



Sveučilište u Zagrebu  
Farmaceutsko-biokemijski  
fakultet

University of Zagreb  
Faculty of Pharmacy &  
Biochemistry



# Sistemiški pogled na problem plijesni u hrani

Ivan Kosalec

ikosalec@pharma.hr

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# Obavjest

Mišljenja i stavovi tijekom predavanja isključivo su Ivana Kosalca, a ne Farmaceutsko-biokemijskog fakultet Sveučilišta u Zagrebu kao niti Europske agencije za lijekove (EMA).

**Sistemski pogled**

**na**

**problem**

**plijesni u hrani**

# Sistemska teorija – opće postavke

- nepredvidljivost **vs.** determinizam
- samo-organizacija na rubu kaosa **vs.** homeostaza
- modeliranje **vs.** kontrola
- sinteza **vs.** analiza
- otvorenost sistema **vs.** zatvorenost sistema
- promatrač je dio sustava **vs.** promatrač je odvojen od sustava

=holistički ili sistemski pristup



# HEALTH AND CONSUMERS

## Food

EUROPA > European Commission > DG Health and Consumers > Overview > Food and Feed Safety

General Food Law | Animal Nutrition | Labelling & Nutrition | Biotechnology | Novel Food | Chemical Safety | Biological Safety | Official controls | Food waste | Food improvement agents

### Food Contaminants - Aflatoxins

#### ► Legislation:

Maximum levels of aflatoxins (aflatoxins B1, B2, G1, G2 and M1) are laid down in [Commission Regulation \(EC\) No 1881/2006](#) as amended by [Commission Regulation \(EU\) No 165/2010](#).

Provisions for methods of sampling and analysis for the official control of mycotoxins including aflatoxins are laid down in [Commission Regulation \(EC\) No 401/2006](#) as amended by [Commission Regulation \(EU\) No 178/2010](#)

#### ► Safeguard decisions as regards aflatoxins

Special conditions governing certain foodstuffs imported from certain third countries due to contamination risks of these products by aflatoxins are laid down in [Commission Regulation \(EC\) No 1152/2009](#)

In order to assist the competent authorities on the official control of aflatoxin contamination in food products which are subject to Commission Regulation (EC) 1152/2009, a guidance document "[Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins](#)" [bg](#) [cs](#) [da](#) [de](#) [el](#) [es](#) [et](#) [fi](#) [fr](#) [hu](#) [it](#) [lt](#) [lv](#) [mt](#) [nl](#) [pl](#) [pt](#) [ro](#) [sk](#) [sl](#) [sv](#) has been elaborated. This document is also applicable for the control of aflatoxins in food products not subject to the safeguard Regulation .

#### ► Approval of pre-export control on aflatoxins in peanuts from US

[Article 23 of Regulation \(EC\) No 882/2004](#) provides that pre-export checks carried out by a third country on feed and food immediately prior to export to the Community with a view of verifying that the exported products comply with the Community requirements may be approved. Such an EC approval of pre-export controls has as consequence that these pre-export controls replace or reduce the documentary, identity and physical controls at import into the EC. An [FVO inspection](#) carried out in September 2006 concluded that the US have a well defined control system for aflatoxin levels in peanuts and well performing approved laboratories. Therefore approval of pre-export control was granted by [Commission Decision 2008/47/EC](#) in December 2007. As these pre-export controls replace effectively and reliably the controls at import this should result in a significant decrease of controls at import.

#### Topics

- Introduction
- Legislation
- Patulin
- Ochratoxin A
- Dioxins
- Heavy Metals
- Chloropropanols/3-MCPD
- Acrylamide
- Information Base
- Fusarium-Toxins
- Sampling, analysis and European Union Reference Laboratories
- Aflatoxins
- Polycyclic Aromatic Hydrocarbons (PAH)
- Contamination incidents

Print



#### Resources

- Speeches
- Press Releases
- Health & Consumer Voice Newsletter
- Publications
- Committees
- Links

#### International Affairs

- Organisations
- Codex
- OIE
- WTO
- Import Conditions
- Pets and Animal Welfare
- Enlargement
- Agreements
- EU - Russia: SPS issues

FOOD AND FEED SAFETY

ANIMAL HEALTH AND WELFARE

PLANT HEALTH

# The specific legislation is within the context of the whole food law

<p><b>General Food Law</b></p> <p>Reg EC 178/2002</p> <p>General food safety requirements Manufacturer responsibilities Notification duty Recall</p>	<p><b>Food Supplements Law</b></p> <p>Dir 2002/46/EC</p> <p>Definition Permitted forms (vitamins/minerals) Maximum levels (vitamins/ minerals) Specific labeling provisions</p>	<p><b>Food Hygiene</b></p> <p>Reg EC 852/2004</p> <p>Rules for hygienic production based on the principles of HACCP Microbiological criteria</p>
<p><b>Novel Foods Regulation</b></p> <p>Reg EC 258/97</p> <p>Pre-marketing approval procedure for novel ingredients</p>	<p><b>General labelling rules</b></p> <p>Dir 2000/13/EC</p> <p>How to label content, composition, etc Quantitative ingredient declaration (QUID) Allergen labelling</p>	<p><b>Health Claims Regulation</b></p> <p>Reg EC 1924/2006</p> <p>Pre-marketing approval procedures for nutrition and health claims</p>
<p><b>Fortification legislation</b></p> <p>Reg EC 1925/2006</p> <p>Risk assessment and risk management procedure in case the use of a substance would result in harmful effects</p>	<p><b>Additives legislation</b></p> <p>Reg EC 1333/2008</p> <p>Pre-marketing approval procedures Allowed additives, including sweeteners and colourings Conditions of use</p>	<p><b>Contaminants</b></p> <p>Reg EC 1881/2006</p> <p>Maximum levels of selected contaminants in ingredients that can be used in foods</p>
<p><b>Pesticides residues</b></p> <p>Reg EC 396/2005</p> <p>Maximum residue levels</p>	<p><b>Extraction solvents</b></p> <p>Dir 2009/32/EC</p> <p>Permitted extraction solvents</p>	<p><b>Irradiation</b></p> <p>Dir 1999/2/EC</p> <p>Permitted ingredients to be irradiated</p>

# Posljedice plijesni/mikotoksina u hrani?

- **Sistemska viđenje problema**
  - Mikotoksini se stvaraju prije žetve i nakon žetve i tijekom obrade hrane, njene pohrane i hranidbe
- **Primjer aflatoksina**
  - Kancerogeni mikotoksin i “pratitelj” čvojeaka
- **Primjer (lošeg) publiciteta u zajednici**
  - Odvajanje biološkog otpada



saprofitizam

Ekološki sustav:  
Ciklus N, C



Su-odnos domaćin-mikrob

alergeni  
patogeni  
oportunisti  
(oko 200)

**Carstvo Gljiva**  
Domena Eukariota  
≈100.000  
Predvidivo 1.5M

Fermentacija  
(hrana)



Fermentacija  
(biogorivo)



## INDUSTRIJSKA VAŽNOST:

- ✓ Fermentacija
- ✓ Antibiotici
- ✓ Enzimi
- ✓ Aminokiseline
- ✓ Organske kiseline
- ✓ Vitamini
- ✓ Masne kiseline
- ✓ Alkoholi
- ✓ Citostatici
- ✓ Imunomodulansi
- ✓ Rekombinantni proteini  
(inzulin, Hb-površinski antigen)

## MIKOTOKSINI

Sekundarni niskomolekularni metaboliti → toksični za nositelja  
(molekularni, stanični, tkivni nivo, organski)

>300

10 ih je povezano sa patologijom





Contaminants in food and feed

Acrylamide

Brominated Flame Retardants

Dioxins and PCBs

Metals as contaminants in food

Mineral oil hydrocarbons

3-Monochloropropane-1,2 Diol Esters (3-MCPD)

Mycotoxins

**Aflatoxins in food**

Aflatoxins in food



Aflatoxins are especially found in nuts and other foodstuffs, and are genotoxic and carcinogenic.

Aflatoxins can occur in foods, such as groundnuts, cereals, and vegetable oils, and cocoa beans, as a result of contamination by Aspergillus species.

Several types of aflatoxins are produced in nature. Aflatoxin B1 is the most common in food and amongst the most potent genotoxic and carcinogenic aflatoxins. It is produced both by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M1 is a major metabolite of aflatoxin B1 in humans and animals, which may be present in milk from animals fed with aflatoxin B1 contaminated feed.

EU framework

The European Union introduced measures to minimise the presence of aflatoxins in different foodstuffs. Maximum levels of aflatoxins are laid down in Commission Regulation (EC) No 1881/2006. Products exceeding the maximum levels should not be placed on the market in the EU. Directive 2002/32/EC lays down maximum levels for aflatoxins B1 in feed materials.

Methods of sampling and analysis for the official control of mycotoxins, including aflatoxins, are laid down in Commission Regulation No 401/2006. This ensures that the same sampling criteria intended for the control of mycotoxin content in food are applied to the same products by the competent authorities throughout the EU and that certain performance criteria, such as recovery and precision, are fulfilled.

EFSA je ocjenila izloženost, opasnosti U procjenama koje su objavljene u EFSA Journalu o slijedećim mikotoksinima:

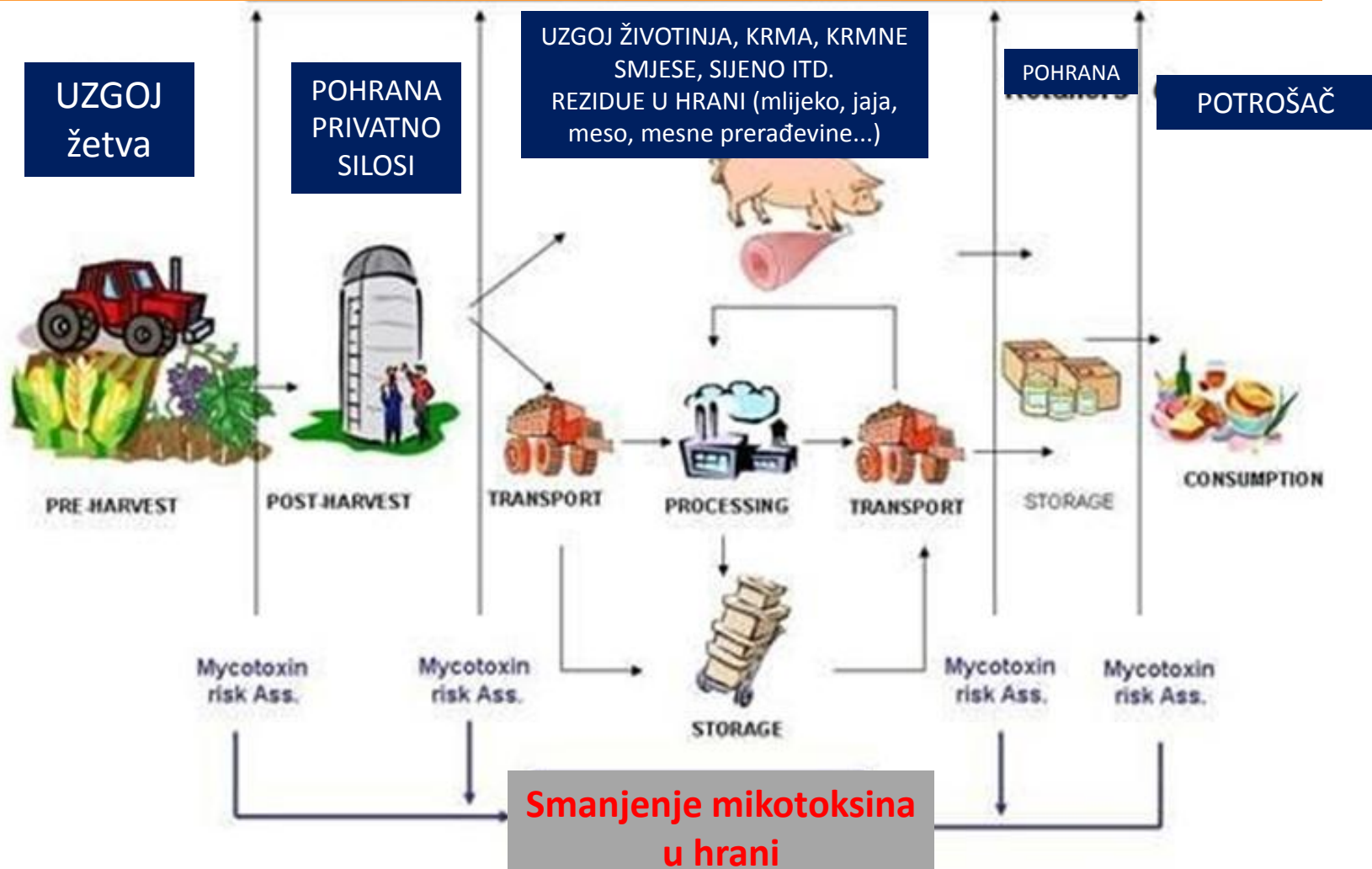
- Aflatoskin B1
- Deoksinivalenol
- Zearalenon
- Oktratoksin A
- Fumonizinima
- T-2
- HT-2



# Kako smanjiti izloženosti mikotoksinima?

kakvoća=sigurnost

Zakonska kontrola kakvoće, edukacija svih čimbenika, širenje informacija



# Zdravstvena važnost aspergila

## Alergeni

- alergije i astma (spore i dijelovi micelija); alergijski gljivični sinusitis (AFS)
- Ratari (zemljoradnici), radnici sa sladom i žitaricama, kompostane...
- hlapljive molekule
- sindrom “bolesne zgrade”

## Infekcije tkiva, organa

- Mikoze ljudi i životinja, sustavne i površinske
- Ptice osjetljivije (kokoši, guske, purani) → od 1960-ih započelo “zlatno doba” istraživanja mikotoksina (**Ainswort i Austwick, 1959**)
- Virulentnije vrste u prednosti (***A. flavus*, *A. fumigatus*, *A niger complex***) → hidrolitički enzimi, sposobnost rasta na razlilitim tenpreaturama, aerodinamičnost spora, tvorba mikotoksina, otpornost činiteljima nositelja → **uspješan oportunistički patogen**
- Angioinvazivnost

## Tvorba niskomolekularnih vanstaničnih metabolita (mikotoksina)

- Različiti biološki učinci i mehanizmi djelovanja
- Kemijski različiti
- Lipofilnost i hidrofilnost različita
- Razgradljivost u prisustvu kemikalija, temperature...
- Različitost u vezanju na nosače (inhibicija transporta kroz jednoslojni epitel)

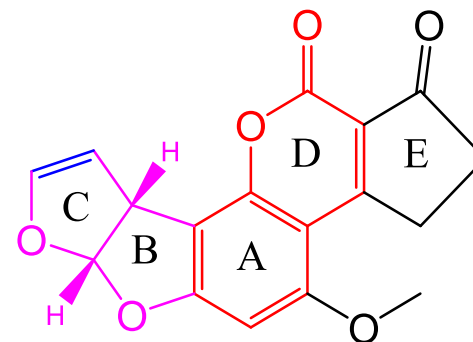
# Primjer: aflatoksinogeni aspergilusi

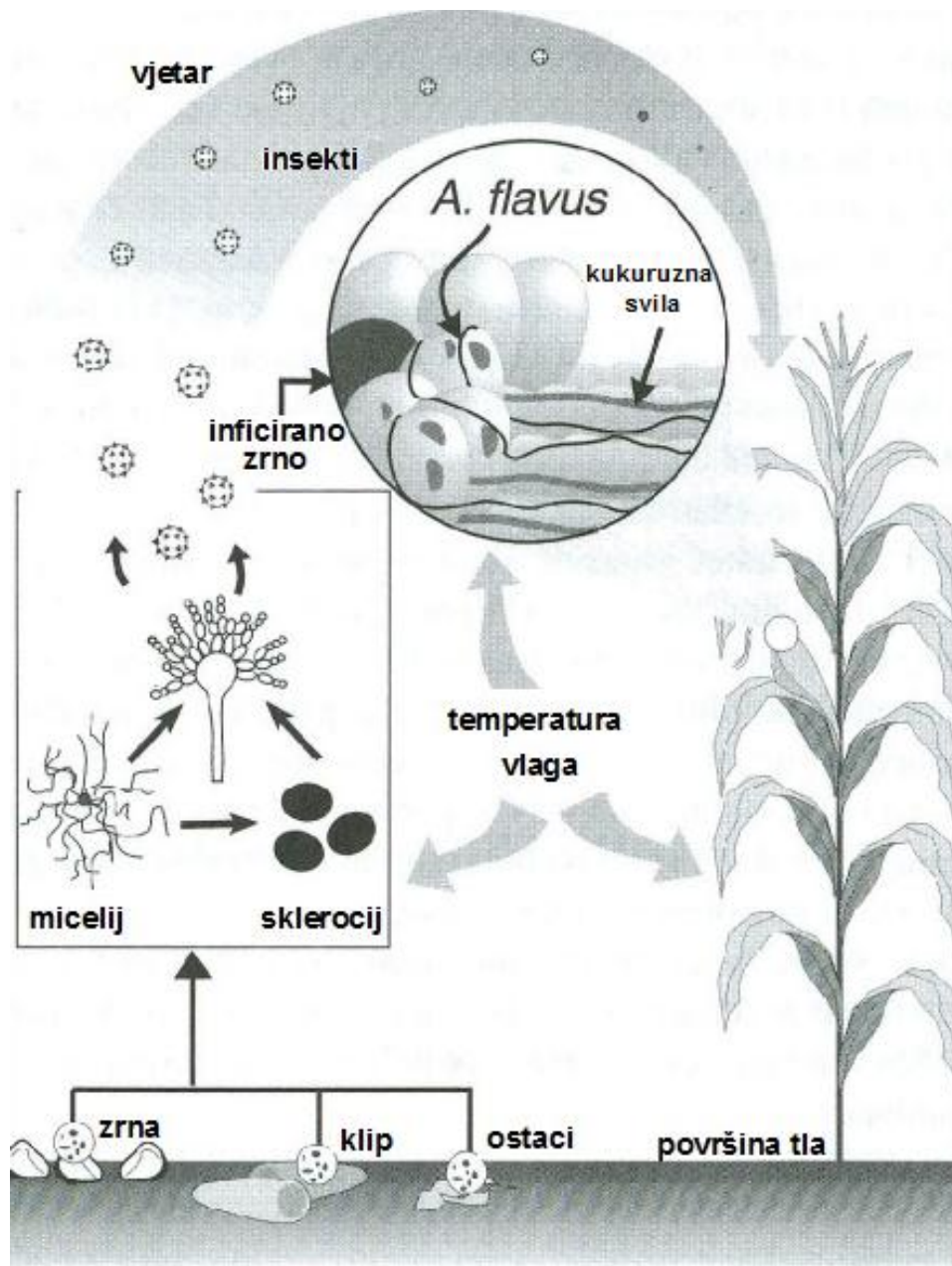
- **AF-producirajuće vrste/sojevi aspergila**

- **sekcija *Flavi*** (*A. arachidicola*, *A. bombycis*, *A. flavus*, *A. minisclerotigenes*, *A. nomius*, *A. parasiticus*, *A. parvisclerotigenus*, *A. pseudocaelatus*, *A. pseudonomius*, *A. pseudotamarii*, *A. togoensis*),
- **sekcija *Nidulantes*** (*Emericella astellata*, *E. olivicola*, *E. venezuelensis*),
- **sekcija *Ochraceorosei*** (*A. ochraceoroseus*, *A. rambellii*)

- ***Aspergillus* sekcija *flavi* : *A. flavus* (zrak), *A. parasiticus* (zemlja)**

- **Ubikvitarnost**
- *A. flavus*: AFB1, B2, G (?), CPA
- *A. parasiticus*: AFB1, B2, G1, G2
- Mlijeko, jaja → AFM1, M2





- Tvorba AF, aw=0,73-0,85
- 8-12% odn. 17-19% vlage
- Silosi: 15% vlage

•Čimbenici stresa (indukacija tvorbe AF)

•**Točkasta kontaminacija**

•Odnos O<sub>2</sub>/CO<sub>2</sub> → 25-50% CO<sub>2</sub> djeluje inhibitorno na rast i tvorbu AF

**Shematski prikaz ciklusa infekcije kukuruza s plijesni *A. flavus***  
(Šarkanj, 2014; EFSA, Battilani i sur., 2012)

dairy cattle, above which the carry over from feed to milk would result in unacceptable levels of aflatoxin M1. The CONTAM Panel concluded that the current maximum levels of aflatoxin B1 in animal feed not only provided an adequate protection from adverse health effects in target animal species, but also prevented undesirable concentration of the metabolite aflatoxin M1 in milk. Among its recommendations, the Panel encouraged monitoring of the presence of aflatoxin B1 in imported feedstuffs and aflatoxin M1 in dairy milk.

#### [Past activities / Completed work](#)

[Latest updates](#) [News \(2\)](#) [Publications \(5\)](#)

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

● 25 March 2013 [Technical report](#)

##### [Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate change](#)

● 23 January 2012 [External Scientific Report](#)

##### [EFSA launches project to predict the effect of climate change on aflatoxin B1 in cereals](#)

● 10 July 2009 [News: News story](#)

##### [Effects on public health of an increase of the levels for aflatoxin total from 4 µg/kg to 10 µg/kg for tree nuts other than almonds, hazelnuts and pistachios - Statement of the Panel on Contaminants in the Food Chain](#)

● 30 June 2009 [Statement of the CONTAM Panel](#)

##### [Aflatoxins in food: EFSA assesses new proposed maximum levels for almonds, hazelnuts and pistachios and advises the European Commission](#)

● 2 March 2007 [News: News story](#)

##### [Opinion of the scientific panel on contaminants in the food chain \[CONTAM\] related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products](#)

● 1 March 2007 [Scientific Opinion of the CONTAM Panel](#)

##### [Opinion of the Scientific Panel on contaminants in the food chain \[CONTAM\] related to Aflatoxin B1 as undesirable substance in animal feed](#)

● 19 March 2004 [Scientific Opinion of the CONTAM Panel](#)

Last updated: 25 March 2013

#### About

[Executive Director](#)  
[Management Board](#)  
[Corporate Documents](#)

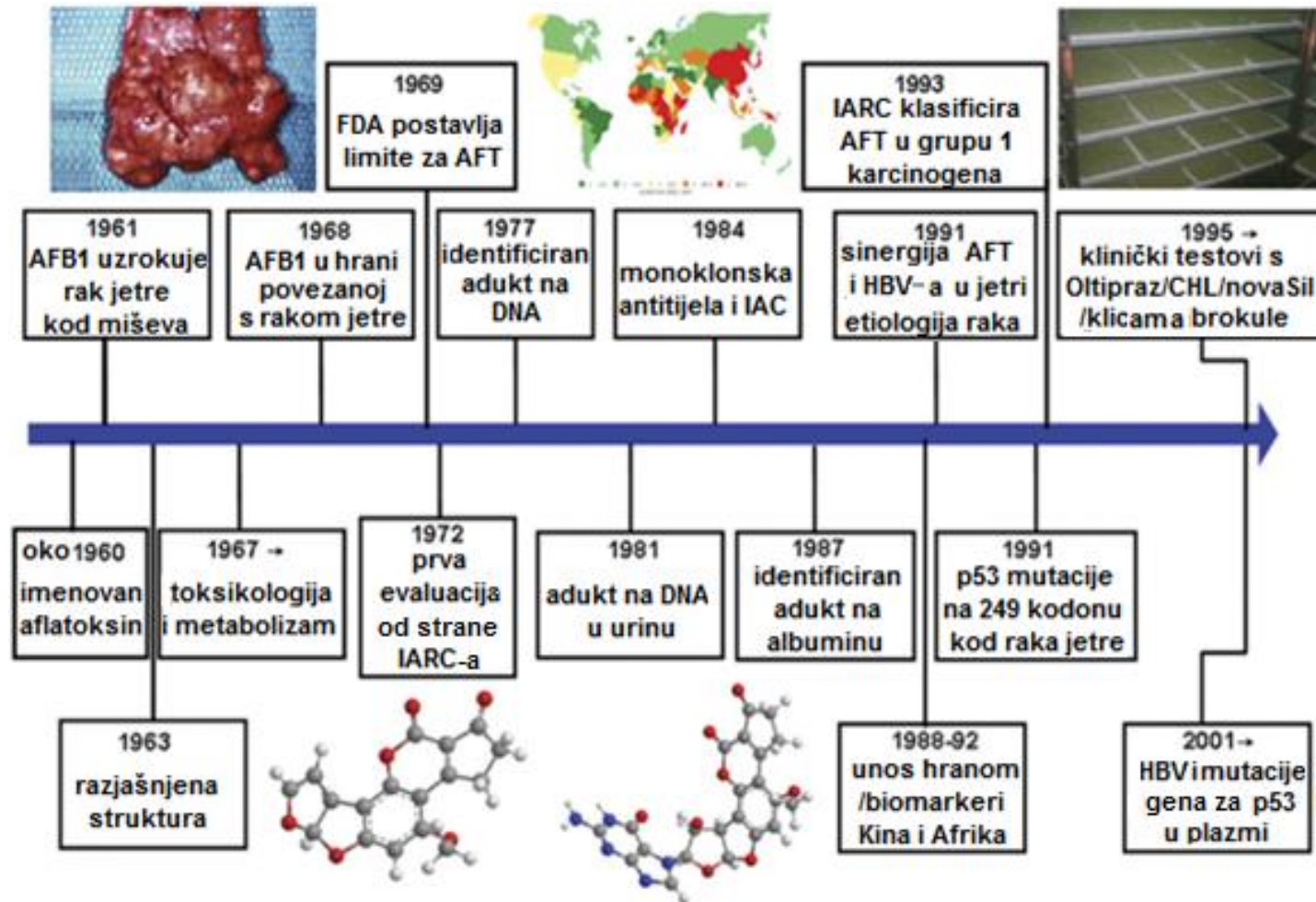
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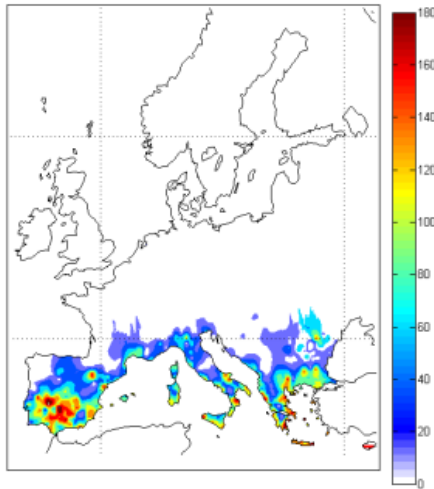
# AF i hepatocelularni karcinom



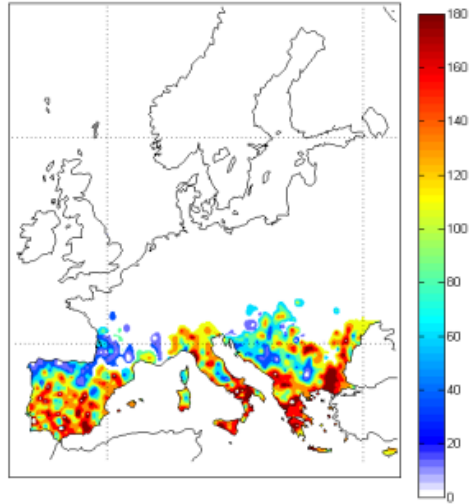
Šarkanj, 2014; Kensler i sur., 2011

# Modeliranje

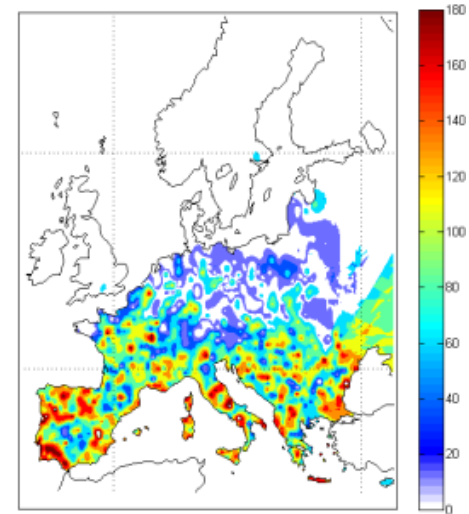
Predviđanja rizika kontaminacije kukuruza s AFB1 kod promjene srednje temperature za 2 i 5 °C  
(Šarkanj, 2014; Battilani i sur., 2012)



Trenutni rizik kontaminacije kukuruza s AFB1



Rizik kontaminacije kukuruza s AFB1 kod porasta od 2°C



Rizik kontaminacije kukuruza s AFB1 kod porasta od 5°C

- ✓ Globalno zagrijavanje → scenario  $\uparrow 2-5^\circ\text{C}$  i emisija  $\text{CO}_2$  sa 350-700 na 900-1000 ppm
- ✓ EU Green Paper: **zagrijavanje je regionalno, lokalne su promjene**
- ✓ Modeliranje vodi ka analizi rizika
- ✓ Kukuruz kao hrana za ljude i životinje, biogorivo
- ✓ Zagrijavanje vodi ka modulaciji ekološkog sustava: **komeptencija sa termofilnim vrstama plijesni**
- ✓ Primjer je *Wallemia sebi* → tvori mikotoksine valeminol i valeminon na vrlo niskim  $a_w$  vrijednostima **0.65-0.75!!!!**

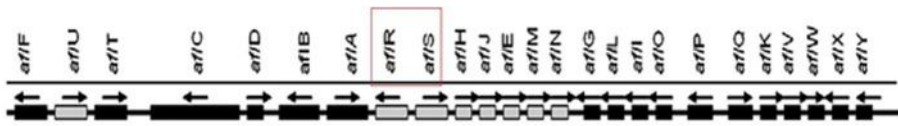


# Iskustva susjeda sa kontaminacijom sa AF i promjene klime na lokalnom nivou

- Sjeverna Italija → ljeta 2012., porast T, suhoća → *Fusarium verticilloides* → *A. flavus*
  - Posljedica pojava AFM1 u hrani preko hrane za životinje (Giorni i sur., 2007)
- Srbija 2012. porast AF-pozitivnih uzoraka (Kos i sur., 2013) usljed suhoće i povišene T
- Mađarska iskustva slična (Dobolyi i sur., 2013)

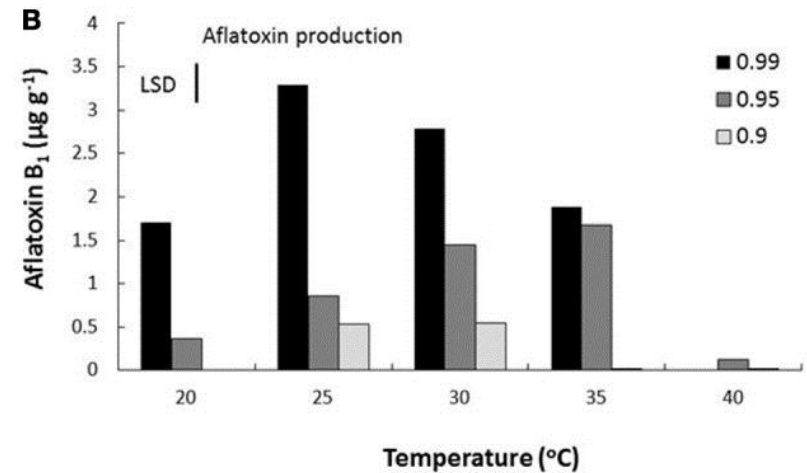
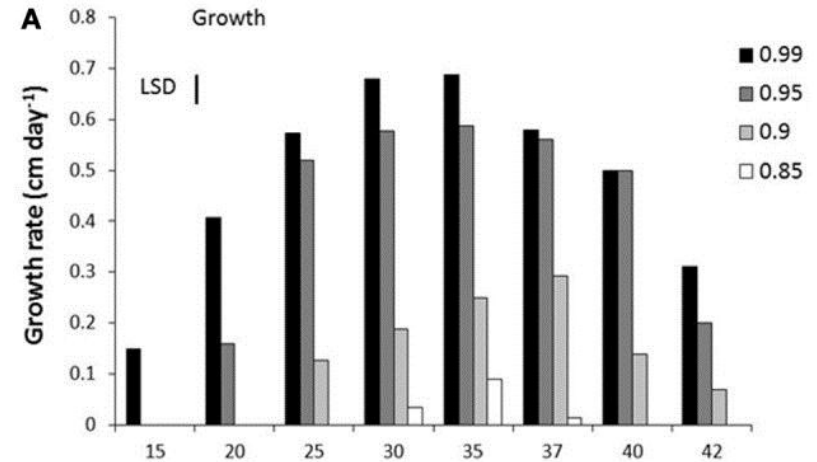
# Učinak $a_w$ i temperature na tvorbu AF

- Potvrđeno da  $a_w$  i  $T$  imaju značajnu ulogu u aktivaciji gena (oko 30) odn. klastera za tvorbu aflatoksina (=korelacija)
- Regulatorni geni *aflS/aflR* i strukturalnih gena → AF
- Učinak kombinacije trigeru  $a_w/T$



## Ratio of *aflS/aflR* in relation to aflatoxin biosynthesis and different parameter combinations

Parameter combination	Ratio <i>aflS/aflR</i>	Aflatoxin [ng/g]
25°C/0.90	0.5	3.7
35°C/0.90	0.3	4.7
25°C/0.95	7.4	830.2
30°C/0.95	7.1	3016.9
25°C/0.99	1.5	1957.3
30°C/0.99	2.7	2758.7



Medina i sur., 2014

# $a_w/T/CO_2$ i učinak na tvorbu AF

		Dijametarski rast soja <i>A. flavus</i> (mm/dnevno)					
temperatura		34°C			37°C		
Aktivitet vode ( $a_w$ )		0.97	0.95	0.92	0.97	0.95	0.92
CO <sub>2</sub> (ppm)	350	12.4	11.7	6.8	10.2	9.8	7.3
	650	12.1	11.6	6.9	11.3	10.7	7.8
	1000	12.1	11.3	6.3	10.9	10.5	7.8

**Odnos  $a_w/T/CO_2$  nema značajnog učinka na rast aflatoksinogenog *A. flavus* soja (*in vitro* uvjeti)**

Medina i sur., 2014

# Učinak $a_w/T/CO_2$ na ekspresiju gena i posljedičnu tvorbu AFB1 = interakcija za pojačanu tvorbu AFB1

Temp.(°C)	$a_w$	CO <sub>2</sub> (ppm)	<i>aflD</i>	<i>aflR</i>	AFB <sub>1</sub>
34	0.97	650	=	=	=
		1000	=	=	=
	0.95	650	=	=	=
		1000	=	↑(×3.6)	=
	0.92	650	=	↑↑(×24.4)	↑(×2.6)
		1000	=	↑(×2.0)	↑(×2.0)
37	<b>0.97</b>	<b>650</b>	↑(×4.6)	=	↑↑(×30.7)
		<b>1000</b>	↑(×6.5)	=	↑↑(×23.8)
	<b>0.95</b>	<b>650</b>	↑(×6.4)	↑↑(×14.6)	↑↑↑(×79.2)
		<b>1000</b>	↑(×3.2)	↑↑(×43.9)	↑↑↑(×78.5)
	<b>0.92</b>	<b>650</b>	=	↑↑(×40.4)	↑↑(×15.1)
		<b>1000</b>	↑↑(×22.5)	↑↑↑(×1680)	↑↑(×23.8)

# Aflatoksinogeni sojevi aspergila i rezidue aflatoksina u hrani/lijeku

## Zakonski okvir (hrana, dodatci prehrani) OPĆA POPULACIJA

- Pravilnik o najvećim dopuštenim količinama određenih kontaminanata u hrani (NN 146/12) → [EC 1881/2006](#); [1650/2010](#)
  - NDK tbl. 2.1. prilog 1
- „Pravilnik o planu uzorkovanja i metodama analiza za službenu kontrolu količina mikotoksina u hrani“ (NN 45/08) → [EC 401/2006](#)
- Naredba o privremenim mjerama u odnosu na sadržaj aflatoksina M1 u mliječnim proizvodima (NN 39/13)ž
- **Kroničan unos**

## Zakonski okvir (biljni lijekovi) PACIJENTI (uglavnom)

- Europska farmakopeja
- Smjernice EMA
- Biljne droge za pripremu TBL i WEU biljnih lijekova
- AF, OTA
- primjer za dodatke prehrani na biljnoj bazi (tzv. *botanical food supplements*)
- Dobra regulativa, regulacija uvoznih sirovina iz Afrike, Azije, Južne Amerike
- **GMP vs HACCP**
- **Primjena u vremenu**

# Sprječavanje ulaska mikotoksina u lanac hrane

## Sistemske mjere

- **SVI sudionici osvješteni o ulozi u sustavu**
  - Zajednički sastanci
  - Jednostavan rječnik mjera
  - Sustavna kontrola u vremenu
- Mala promjena ima dalekosežne posljedice za cio sustav
- Kontrola i nadzor
- Lakoća promjene
- Edukacija

## Ubikvitarnost toksinogenih plijesni kao veliki izazov

- Nemogućnost kontroliranja
- Mogućnost kontroliranja

# Primjer grada Zagreba

- Dobre strane gospodarenja otpadom
- Biološki materijal degradira enzimatski u vremenu
- Supstrat za razvoj mikroba
- Alergološki rizik u zajednici se povećava
- Organoleptički neprihvatljivo (smrad!)



- ✓ Termofilne i termotolerantne plijesni
- ✓ *Aspergillus fumigatus* prednjači
- ✓ Učinak T i ispiranja a kontejnera razrijeđenim octom
- ✓ **Nedovoljan dokaz POZITIVNI H VS NEGATIVNIH učinaka**



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130  
godina  
FARMACEUTSKO-BIOKEMIJSKOG  
FAKULTETA

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**Zahvaljujem na pozornosti**