission Regulations (EC) No. 1881/2006

Section 2: Mycotoxins

Foodstuffs (1)		Maximum levels (µg/kg)		
2.1.	**Aflatoxins**	B_1	Sum of B_1, B_2, G_1 and G_2	M_1
2.1.1.	Groundnuts (peanuts) and other oilseeds (**), to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs, with the exception of: — groundnuts (peanuts) and other oilseeds for crushing for refined vegetable oil production	8,0 (*)	15,0 (*)	—
2.1.2.	Almonds, pistachios and apricot kernels to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	12,0 (*)	15,0 (*)	—
2.1.3.	Hazelnuts and Brazil nuts, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	8,0 (*)	15,0 (*)	
2.1.4.	Tree nuts, other than the tree nuts listed in 2.1.2 and 2.1.3, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	5,0 (*)	10,0 (*)	—
2.1.5.	Groundnuts (peanuts) and other oilseeds (**) and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs, with the exception of: — crude vegetable oils destined for refining — refined vegetable oils	2,0 (*)	4,0 (*)	—

Commission Regulations (EC) No. 1881/2006

Legislative Reference	*Matrix*	*ML[1] (Y/N)*	*Compound*
Commission Regulation (EC) No 1881/2006	Food	N[4]	3-acetyl deoxynivalenol 15-acetyl deoxynivalenol Diacetoxyscirpenol Fumonisin B3 Fusarenon-X Monoacetoxyscirpenol Neosolaniol T2-triol Verrucol
		N (TDI)[3,4]	Trichothecenes (NIV + T-2 &HT-2 + DON)
		Y	Aflatoxins (Sum of B1, B2, G1 and G2) Aflatoxin M1 Deoxynivalenol Fumonisins (B1 and B2) Ochratoxin A Patulin T-2 and HT-2 toxins Zearalenone

Commission Recommendation of 27 Mar 2013 (2013/65/EU)

- Presence of T-2 and HT-2 toxins in cereal and cereal products

Member States should encourage that samples are simultaneously analysed for the presence of T-2 and HT-2 and other *Fusarium*-toxins such as deoxynivalenol, zearalenone and fumonisin B1 + B2 to allow the extent of co-occurrence to be assessed.

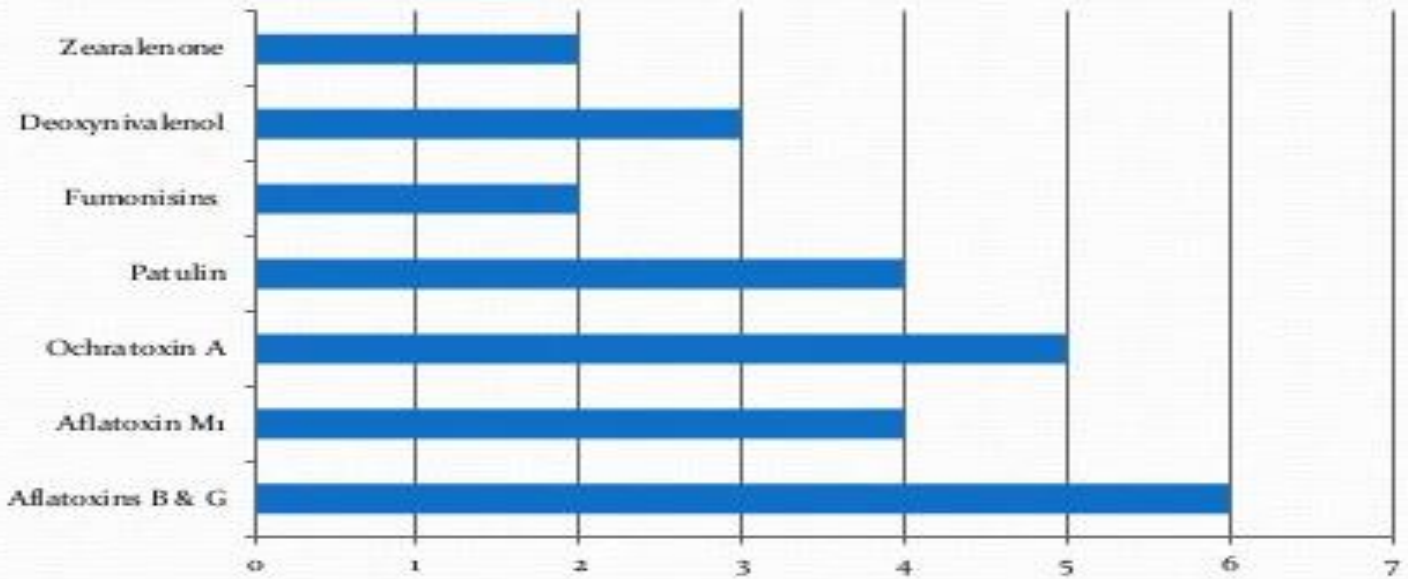
In case the used method of analysis enables it, it would be appropriate to analyse also the masked mycotoxins in particular the mono- and di-glycosylated conjugates of T-2 and HT-2 toxin.

Indicative levels for cereals and cereals products (*) (**)

	Indicative levels for the sum of T-2 and HT-2 (µg/kg) from which serendipitous/above which investigations should be performed, cautiously in case of repressive findings (*)
1. **Unprocessed cereals** (***)	
1.1. barley (including malting barley) and maize	200
1.2. oats (with husk)	1 000
1.3. wheat, rye and other cereals	100
2. **Cereal grains for direct human consumption** (****)	
2.1. oats	200
2.2. maize	100
2.3. other cereals	50
3. **Cereal products for human consumption**	
3.1. oat bran and flaked oats	200
3.2. cereal bran except oat bran, oat milling products other than oat bran and flaked oats, and maize milling products	100
3.3. other cereal milling products	50
3.4. breakfast cereals including formed cereal flakes	75
3.5. bread (including small bakery wares), pastries, biscuits, cereal snacks, pasta	25
3.6. cereal-based foods for infants and young children	15
4. **Cereal products for feed and compound feed** (*****)	
4.1. oat milling products (husks)	2 000
4.2. other cereal products	500
4.3. compound feed, with the exception of feed for cats	250

Mycotoxin regulations

- Legislation established on regulatory limits of mycotoxins in ASEAN countries.



Mycotoxin regulations

- Regulated levels of mycotoxins differ from nation to nation.

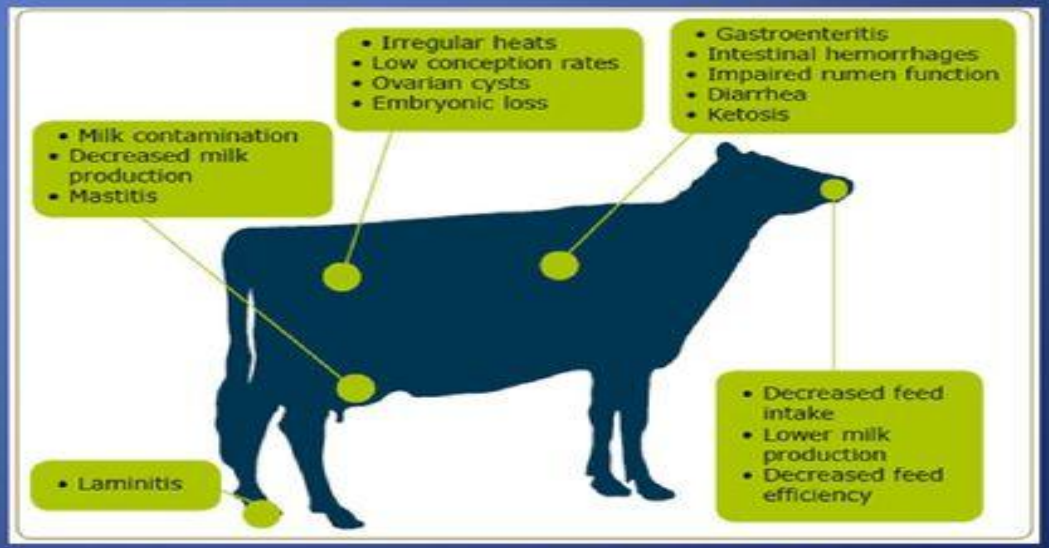
Country	Limits for Patulin	
Indonesia	Fresh apple, canned apple, apple extract, nectar, alcoholic drinks	50µg/kg
	Apple puree	25 µg/kg
	Apple puree for infants and children	10 µg/kg
Malaysia	Apple juice (includes apple juice as ingredients in other beverages)	50 µg/kg
Singapore	Juices	10 µg/kg
Vietnam	Fruit and fruit juices	50 µg/kg
	Concentrated fruit juices and all product derived from them[5]	50 µg/kg

Mycotoxin Health Hazards

- Generally lower risk in well developed countries due to improved standards of living.
- High intake of affected product, usually in conjunction with limited amounts of other food sources.
- Greatest threat comes from long term exposure due to eating spoiled food or meat from animals fed contaminated feed.

Mycotoxin Effects on Animals

- Feed refusal.
- Impaired animal health, resulting in reduced production of eggs, milk, weight gain, etc.
- Metabolites are passed through the milk in cheese, dry milk, and yogurt.
- Disease.
- Death in animals.



http://www.thecattlesite.com/articles/contents/09-02-24Mytotoxin.gif

Regulatory Control

- In 1965, the Food and Drug Administration (FDA) set the first mycotoxin limit of 20 parts per billion (ppb) for aflatoxin in all foods and feed.
- But, this toxin can appear at varying levels of food production, so multiple testing at different points in the food chain is necessary.
- Using ELISA (enzyme-linked immunosorbent assay) technology, testing can be done cheaper and faster than previously.
- The FDA does not do the testing, various other agencies do, such as the Grain Inspection Packers and Stockyards Administration; but, toxic levels must be reported to the FDA.

Toxin-Producing Organism Classes

- **Toxins: substances produced during metabolism and growth of certain microorganisms and some plant and animal species**

Primary factor – pathogenicity

- Viruses (e.g. cytotoxins, lysins)
- Bacteria (e.g. endo and exotoxins)
- **Fungi (e.g. mycotoxins)**
- Protozoa (endotoxin, phospholipase, protease)
- Algae (microcystins, ……)
- Plants (alkaloids, tannins, cyanogenic glycosides)
- Higher Animals (fish, insects, snakes, frogs)

Occurrence data (EFSA chemical occurrence database)

efsa

TECHNICAL REPORT

Aflatoxins (sum of B1, B2, G1, G2) in cereals and cereal-derived food products

- 2,183 samples retrieved from EFSA chemical occurrence database on 15 Mar 13.
- Samples collected between 2007-2012.
- Analytical data on Aflatoxins (B, B1, G, G1).
- Samples include cereal and milling products, processed cereal products.
- Sampling carried out in 16 European countries.

انواع مایکوتوکسین ها و مواد غذایی در معرض آنها

پاتولین

- تشکیل دهنده کوچکترین گروه متابولیت های سمی و تولید توسط آسپرژیلوس، پنی سیلیوم اکسپانزوم و فاسیلومیسس و بیسو کلامیس (بیسوکلامیس فلوا و نیوا)
- خطرناک ترین توکسین در میوه ها به ویژه سیب، هلو و محصولات آنها

سیترینین

- تولید توسط پنی سیلیوم اکسپانزوم و بعضی گونه های آسپرژیلوس و موناسوس
- حضور در میوه ها، جو، ذرت، پنیر و مکمل های رژیمی

توکسین های آلترناریا

- تولید توسط گونه های آلترناریا آلترناتا، آلترناریا دوکی، آلترناریا کیوکامرینا، آلترناریا سولانی و آلترناریا تنویسیما
- حضور در غلات، دانه آفتابگردان، دانه کلزا، زیتون و میوه ها
- آلترناریول و آلترناریول مونو متیل اتر سمی ترین توکسین های آلترناریا و توکسین های دیگر شامل تنوزونیک اسید، آلتنون و آلترتوکسین

Bhat et al, 2010

اثرات سمی مایکوتوکسین های اصلی

- **پاتولین**

در مصرف طولانی مدت توسط حیوانات ایجاد اثرات نوروتوکسیک، ایمونوتوکسیک و تاثیرات گوارشی در نشخوار اکنندگان و نگرانی از ایجاد تاثیرات مشابه در انسان

در گروه 3 از لحاظ سرطانزایی قوانین اتحادیه اروپا برای پاتولین (جدول 7)

تاثیرات حاد شامل تهوع، استفراغ و سایر علائم گوارشی همراه با صدمه به کلیه

Marin et al, 2013

شرایط لازم جهت رشد قارچ و تولید مایکوتوکسین

- رطوبت نسبی هوا بالاتر از ۵۰ درصد
- دمای هوای محیط در حدود ۶ تا ۵۰ درجه سانتی گراد (۲۵ تا ۳۵ بهترین دما)
- رطوبت خوراک بالاتر از ۱۰ تا ۱۲ درصد
- سطح اکسیژن کمتر از ۰/۵ درصد
- عدم تهویه مناسب در محل ذخیره مواد غذایی
- آلودگی محل ذخیره یا عدم کف سازی مناسب محل (جنس کف از خاک باشد)
- ذخیره سازی نامناسب (دپو کردن مواد غذایی با عمق زیاد)
- انبارداری طولانی مدت مواد غذایی

-خواص بیولوژیکی :

- سرطانزایی(ایجاد تومورهای کبدی)
- ایجاد ناهنجاری در جنین
- جهش زایی
- اثرات بیوشیمیایی :
  - اثرات متقابل با DNA
  - جلوگیری از سنتز DNA
  - کاهش سنتز RNA
  - تغییرات مورفولوژی هستک
  - کاهش در بیوسنتز پروتئین
- ممانعت از سنتز چربیها :