

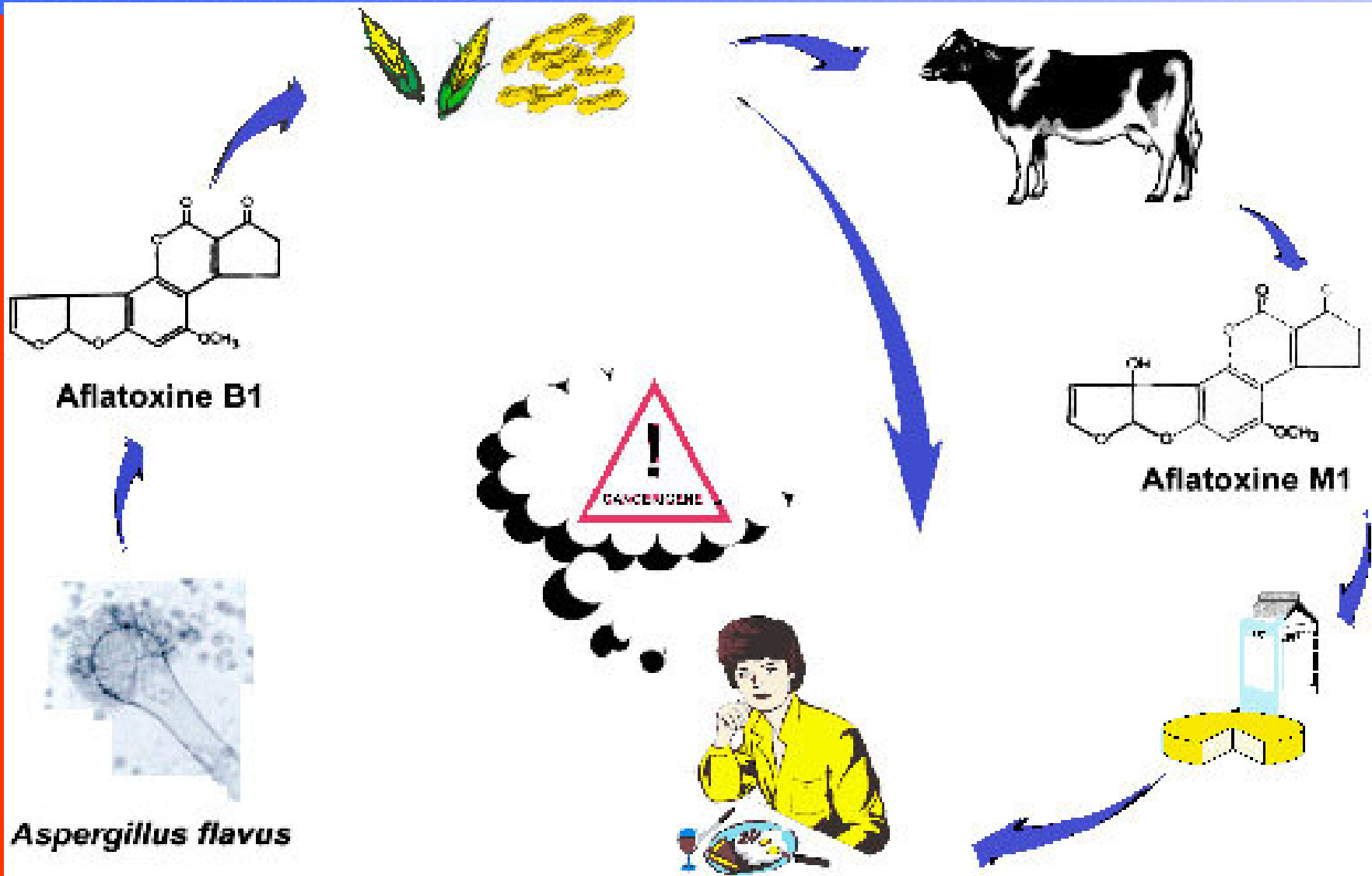
ANALYSIS OF MYCOTOXINS

Reliability

Quality

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CONTAMINATION DE LA CHAÎNE ALIMENTAIRE PAR L'AFATOXINE B1

TOXICITY >>> RISK ASSESSMENT

JECFA (OMS/FAO)

IARC (OMS)

NNATURAL TOXICANTS

(hépato-, néphro-, immuno-toxicities)

- ♦ *Chronic (mostly humans)*

carcinogenic: AFs, OTA, Fs, T2 / HT2(?)

- ♦ *Acute (mostly animals)*

DON, ZEA, PAT, T2 / HT2

RISK MANAGEMENT >>> REGULATORY LIMITS

EU: Regulation CE 466/2001; 472/2002; 856/2005

- AF B1, total AFs (B1+B2+G1+G2), AF M1
- OT A
- Patulin
- some *Fusarium* toxins

ANALYSIS OF MYCOTOXINS : *FACTS AND DIFFICULTIES*

Heterogeneity of contamination

☞ *various sampling methods*

Various and complex matrices

☞ *various analytical methods*

Small contents (ppb = $\mu\text{g}/\text{kg}$; ppt = ng/kg)

☞ *low limits of detection and/or quantification*

Particular physico-chemical Properties

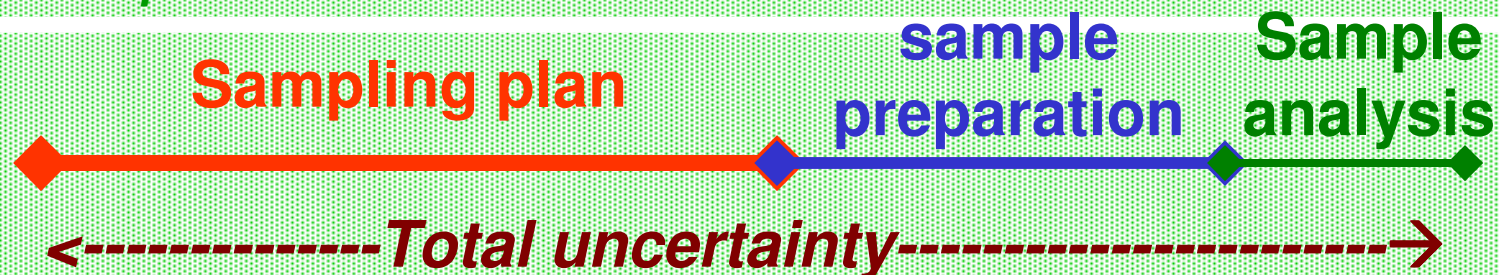
☞ . *low molecular weight*

. *Various and limited spectroscopic properties*

SAMPLING

EU: Regulation CE 401/2006

- ☞ *Cereals and cereals products*
- ☞ *Dried fruit*
- ☞ *Dried figs, groundnuts and nuts*
- ☞ *Spices*
- ☞ *Milk and milk products (including infant formulae)*
- ☞ *Coffee and coffee products*
- ☞ *Fruit juices, wine and cider*
- ☞ *Apple products (including infant formulae) and apple juice*
- ☞ *Cereals products for infant*



SAMPLE ANALYSIS:
RELIABILITY and QUALITY of DATA

Analytical methodology

Analytical Laboratory

Result Expression

Analytical methodology

some key availabilities:

Analyte Standard

Certified Reference Materials

Validated Methods

STANDARDS of MYCOTOXINS

- Commercial availability for the major toxins
- 98 % purity in freeze dried film or powder
- Necessity of checking both concentration (quantity) and purity (quality) by spectrophotometry

REFERENCE MATERIALS

3 categories (définitions)

ISO 30 (1992), Eurachem (2002)

- ***Certified Reference Materials***
- ***External Reference Materials (or Quality Control Materials)***
- ***Internal Reference Materials***

REFERENCE MATERIALS

Organisations

Data base>Virtual Institute for Reference Materials

- *Institute for Reference Materials and Measurements (IRMM/JRC, CE)*
- *BAM (Germany)*
- *International Atomic Energy Agency (IAEA, Austria)*
- *National Institute for Standards and Technology (NIST, USA)*

CERTIFIED REFERENCE MATERIALS

I R M M (JRC EC)

Mycotoxins	Matrices	Content ($\mu\text{g}/\text{kg}$)
Aflatoxin B₁	Peanut Butter	1 2
		7,0 \pm 0,8 < 0,2
	Animal Feed	1 2
		9,3 \pm 0,5 < 1
Aflatoxin M₁	Milk Powder	1 2 3 4
		0,76 \pm 0,05 0,31 \pm 0,06 0,09 + 0,04 et - 0,02 < 0,05
Déoxynivalenol (DON)	Wheat Flour	1 2
		0,67 \pm 0,02 < 0,05
	Corn Flour	1 2
		0,43 \pm 0,04 < 0,05
Ochratoxin A	Wheat	1 2
		8,2 \pm 1,0 < 0,6

CATEGORIES of ANALYTICAL METHODS

- **Published** (*characterised, and/or peer reviewed*)
- **Validated** (*intra-laboratory or inter-laboratories*)
- **Standard** (*subject to comments and/or approval and/or vote*)
- **Official**, (*recognised by an official body and/or organism*)
- **Reference**, (*recommended by a reference laboratory*)

VALIDATIONS of ANALYTICAL METHODS

- **intra-laboratory** (IUPAC-AOAC Doc >>> CCMAS)

linearity, LOD, LOQ

recovery rate

Intra lab trueness

repeatability

- **inter-laboratories** (ISO 5725)

precision (repeatability + reproducibility)

Inter labs trueness

V A L I D A T E D S T A N D A R D M E T H O D S

(Harmonized standard protocol ISO 5725)

AOAC

ISO

IDF

NMKL

CEN

National

PERFORMANCE CRITERIA for the ANALYSIS of MYCOTOXINS

Recommended by CEN/TC 275 WG 5 Biotoxins

Recovery rates

60 - 120 or 70 – 110 %

Reproducibility

40 - 30 %

Limit of Quantification

the lowest validated level

Selection of full validated methods (ISO 5725) for mycotoxins EN and AOAC (except *)

Mycotoxins	Matrices	LOQ µg/kg	Recov %	R _{inter} %
Aflatoxins B&G	Peanut Butter, Pistachios, Figs, Paprika	AFB ₁ : 0,2 µg/kg AFs Total : 0,4 µg/kg	82 - 109	9 – 32
Aflatoxins B&G	Baby Food	AFB ₁ : 0,02 µg/kg	92-101	9 – 23
Aflatoxin M ₁	Whole Liquid Milk	0,005 µg/L	70 - 95	21 - 26
Ochratoxin A	Barley	0,6 µg/kg	65 - 113	17 - 33
	Coffee	0,3 µg/kg	65 - 97	14 - 26
Ochratoxin A*	Baby Food	0,008 µg/kg	108	29 - 45

Selection of full validated methods (ISO 5725) for mycotoxins

Mycotoxins	Matrices	LOQ µg/kg	Recov %	R_{inter} %
Patulin	Apple Juice	15 µg/kg	69 – 109	11 – 33
	Apple Puree		80 - 104	29 - 36
Fumonisin	Corn Flour, corn flakes	-	75 - 110	21 - 31

RELIABILITY and QUALITY

Analytical methodology

Analytical Laboratory

Result Expression

QUALITY ASSURANCE OF LABORATORY

Int'l Standard ISO 17025, revised September 2005

Basic Principle

- a global quality approach handled and by a specific responsible manager
- a quality assurance system

Quality Assurance

- ☒ Use of validated methods or standards
- ☒ Use of internal control figures (CRMs)
- ☒ Satisfactory results in proficiency tests
- ☒ Accreditation as a recognition

Proficiency Testing

Raw data from laboratories are analysed according to appropriate statistical tests

Lab data are checked for trueness and inter-laboratory reproducibility

Laboratories which do not pass the statistical tests should carry out corrective actions to improve their analytical competency (to keep their agreement)

PROFICIENCY TESTING

FAPAS

Aflatoxins / Corn / Peanut cakes

Aflatoxin M₁ / Milk

Patulin / Apple Juice

Ochratoxin A / Wheat Flour / Coffee

RECOGNITION = ACCREDITATION

The Five Keys

 **Instrumentation**

 **Materials, Products and Reagents**

 **Methods**

 **Laboratory Facilities**

 **Laboratory Staff**

RELIABILITY and QUALITY

Analytical methodology

Analytical Laboratory

Result Expression

WHICH ANALYTICAL RESULTS ?

Analytical protocol

RAW RESULT

Recovery Rate

CORRECTED RESULT

Uncertainty

RESULT and M U

USE OF RECOVERY RATE

Working document IUPAC-ISO-AOAC

- Various use according countries

- Disturb Comparison of results of analysis between labs from countries with different rules
- Which consequences by comparing corrected result to MLs?

EXPRESSION OF UNCERTAINTY

(CGMA/AFNOR: FD X 07-021)

$U = 2 S_{Rintra}$ intra lab reproducibility

$U = 2 S_{Rinter}$ inter labs validation

$U = 2 S_{Rpt}$ Proficiency testing

$U = 2 S_{RHor}$ Horwitz value (can be used for most of contaminants >ppb)

UNCERTAINTY CONSEQUENCES

Should the « Uncertainty » be taken into account for the interpretation of the results?

Discussion initiated
at *Codex Alimentarius*

The End