SAFE FOODS SEMINAR

Lisbon, 1 – 2 October 2007

Review on mycotoxin risk

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‘MYCOTOXIN’

Secondary toxic chemical substances produced by fungi

Potential threat to human and animal health (inhalation, absorption, ingestion of contaminated food products)

One or more fungal species ⟺ one or more mycotoxin

Major mycotoxins

▪ Aflatoxins	*Aspergilli – Penicillium verrucosum*
▪ Ochratoxins	*Aspergillus ochraceus – P. verrucosum*
▪ Deoxynivalenol	*F. graminearum – F. culmorum*
▪ Fumonisins	*Fusarium* spp.

Safe Foods

MYCOTOXIN EFFECTS

- Manifest effects with doses close to acute toxicity
- Belated symptoms
- Long-term effects after suspension

>> Symptoms frequently confused by multi-presence and conventional induced pathologies

SUSPECTED SYMPTOMS

- **reduced ingestion**
- **reproductive problems**
- **nephropathy**
- **breathing syndrome**
- **worsening of performance**

Other effects

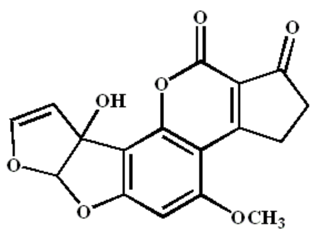
Economic losses

- FAO values in many milions of $ per year the losses due to MYCOTOXIN contaminations (about 25% of world cereal harvests)
- Losses had been shown in the entire food chain, from field to production process and distribution, including losses in animal breedings
- Excluding sanitary costs for human health

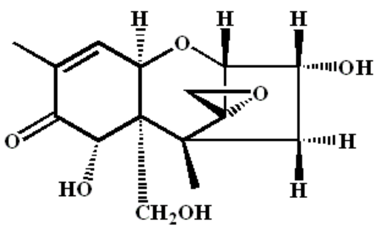
Mycotoxins were associated to food problems in:

France (16th)
England (17th), Central-Eastern Europe
Ethiopia and India (last 30 y), Africa, Asia
Italy (1970's)
Denmark (mid-1980s)
Malaysia and the Netherlands (1990's)
Eastern Kenia (2004) ….

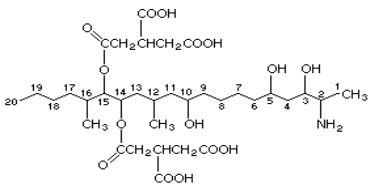
The secondary toxic metabolites are formed in the final exponential growth phase of fungi



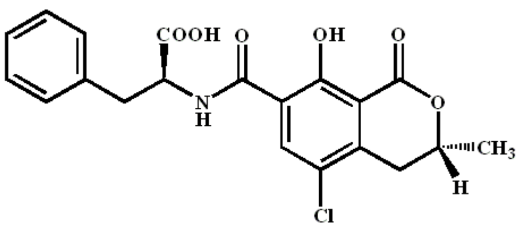
AFM_1



DON



FM B_1



OTA

MYCOTOXIN CONTAMINATION

In field

stresses predispose plants to infestation and colonization by toxigenic fungi

In stored grain

the contamination is influenced by:

- moisture (0.7 a_w)
- temperature (10° - 40℃)
- substrate
- oxygen (O_2; 1-2%) and carbon dioxide (CO_2) concentration
- pH (4-8)

→ Quality loss and mycotoxin formation is of particular concern when toxins enter human food chain by direct consumption

MYCOTOXINS

Stable substances

after the fungal death

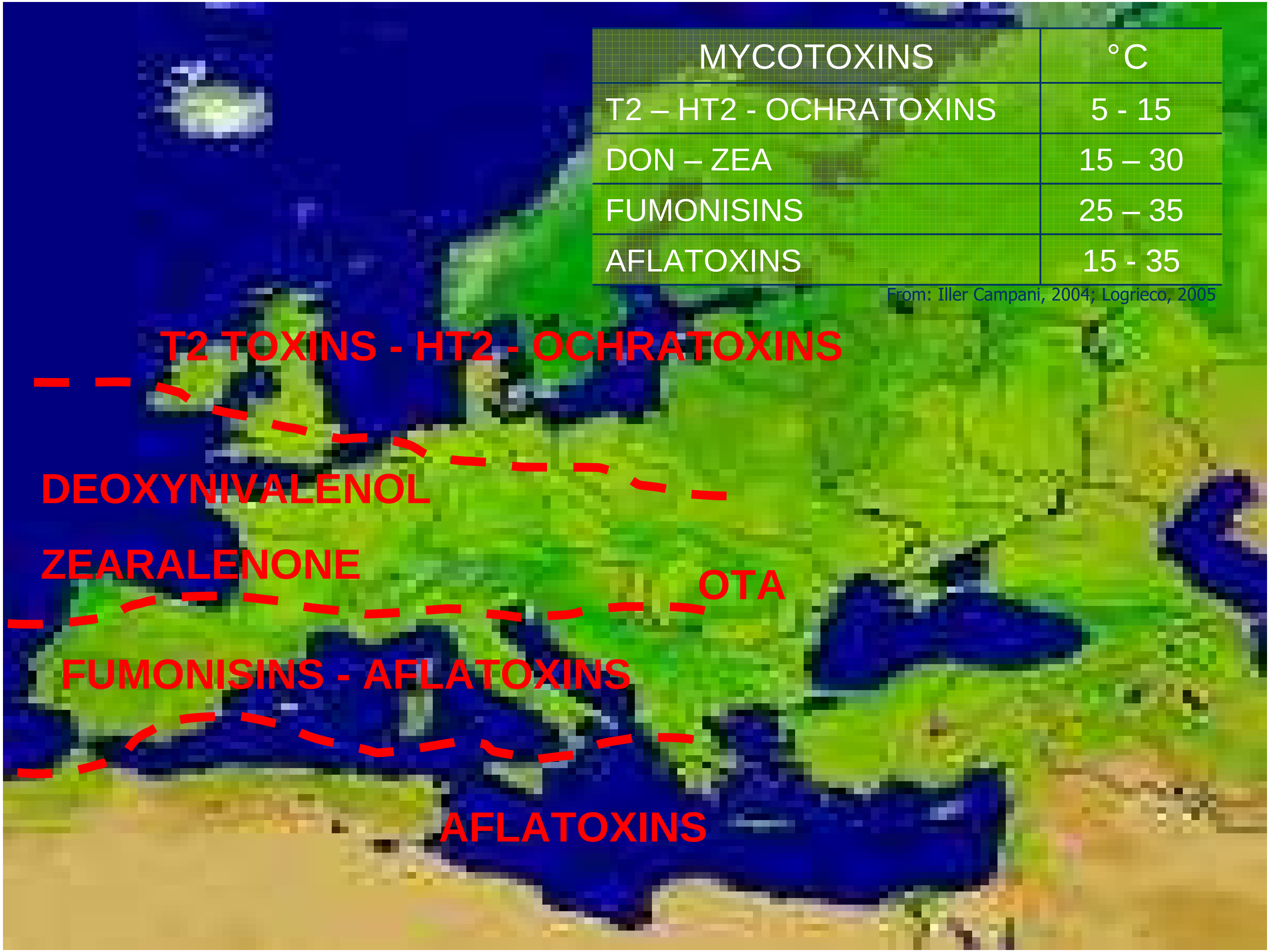
PREVENTION OF CONTAMINATION

=

PREVENT FUNGAL GROWTH

=>

CONTROL THE EFFECTS

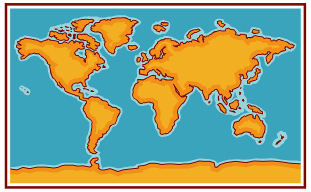


MYCOTOXINS	°C
T2 – HT2 - OCHRATOXINS	5 - 15
DON – ZEA	15 – 30
FUMONISINS	25 – 35
AFLATOXINS	15 - 35

From: Iller Campani, 2004; Logrieco, 2005

MYCOTOXIN RISK

- Limited effect of acute mycotoxin exposure
- Pre-harvest control of fungi growth during crop development
- Changes in global climatic conditions



FOOD QUALITY AND FEEDING NECESSITY

New EU Membres States increase demand for food

- Adequate growth of agricultural industry in particular contexts
- Implementation of trading system inside an enlarged pan-European market

and …..

- Improvement of analytical methods to detect toxins

It is necessary to fulfill the requirements of CEN (European Committee for Standardization)

LEGISLATION

EU REG. n. 1881/2006 set maximum limits for contaminants

Article 3

Prohibitions on use, mixing and detoxification

4. Foodstuffs containing contaminants listed in section 2 of the Annex (Mycotoxins) shall not be deliberately detoxified by chemical treatments

EU RECC. n. 2006/576/EC on the prevention and reduction of *Fusarium* toxins in cereals and cereal products

RASFF

		2005	2006	
Mycotoxins:		989	892	
▪ Aflatoxin		939	812	
(country of origin)	Iran	452 (440*)	240 (233*)	*pistachios
	China	80 (79**)	69 (66**)	**peanuts & deriv.
	Brazil	37 (32**)	26 (20**)	
	Argentina	23 (22**)	45 (42**)	
▪ Ochratoxin A		16	26	dried vine fruits
		12	4	spices
		10	13	coffee & its products
		3	11	cereals & their products
▪ Patulin		6	7	apple & derived
▪ Fumonisin		2	17	cereals
▪ Zearalenone		0	1	cereals

ITALY

	2005	2006
Mycotoxins:	13	14
Aflatoxin	10	4 maize flour & pistachio
Ochratoxin A	2	1
Patulin	1	0
Fumonisin	1	9 maize flour
Zearalenone	0	0

and Deoxynivalenol?

RASFF have began notification from 1st May 2007 (1 in maize flour, Italy)

France	DON (cereals)	low level (2001)
		high level (1999, 2000-2)
Germany	AFs (dried fruits, spices)	50-70% (<20%)
	OTA (coffee, beer, sausages)	50-100% > limits
	DON (cereals in baby foods)	13% > 100 µg/kg
	ZEA (maize)	in trace
Portugal	AFs (dried fruits, spices)	exceeded allow. limits
	OTA (spices, wine)	< allow. limits
Spain	$AFB_{1,2}$ (maize, dried fruits)	+ > allow. limits
	DON, ZEA (maize)	< allow. limits
	OTA (coffee, wine, beer)	100% > allow. limits
UK	DON, ZEA (breakfast cereals)	80% < limits
	OTA (wheat, barley)	2-3% > limits

MYCOTOXIN - CONCLUSION

Awareness to reduce occurrence of mycotoxin in the years to come (National authorities should educate producers)

Good Agricultural Practices (GAPs) represent the primary line of defense against contamination of cereals with mycotoxins

'Know how' of the storage conditions to limit mycotoxin production may make it possible to control further increases in mycotoxin levels during storage (GMPs)

… more information

European Mycotoxins Awareness Network (EMAN)
http://www.mycotoxins.org

Scientific Committee for Food (SCF)
http://europa.eu.int/comm/food/fs/sc/scf/reports/

Codex Alimentarius Commission
www.codexalimentarius.net/download/report/28/Al03_12e.pdf

European Food Safety Authority (EFSA)
http://www.efsa.europa.eu/en/science/contam/contam_opinions.html

Scientific Cooperation (SCOOP)
http://ec.europa.eu/food/fs/scoop/index_en.html

European Commission Regulation (CE) n. 1881/2006
http://europa.eu.int/eur-lex/lex/JOIndex.do?

year 2006 OJ series L OJ number (mandatory) 364

A case study on the occurrence of Aflatoxin M_1 in milk and dairy products

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AFLATOXINS

- ***Aspergillus flavus***
- ***Aspergillus parasiticus***
- ***A. nomius, Penicillium verrucosum***

FUNGUS SPECIES	MYCOTOXINS	TEMPERATURE RANGE (°C)	WATER ACTIVITY (a_w)
Aspergillus flavus	**AFB_1 AFB_2**	**6 - 48** **optimum 36 - 38**	**>0.78**
Aspergillus parasiticus	**AFB_1 AFB_2 AFG_1 AFG_2**		

(Lacey & Magan, 1991; Battilani, 2002)

Contaminated commodities:
cereals, tree nuts, spices, oilseeds

Major hazard: most widely occurrence (CAST, 1989)
and our inability to detect them biologically

CONTAMINATION

Direct:

- Fungal growth for fermentation (cheese, *Penicillium*)
- Unintentional fungal growth (uncorrect manifacturing)

Indirect:

- Contaminated rations for cows (AFM_1 in milk)

AF: immunosuppressive, liver tumor inducing and carcinogenic potency

AFB_1: class 1 (human carcinogenic IARC, 1993)

AFM_1: class 2B (possible human carcinogenic IARC, 1993)

- Continued low-dose exposure brings to chronic effects

Milking animals that ingest aflatoxin B_1 (AFB_1) by contaminated diets, excrete the 4-hydroxylated metabolite aflatoxin M_1 (AFM_1) into milk with a proportion of 1-3%

LEGISLATION

The Commission set limit for AFB_1 of 5 µg/kg for supplementary feedstuffs for lactating dairy cattle (European Commission, 1991)

<u>tolerance level is difficult to be observed</u>

to produce bulk milk <50 ng AFM_1 per kg = the daily average individual intake in a herd is <40 µg AFB_1 per cow

The EU Commission fixed a limit for AFM_1 of 50 ng/kg for milk and a variable limit for cheese

In particular to protect babies and children: AFB_1 transfer directly in breast milk

Aflatoxins in milk are stable during heating treatments i.e. pasteurization and sterilization

- EU limit: 50 ng/kg of AFM_1

Variation of pH during fermentation (yoghurts, cheeses) cause coagulation of proteins that adsorb or occlude the toxins

- EU limit: variable

RISK FACTORS ANALYSIS

AFB_1 analysis in corn is necessary to evaluate risk of AFM_1 contamination in milk and dairy products

If stresses affect plant growth during pollination
Aspergillus fungi increase AF level

Risk factors of AFB_1 contamination in corn

To prevent fungi and AFB_1 ← To reduce AFB_1 ← To control AFM_1

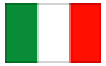
maize → feedstuffs → milk

Risk factors of AFB_1 contamination in corn silage production

PRE-HARVEST	HARVEST	SILAGE AND STORAGE
▪ Choice of seeding time and density ▪ Lack of irrigation and weed killing ▪ Phytophagous damages ▪ Excessive or not balanced fertilization	▪ Low silage moisture ▪ Long and irregular cutting up	▪ Unsuitable filling and closing of storage silo ▪ No use of organic acids and/or preservatives

Risk factors of AFB_1 contamination in corn grain production

PRE-HARVEST	HARVEST	POST-HARVEST	PRE-PROCESSING
Such as corn silage production and ▪ Drought and high temperature (>25-30℃) ▪ Minimum tillage or sod seeding ▪ Choice of hybrids ▪ Unsuitable crop rotation	▪ Mechanical damages ▪ Prolonged drying in field	▪ Wet grain storage before drying process ▪ Grains storage with moisture > 14% ▪ Unsuitable driers ▪ No kernels cleaning ▪ No refrigeration ▪ No phytophagous control	▪ Grain heating ▪ Grains re-humidification

Particular climatic conditions (0.78 a_w: closely interrelated with AFB_1 incidence and contamination levels) for the first time in Italy  during summer 2003 conduced to a significant diffusion of aflatoxins:

Year	AFB_1(>0.2 ppb)	Year	AFB_1 (>0.2 ppb)
1995	1.9	2000	0.0
1996	0.3	2001	6.3
1997	1.5	2002	2.1
1998	1.5	**2003**	**14.3**
1999	4.1	2004	3.3

% contaminated corn samples superior to the instrumental limit in Pianura Padana (Po Valley)

(Pietri et al, 2004; Reyneri, 2006)

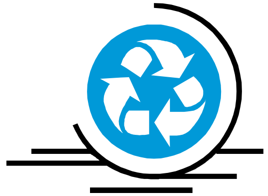
PREVENTION OF RISK IN MILK

With high temperature and high humidity in field it is difficult to prevent the formation of aflatoxins

- SCOUTING: land control of ‘stressed’ culture
- Essential to be careful in post-harvest storage (12-12.5% RH)
- Suppliers careful of mycotoxin risk, in particular of aflatoxin risk (HACCP)
- Choice alternative feedstuffs without hazardous raw materials

What can we do with contaminated material?

Detoxification (elusive goal)



- **Feedstuffs**
  - ammonia (gaseous phase): alteration of molecular structure
  - sodium bentonite and aluminosilicates: binding agents
  - sodium-, potassium-, calcium-hydroxide (with formaldehyde)
  - ADSORBENT

- **Milk**
  - acidification of milk with organic acid (lactic, citric or acetic)
  - addition of formaldehyde (0.5%)
  - via Nocardiaceae (*Flavobacterium aurantiacum = Nocardia corynebacterioides*)

New utilization

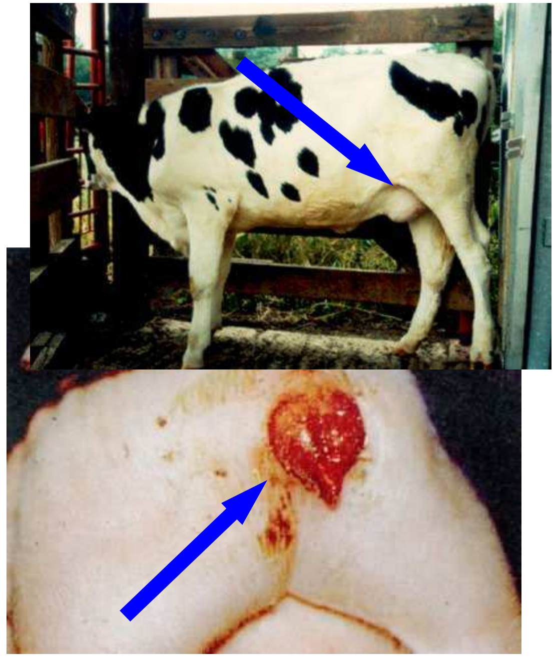
- corn: production of ethanol or wet milling
- milk: meal for swine

Destruction (following national impositions, Italy – 2003)

WORST CASE SITUATION

for ruminants (cattle, buffalos, sheep, goats)

- Occurrence of AFB_1 at maximum permissible level in feed <u>concentrates</u>
- Maximum concentrate intake of high yielding dairy cow
- Contamination of <u>basic feedstuffs</u> in rations of dairy cows at maximum permissible level



→ Climate change in Southern Europe

AFM$_1$ - CONCLUSION

- Controlling critical point for fungal growth and mycotoxins production such as cultural phases (HACCP)

- Primary strategy to protect: monitoring by reliable analyses → surveillance in field could be appropriate

- Spreading news about risks linked to unsuitable farming management systems

On the occurrence of Deoxynivalenol and Ochratoxin A in wheat

Prandini, A., Sigolo, S. and Filippi, L.
Institute of Food Science and Nutrition - Catholic University of Piacenza

DEOXYNIVALENOL (DON)

- ***Fusarium graminearum*** (maize)
- ***Fusarium culmorum*** (wheat)

FUNGUS SPECIES	TEMPERATURE RANGE (℃)	WATER ACTIVITY (a_w)
Fusarium graminearum	**25 - 30**	**>0.88**
Fusarium culmorum	**21 - 25**	**>0.87 → >0.88**

Contaminated commodities:
all species of cereals

Major hazard: occurrence at undetectable level in a wide variety of food

CONTAMINATION

Direct:

Ingestion of contaminated cereals and grains

The exposure varies with supplies in different geographical regions:

- in Europe major source is wheat
- in Asia major sources are rice and wheat

DON: strongly immunosuppressive, neural disturbance, haemorrhaging, necrosis of tissue, vomiting and feed refusal (= vomitoxin)

DON: a class 3 (not classifiable as to its carcinogenicity to humans IARC, 1993)

Stable during the processing of cereal products (<u>bread</u>, noodles, infant food, beer)

DON contamination is located at the surface of the kernel: milling practice is a physical technique accepted from **EU REGULATION n. 1881/2006**

LEGISLATION

- The Commission set limit of DON µg/kg :

500 (bread) - 1750 (not manifacturated wheat and corn)

- Canada, Russia and the USA set statutory or guideline limits :

500 – 2000 µg/kg (wheat)

RISK FACTORS ANALYSIS

DON analysis in wheat is necessary to evaluate risk of DON contamination in by- products

Incidence of FHB → DON contamination of wheat

Infection depend on:

- **Rainfall and relative humidity (RH)**
- **Duration of canopy wetness**
- **Temperature related to the stage of wheat development**

To prevent in field, condition for DON contamination

Risk factors of DON contamination in wheat and bread

WEATHER	AGRICULTURAL PRACTICES	POST-HARVEST	MILLING AND BREAD PROCESSING
▪ High temperature ▪ High moisture ▪ Rainfall (among flowering and early dough stage)	▪ Unsuitable crop rotation ▪ No removal of crop debris ▪ Excessive N fertilization ▪ Choice of variety ▪ No chemical or biological control	▪ Unsuitable conditions of conservation (SMC) ▪ Damaged kernels ▪ No chemical or alternative control ▪ Unsuitable conditions of transport	▪ Wholemeal bread production

PREVENTION OF RISK IN FIELD

Conventional as well as organic agriculture

good agricultural, handling, and storage practices

minimize the risk of mould growth
and mycotoxin contamination

- Long crop rotations (crop/species-specific mould)
- Low/different nitrogen fertilization rates (rough plants)
- Tilling as weed control (minor fungal inoculum)

DON - CONCLUSION

- Scouting critical points during most sensitive crop period (among flowering and early dough stage) to reduce the risk of FHB outbreaks

- Under good condition of storage (25°C, 62% RH) seeds mantain good quality and *Fusarium* species do not compete with other storage-fungi

OCHRATOXIN A (OTA)

- ***Penicillium verrucosum*** (temperate climate)
- ***Aspergillus ochraceus*, *A. carbonarius*** (warm climate)

FUNGUS SPECIES	TEMPERATURE RANGE (°C)	WATER ACTIVITY (a_w)
Penicillium verrucosum	**0 - 31** **Optimum 20**	**< 0.80**
Aspergillus ochraceus	**12 - 37** **Optimum 24-31**	**> 0.80** **Optimum 0.95 – 0.99**

Contaminated commodities:
cereals, grapes, dried and stored foods

Major hazard: occurrence at low level in a wide variety of food

CONTAMINATION

Direct:

- Ingestion of contaminated cereals, juices
- Inhalation in people working on waste fields (rarely)

Indirect:

- Contaminated feed for monogastric animals (OTA in meat, liver paté)

OTA: immunosuppressive, embriotoxic, carcinogenic and teratogenic, genotoxic, nephrotoxic in mammalian species (BEN = Balkan Endemic Nephropaty, 1991)

OTA: class 2B (possible human carcinogenic IARC, 1993)

Carry-over:

- limited in ruminant probably due to detoxification activities of microflora
- depend on tissue in meat production (swine, poultry)

Longest half-life of OTA (35 days) known for living mammals

LEGISLATION

The Commission set limit for OTA of 2 - 10 µg/kg from not manifacturated wheat and maize to soluble coffee

- EU limit: 5 µg/kg of OTA (wheat and corn)
- EU limit: 5 µg/kg of OTA (roasted coffee)

According to the occurrence in many foods a provisional Tolerable Daily Intake (TDI) was established of:

- 14 ng OTA /kg b.w./day (JECFA, accumulation)
- 5 ng OTA /kg b.w./day (EU SCF, carcinogenity)

Ochratoxin A is stable to heat (bread-making), to roast (coffee), to fermentation (wine, beer):

- EU limit: 2 µg/kg of OTA (wheat and corn by-products)
- EU limit: 0.5 µg/kg of OTA (baby food, infant formula with cereals)

RISK FACTORS ANALYSIS

OTA analysis in wheat is necessary to evaluate risk of OTA contamination in bread

A. ochraceus

P. verrucosum

→ **OTA occurrence in stored grains**

↓

Infection depends on:

- **Moisture of grains at harvest: 0.77 a_w**
- **Temperature**
- **Controlled atmosphere during storage**

A good storage practice to prevent OTA production

Risk factors of OTA contamination in bread

POST-HARVEST	WHEAT MEAL PRODUCTION	BREAD PROCESSING
Unsuitable conditions of conservation: ▪ Insufficient drying ▪ Over-long storage before drying ▪ No chemical or alternative control ▪ Damaged kernels	▪ No cleaning of kernels ▪ No scouring of kernels	▪ Wholemeal bread production

WHAT COULD WE DO WITH CONTAMINATED MATERIAL ?

- Detoxification of

SOLID MEDIA / AGRICULTURAL PRODUCTS

• a strain of *A. niger* (normally use in fermentation of food) and *A. fumigatus*

• ADSORBENT (stop carry-over in pig and chick)

LIQUID MEDIA / MILK

• *Lactobacillus*, *Streptococcus* and *Bifidobacterium* detoxify milk

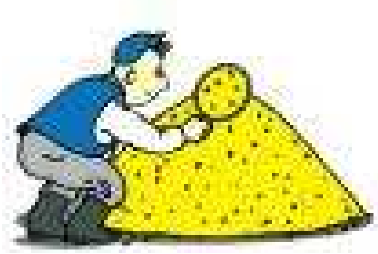
• A. Nigri and *A. fumigatus*

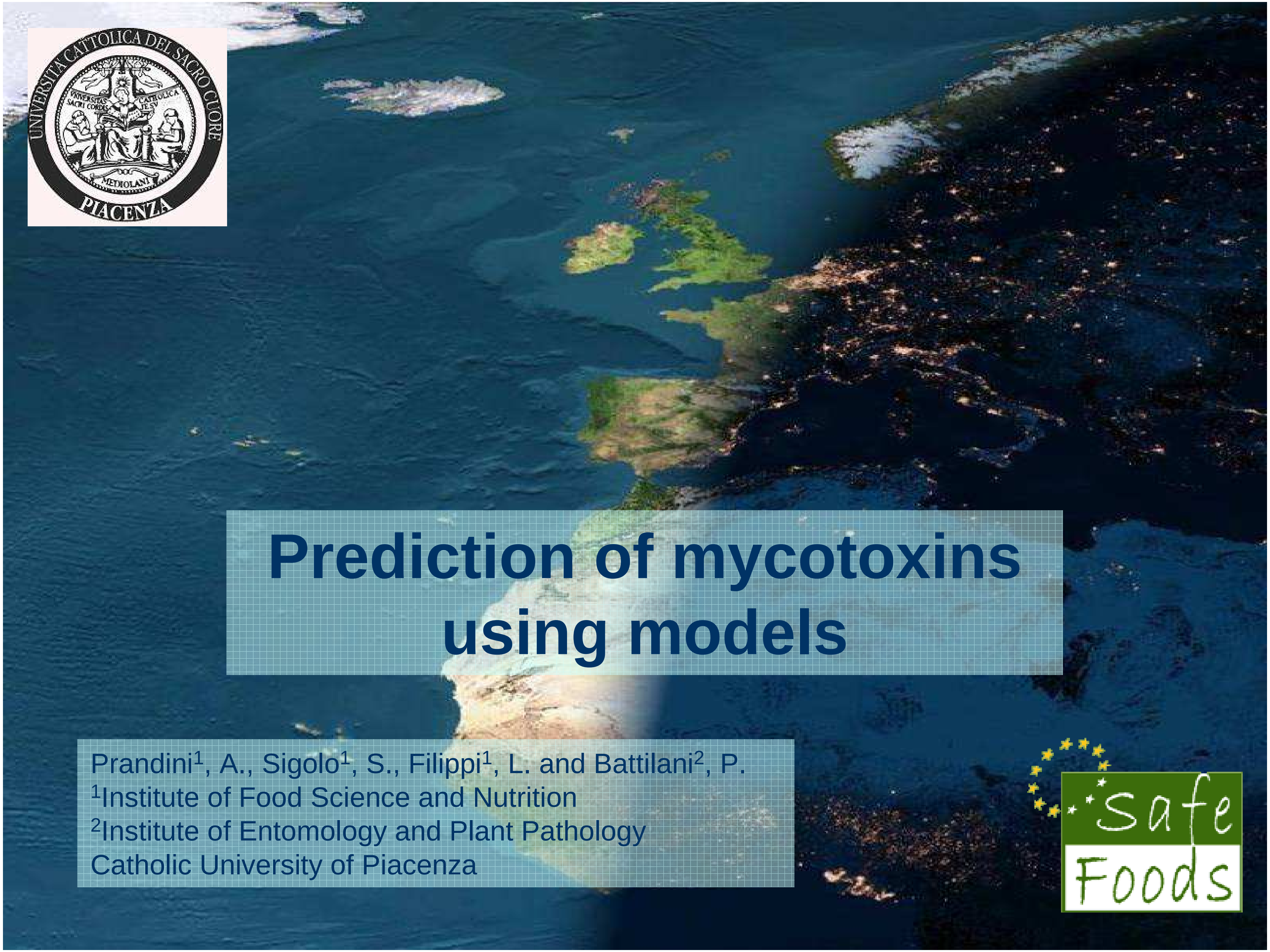
Such methods must be :

- compatible with existing national and european food safety legislation (Reg. CE 1881/2006)
- protective of the functionality/quality of cereals

OTA - CONCLUSION

- Exposure to OTA is worldwide (detected in human sera in many countries) with high incidence, at low level
- Difficulty of eliminating OTA from food chain makes it essential avoidance in raw materials, and protection from further contamination occurs
- Controlling critical points for fungal growth and mycotoxin production such as storing techniques





Prediction of mycotoxins using models

Prandini[1], A., Sigolo[1], S., Filippi[1], L. and Battilani[2], P.
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[2]Institute of Entomology and Plant Pathology
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Forecasting AFB_1 and AFM_1 contamination

- Linear relationships to estimated the carry-over

AFM_1 (ng/kg milk) = [1.19 x AFB_1 intake (µg cow^{-1} day^{-1})] + 1.9 **(Veldman)**
AFM_1 (ng/kg milk) = 10.95 + 0.787x AFB_1 intake (µg day^{-1}) **(Pettersson)**

No predictive models for the risk of AFB_1 contamination in corn or AFM_1 contamination in milk and dairy products

Prevention in field

1. Control insects and weeds
2. Scouting (EC Recc. 2006/576)
3. Minimize damage kernels

Forecasting FHB epidemics

FHB is an IDEAL disease, given :

- prevalence of FHB epidemics in wet growing season
- short period (anthesis) of susceptibility to infection

A generalized forecasting system is difficult to apply in a field situation

Measures of control as:

- use of cultural control techniques
- growing of resistant cultivars
- use of fungicides or biological antagonists

to reduce the risk of FHB epidemic

Forecasting OTA contamination

OTA production in grains is a IDEAL phenomenon, given:

- occurrence of *A. ochraceus* and *P. verrucosum* primarily in stored grains

In a predictive model based on:

temperature X a_w ⟷ different species

Measures of control as:

- Cleaning and removal of damaged kernels
- Use of chemical control (fumigants)

ARGENTINA (2001)

Empirical equation for predicting FHB incidence:

- Temperature
- Moisture variable

associated to head blight in many wheat cultivars

The equations should be carefully used for prediction in other geographic areas, with few changes in temperature thresholds.

ITALY (2002)

FHB dynamic simulation model:

daily infection risk based on

- Sporulation
- Spore dispersal
- Infection of host tissue

Main factors affecting the risk for DON and ZEA

- Air temperature
- Relative humidity and rainfall
- Fungal species and host growth stage

Model produce one index for FHB risk, and one for mycotoxin level in grain

CANADA (2002)

DON prediction in mature grain using:

- Rainfall
- Temperature (4 -7 days before heading and from 7 days before to 10 days after heading)

Predictive model for timing use of fungicides

A web site (http://www.ownweb.ca) provide predictions of DON across the province of Ontario in Canada *(Hooker et al., 2002; Hooker et al., 2003)*.

USA (2004)

Model for FHB disease based on:

- Weather (hourly temperature, humidity and rainfall)
- Crop growth stage
- Disease observations

Predict the risk of disease severity greater than 10% (model accuracy ~ 80% using validation data)

With integration of empirical observation, the model is given for prediction of DON level in grain

A web site (www.wheatscab.psu.edu) provide data from 23 states both spring and winter wheat areas

EUROPE (2003)

Predictive model about ochratoxin A production in stored grain (OTA PREV project)

Fungal growth and OTA production are influenced by:

abiotic (mainly water availability and temperature) and

biotic factors.

Mathematical model use:

- numbers of *P. verrucosum* colonies
- moisture content during storage

They are significantly related to the risk of exceeding the '5 µg OTA kg^{-1} grain' legislative limit

Predictive models on *Fusarium verticillioides* and fumonisin contamination in maize

Variables used:

* relative humidity
* free water in plant tissue
* air temperature
* wetness
* corn growth stage

and mathematical equations related to spore production, infection, invasion and mycotoxin production.

Italy: conceptual model for the dynamic simulation of the life cycle of F. verticillioides in maize and production of fumonisin B_1 in grain in dry and warm climates of southern Europe.

To produce an operative model, some aspects of the disease cycle need to be investigated further

'PREDICTIVE' - CONCLUSION

- Implementation of food safety measures (GAPs, GMPs, HACCP system) to reduction of mycotoxins in first steps of commodities production
- Timing use of fungicides (to reduce hazards for human and animal health) could be improve with better meteorological predictive models

OVERALL CONCLUSIONS

- Mycotoxins can be produced in field as well as during food storage, and a variety of climatic, environmental and agronomic factors determine their production
- Prevention of growth and mycotoxin production of fungi on plants and in feedstuffs is the best approach to impede the harmful effects on animal and human health
- Contribute to frequency and worldwide diffusion of mycotoxin contamination is the global transportation and food conservation systems

OVERALL CONCLUSIONS (continued)

- It's difficult to forecast the occurrence of fungal diseases and toxins contamination in food grains
- Predictive models are limited to climatic variables (no use of field specific effects such as crop rotation, crop variety, tillage, etc)
- They are usually site-specific and do not provide acceptable accuracy when applied to diverse and complex environments (also human behaviour influence mycotoxin problem but is difficult to quantify)

IN THE FUTURE?

- Researches about genetic resources for improvement of resistance or less susceptibility of plants to contamination
- Integration of predictive models with GAPs and GMPs to prevent contamination risk
- Disclosure of guidelines (‘know how’) to harmonize the storage centres about the best way for managing and storing commodities
- National storage centres have due to follow guidelines for good conservation of commodities

⇒ Bigger pertaining areas are favourable for better management

Thank you for your attention