CODEX ALIMENTARIUS International Food Standards





Food and Agriculture Organization of the United Nations



Hotel Aquincum, Budapest

Introduction



Status of Food Safety in the Light of Analyses of Wide Range of Foods

Examples: ATO M1, B1, Glyphosate and Salmonella confirmation using MALDI-TOF-MS

Dr. Tamás János Szigeti Business development manager **WESSLING Hungary Ltd.** H-1047 Budapest Fóti street 56.











The WESSLING Group in numbers (2015)





1983: Foundation of the firm, Altenberge, Germany in a wind mill...(Erwin Wessling);

- **1992:** Foundations of Wessling Hungary, 220 employees;
- 10 million € turnover (Hungary)
- For 24 years: leader of the commercial laboratory market in Hungary;
- 8 Countries, 18 laboratories, 30 offices;
- >1200 employees;

78 million € turnover (all labs.);

Site of our laboratories and offices

Quality of Life

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Germany: Altenberge Berlin Bochum Bremen Darmstadt Dresden Halle/Saale Hamburg Hannover Ingelheim Köln Mannheim München Münster Spremberg Stuttgart Walldorf

France: Lyon Paris **Italy:** Bologna **Poland:** Krakow Posen Warsawa Románia: Bucurest Marosvásárhely **Russia:** Moscow Switzerland: Bern Lyss

Hungary: Budapest & Gödöllő (WESSLING / BIOMI)



Out of Europe: China (Shaghai)

WESSLING Hungary Ltd. Fóti út 56. Budapest, Hungary





Investigated samples (>280,000 sample in 2015)





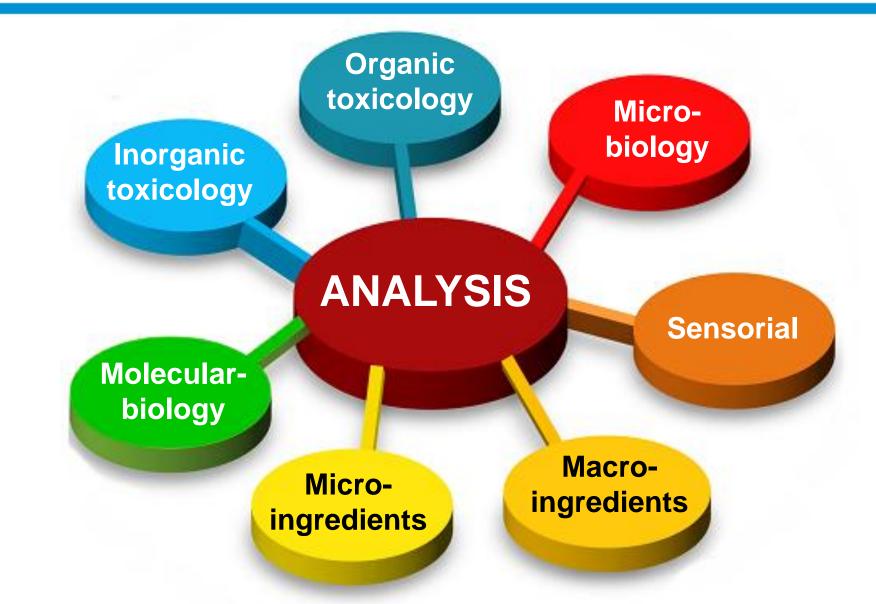
>250,000 sample, more than 1,000,000 results





Food and feed investigations





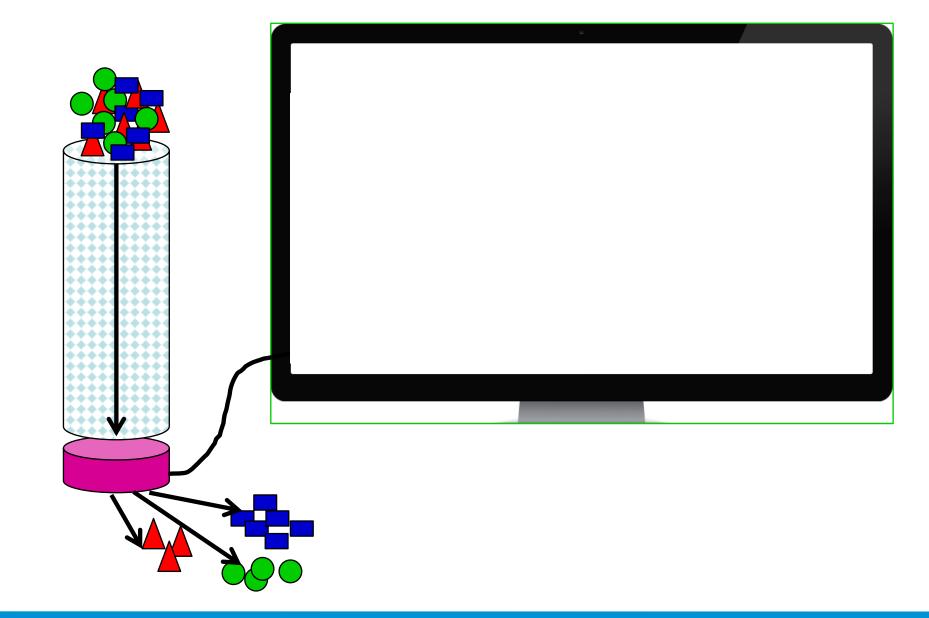
Analytical technics, instrumentation



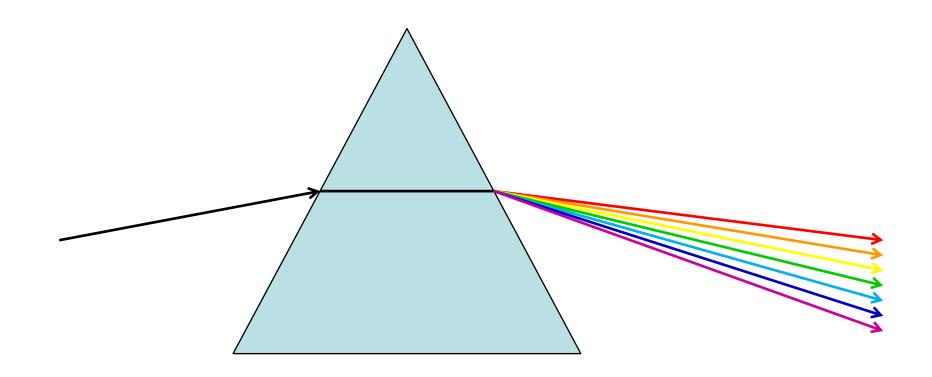


Separation technics (Chromatography: GC, LC, MS etc.)





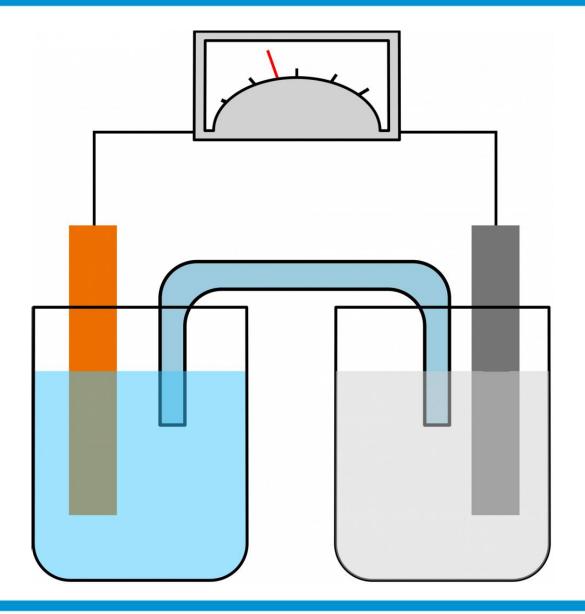




Electrochem. (potenciometry, culometry, conductometry)

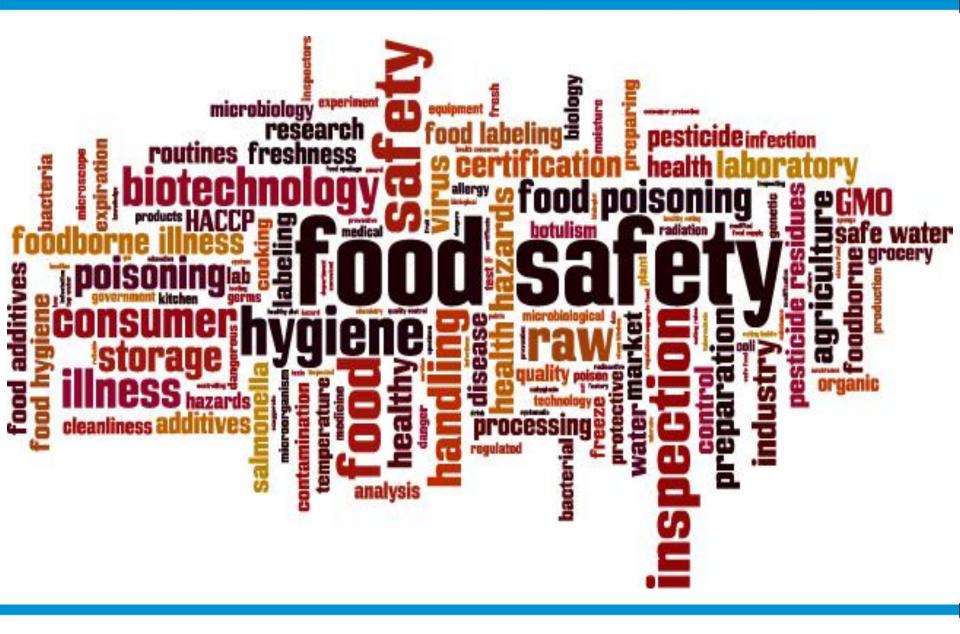
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Examples from the measurements of food safety





Instrumental food safety analytics – just exmples





Climate change: 2000 and 2010 (scenarios +2 or +5 °C)





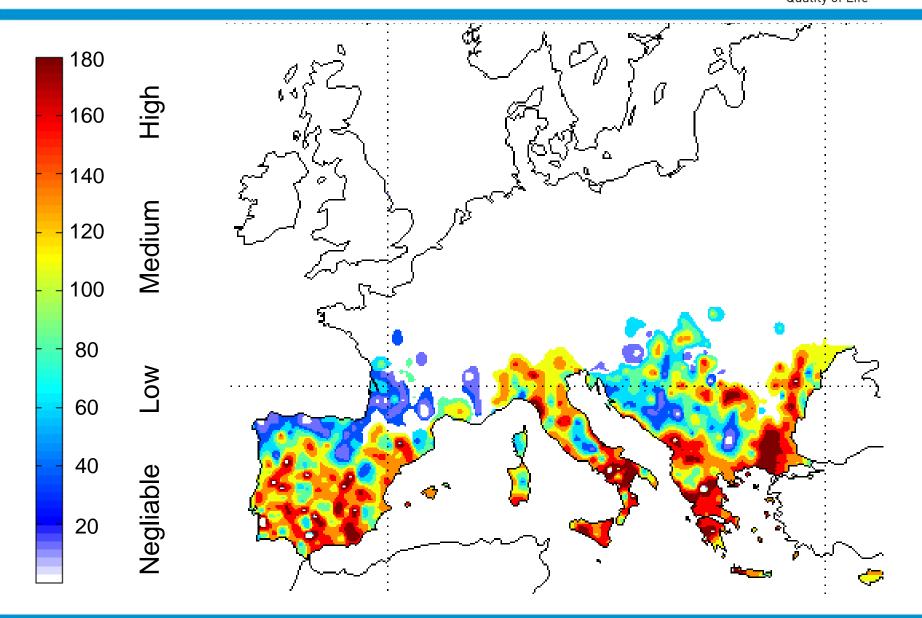
About aflatoxines





Risk index of aflatoxin B1 contamination (+2 °C)

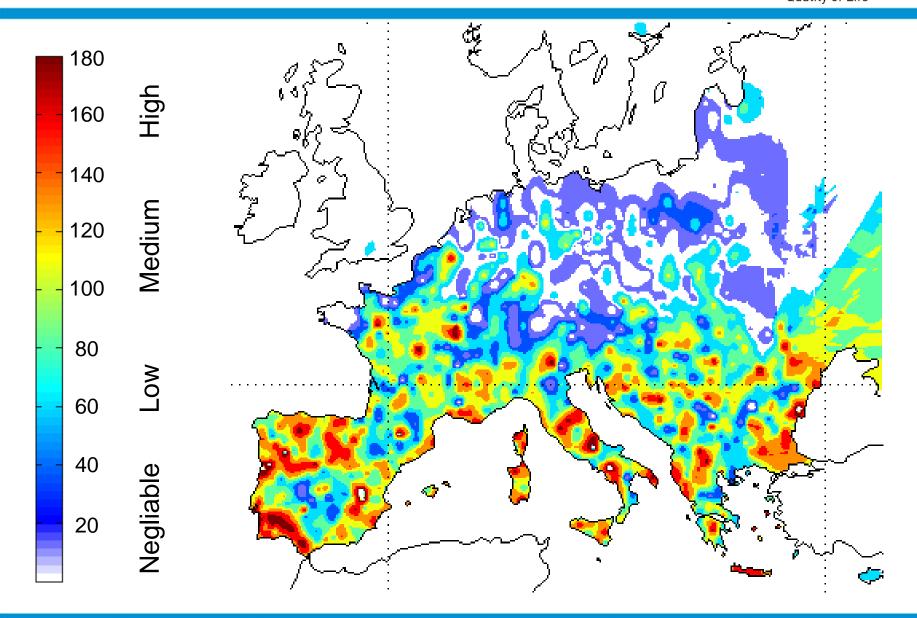




Battilani P. et al. (2012): Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate (Question No EFSA-Q-2009-00812) – After Szeitzné Sz. M.

Risk index of aflatoxin B1 contamination (+5 °C)





Battilani P. et al. (2012): Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate (Question No EFSA-Q-2009-00812) – After Szeitzné Sz. M.

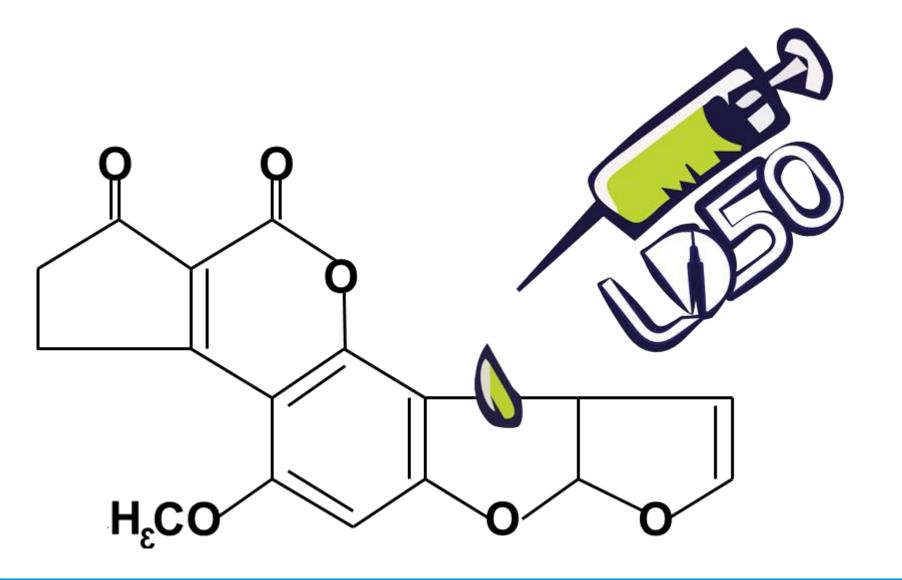
Aflatoxin-group: substitued cumarin derivatives

- aflatoxin B1
- aflatoxin G1
- aflatoxin M1
- aflatoxin B2
- aflatoxin G2
- aflatoxin M2
- aflatoxin B3

Aspergillus flavus, Asp. parasiticus

B1, G1, M1 : 7,8-dihidrofurano(2,3-b)furane B2, G2, M2: 2,3,7,8-tetrahidrofurano(2,3-b)furane Aflatoxin B1 – Acut toxicity on different animals po.





Aflatoxin B1 LD₅₀ po. mg/bwkg





Rat: 2.71



Pig: 0.62



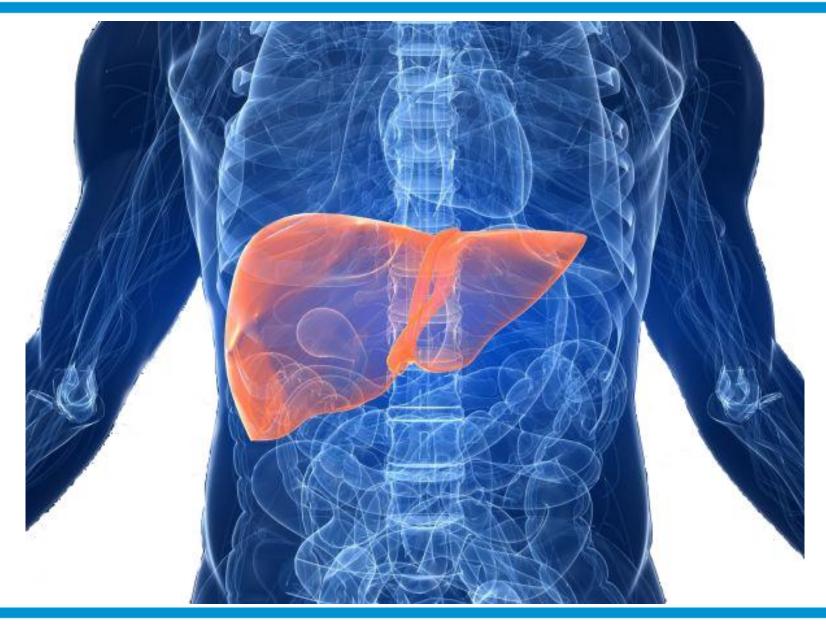
Monkey: 2.20



Safety Data Sheet Aflatoxin B1 - https://www.caymanchem.com/msdss/11293m.pdf (Aquired 20.02.2016)

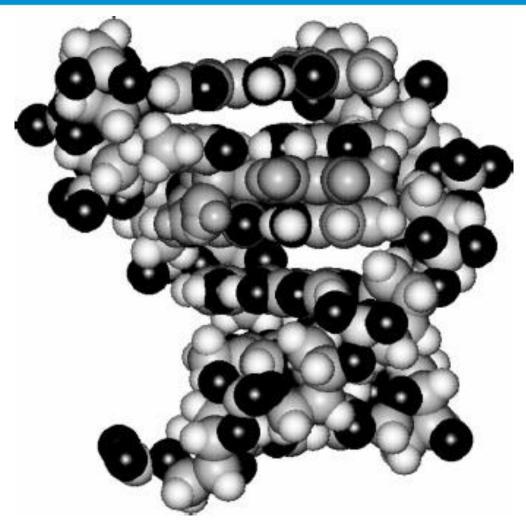
The target organ: the liver





The target organ: the liver



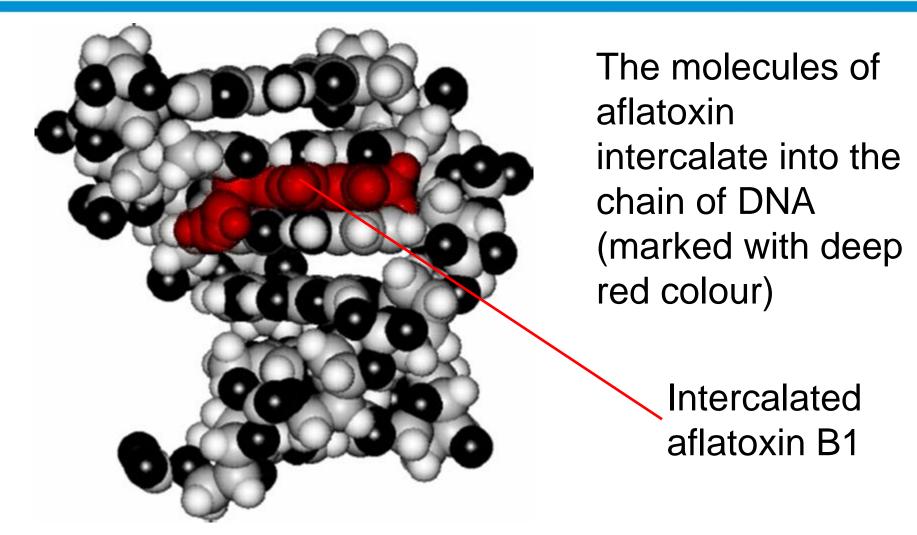


The molecules of aflatoxin intercalate into the chain of DNA (marked with deep red colour)

[d(ATC^{ATOB1}GAT). d(ATCGAT) and d(AT^{ATOB1}G-CAT)₂]

The target organ: the liver





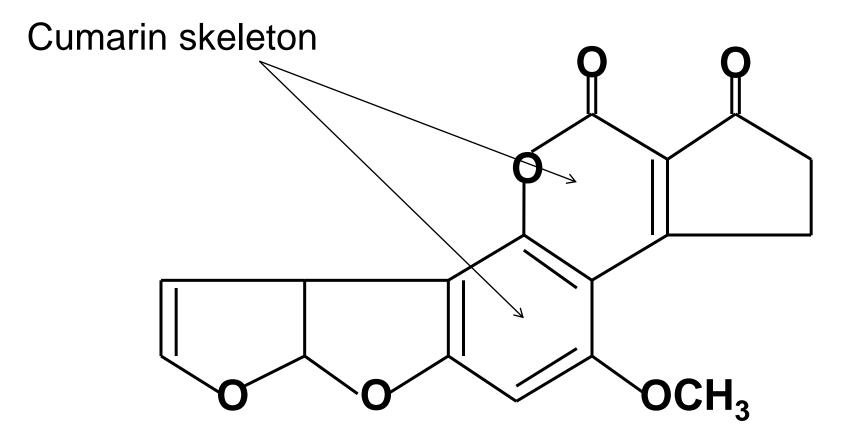
[d(ATC^{ATOB1}GAT). d(ATCGAT) and d(AT^{ATOB1}G-CAT)₂]



Woodruff (*Galium odoratum;* spirit industrial application) Cinnamon (*Cinnamomum verum;* Ceylon, Cassia) Brazilian oak (*Quercus*) Sweet clover (*Melilotus sp.*)

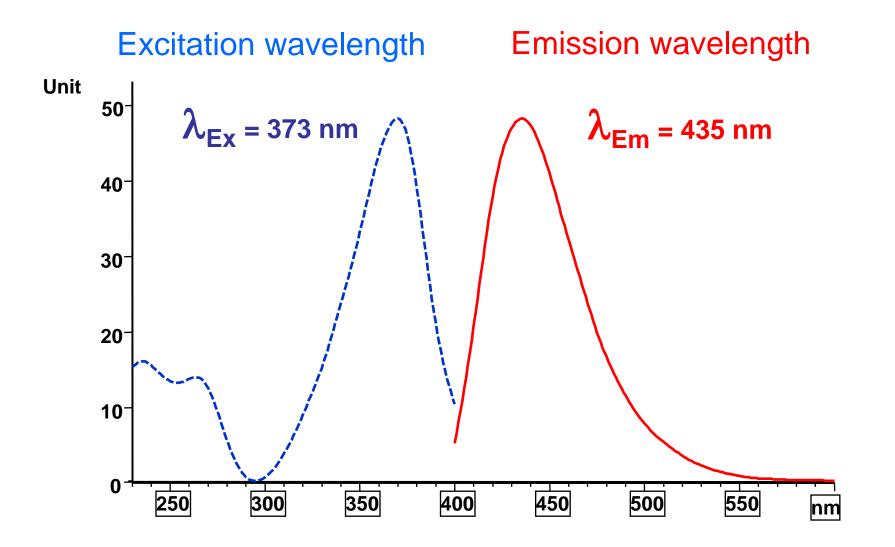






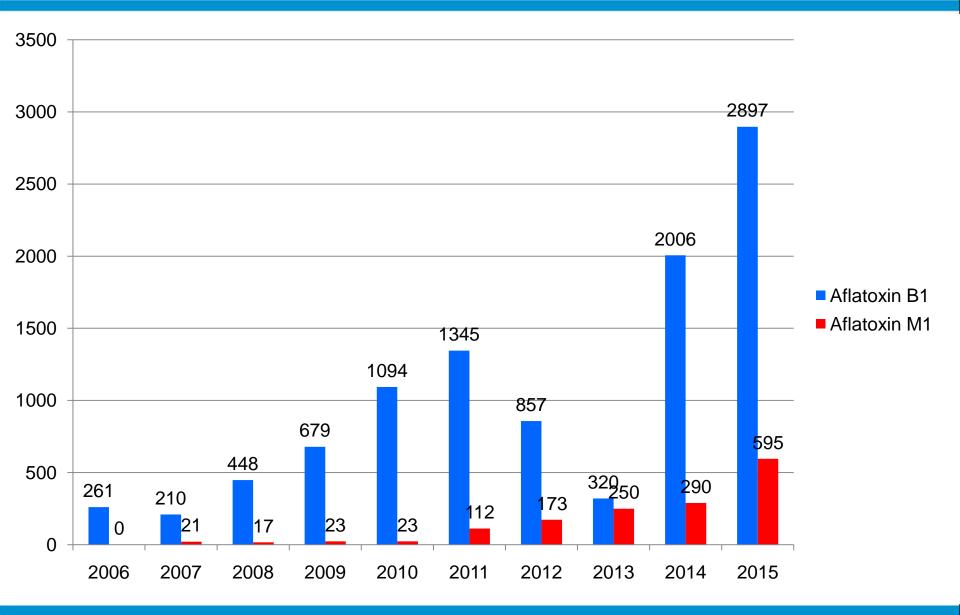
Underhand, definitely toxic compound





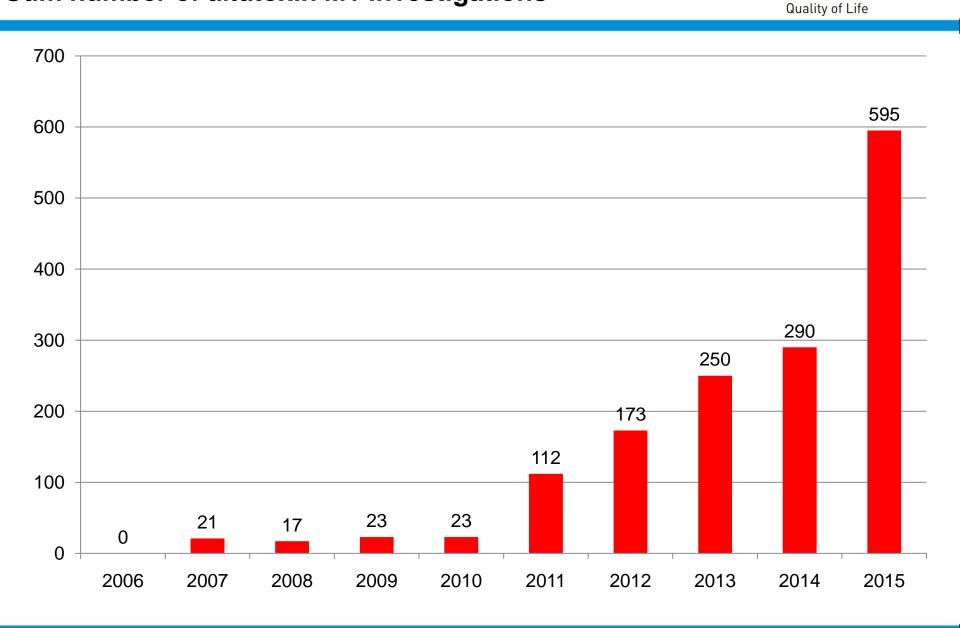








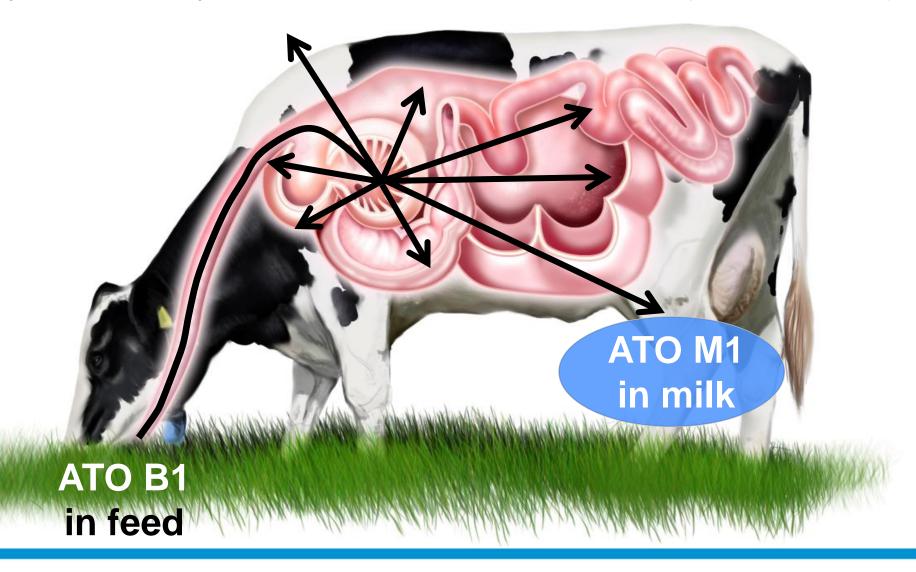
Sum number of aflatoxin M1 investigations



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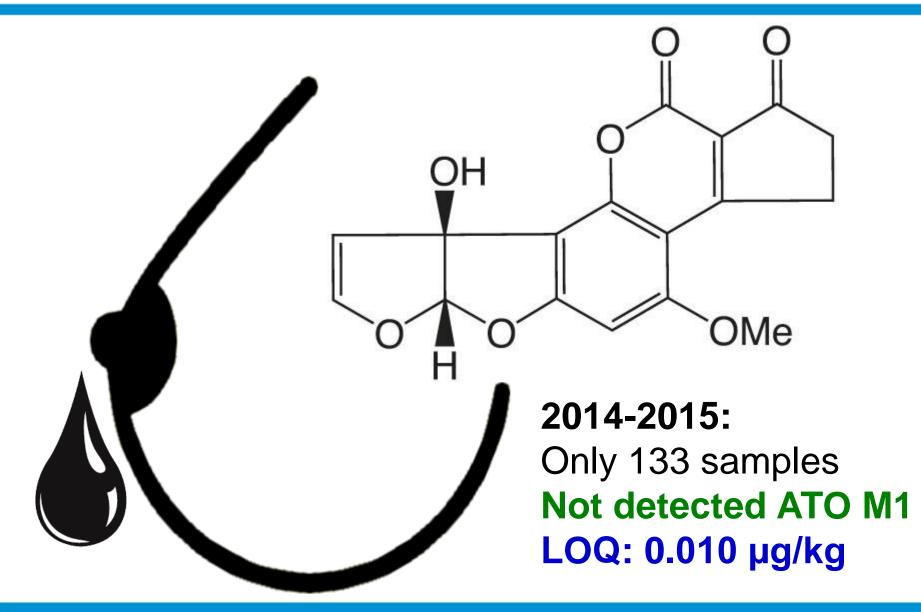


Mytochondrial cytochrom: P450: ATO B1 \rightarrow ATO M1 (15-30 minutes)



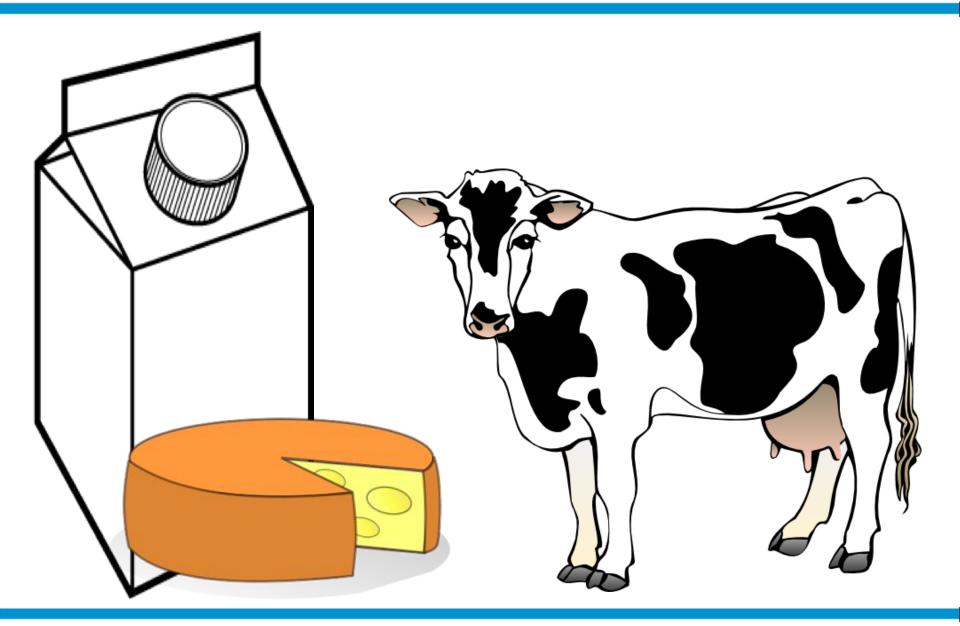
Aflatoxin M1 in breast milk – below the LOQ





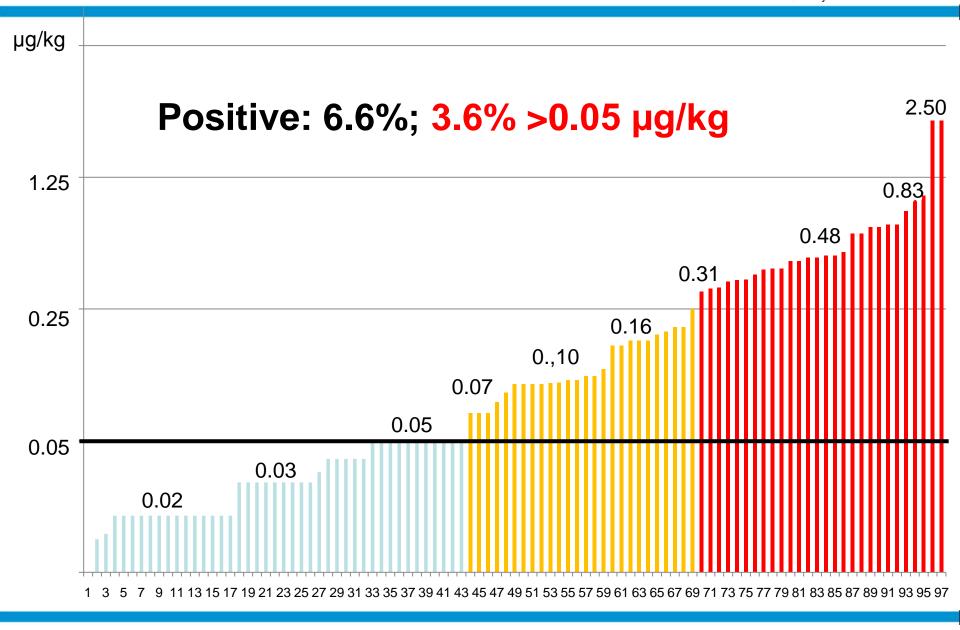
Aflatoxin M1 in cow milk and milk products (2006-2015)





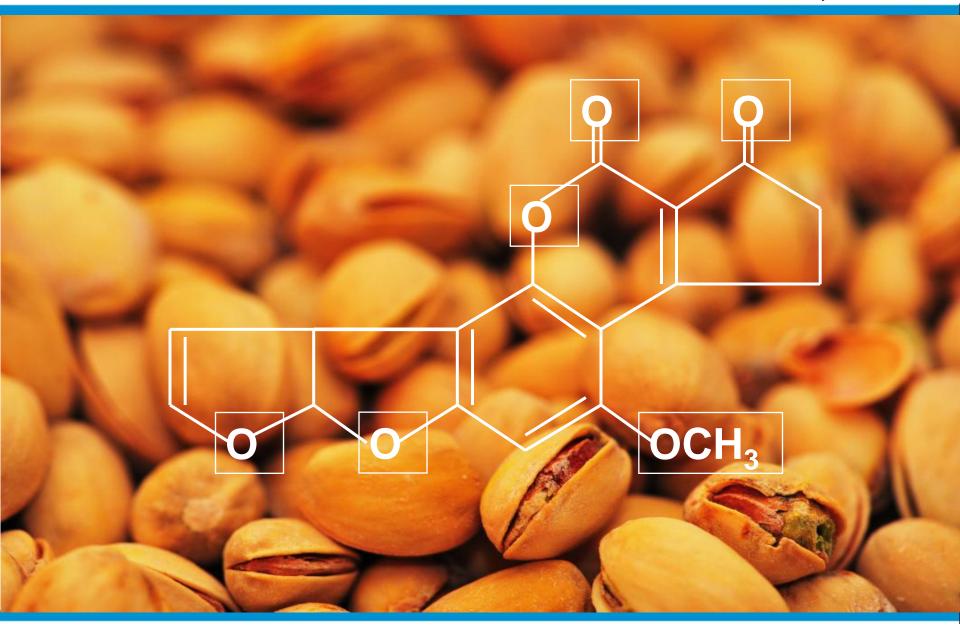
Aflatoxin M1 in milk products 2006-2015 (1493 samples)

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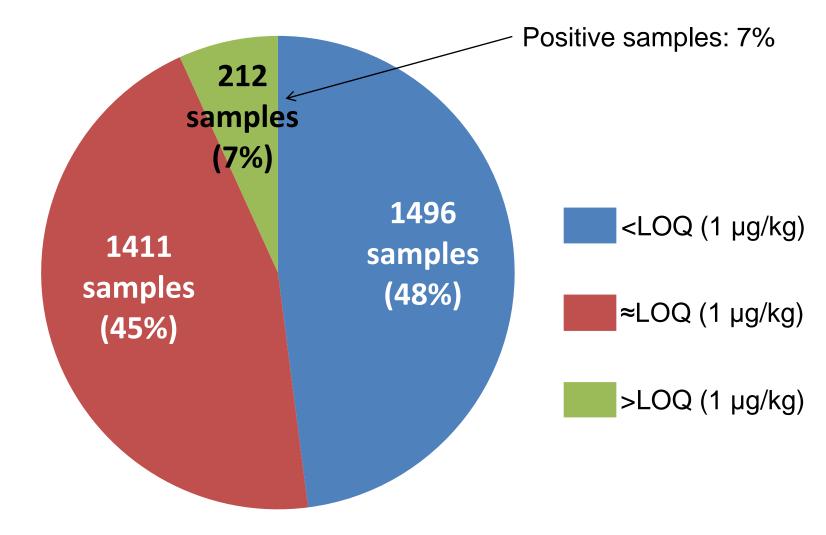


Aflatoxin B1 in several food samples

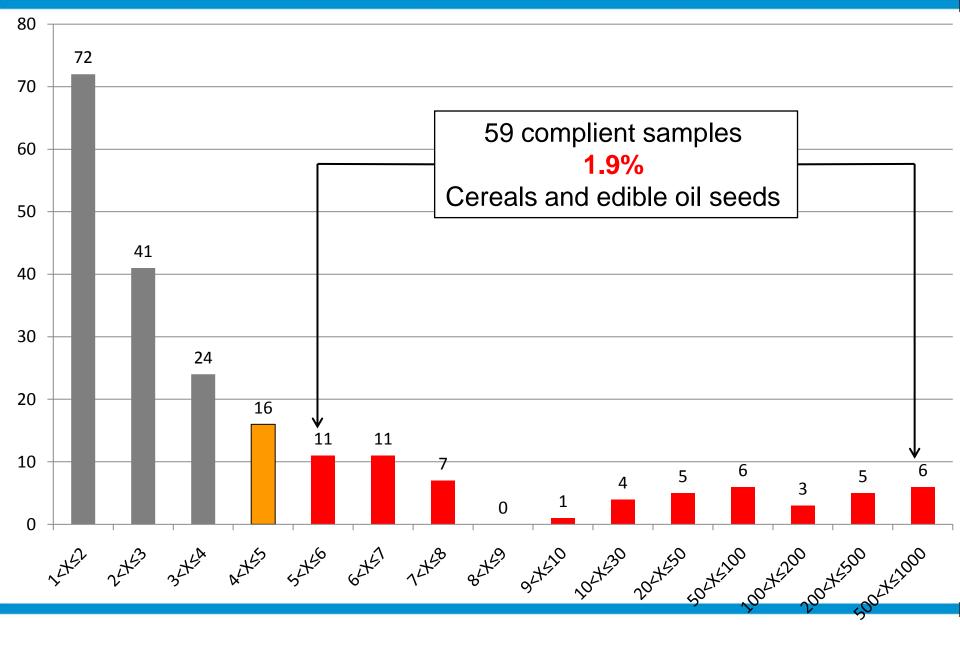




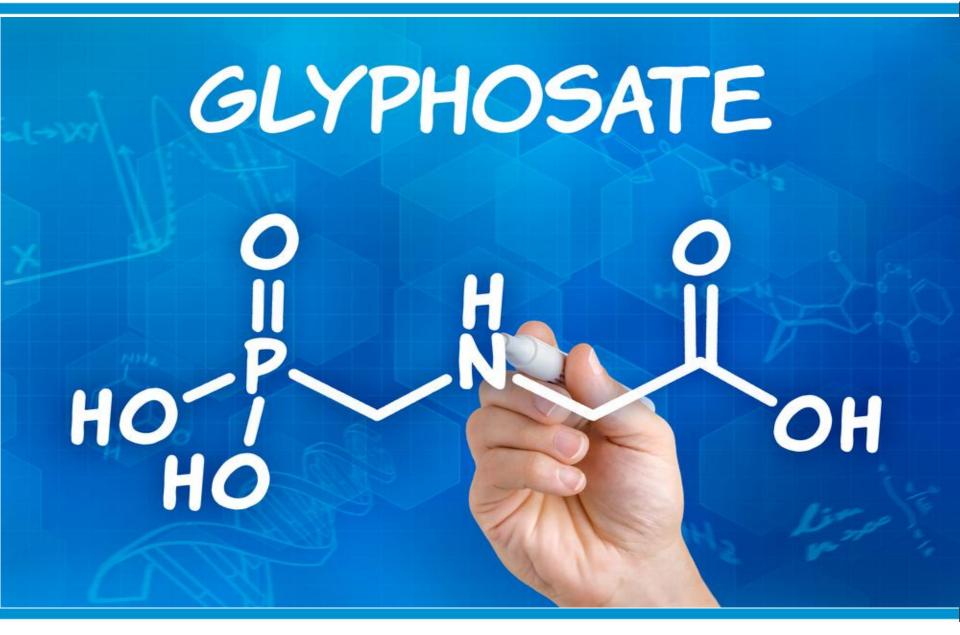












Debate on topic glyphosate is carcinogen or not?







- Decreased head size,
- Lesions in central nervous system,
- Damage of skull forming cells,
- Deformation of cartilage in joints,
- Human defects during child-birth,
- Chelate forming with metal ions,
- The primer human glyphosat source is the food;

African clawed frog (Xenopus laevis)





Medizinisches Labor Bre	emen Gemeinschaftsproxis für Laboratoriumsmedizin, Mikrobiologie und Infektionsepidemiologie, Biochemie; Umweltmedizie Haferswerde 12 • 28357 Bremen	174 voluntary person's urine was investigate from the EU:
Document Title	Determination of Glyphosate residues in human urine samples from 18 European countries	
Test Compound	Glyphosate and AMPA	Positive samples: Glyphosate 42.5 % AMPA 34.5 %
Study Initiation Date	March 2013	
Study Completion Date	June 6, 2013	LOQ: 0.150 µg/L for glyphosate and AMPA
Test Facility	Medical Laboratory Bremen, Haferwende 12, 28357 Bremen, Germany	
Sponsor	BUND, FoE	
		Range: 0.155-0.260 µg/L
Date of the Document	June 12, 2013	



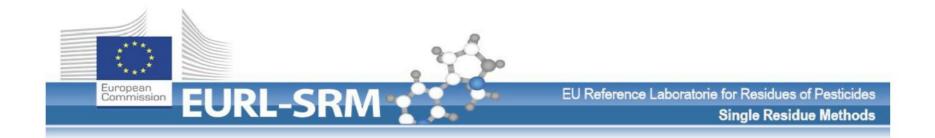
- Strongly polar compound,
- Small molecule,
- Despite of it non volatile,
- Week light absorbance,
- No fluorescency,
- Poor retention in reversed phase;

Glyposate needs specific method, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method isn't appropriate to extract from the different samples!



- Homogenisation, weighting 5 g sample
- Extraction with ethanol acidified with formic acid,
- Cenrifuge, filtration,
- Adjusting the sample volume,
- Sample dosage into the auto sampler of HPLC
- Separation on Hypercarb GCB column,
- LC-ESP MS/MS (TripleQuad) detection,
- Internal standards: ¹³C₂ és ¹⁵N nuclide marked glyphosate standard;
- Transitions: 168/63 m/z, or in the case of nuclide marked standards: 171/63 m/z;
- LOQ: 10 ng/g = 10 µg/kg



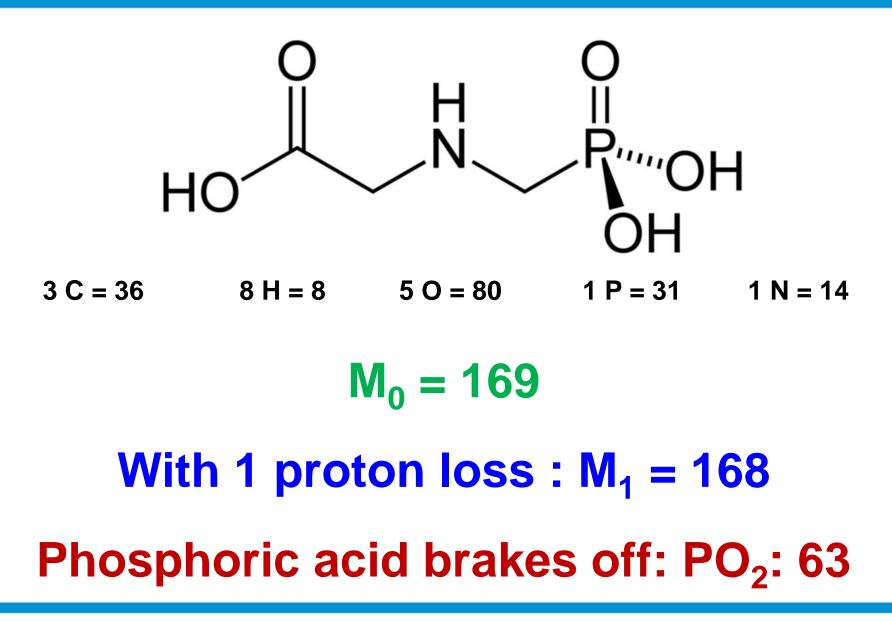


Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPe-Method)

- Version 7 (Dec 2012, Document History, see page 42) Authors: M. Anastassiades; D. I. Kolberg; D. Mack; Chr. Wildgrube; I. Sigalova; D. Roux; D. Fügel

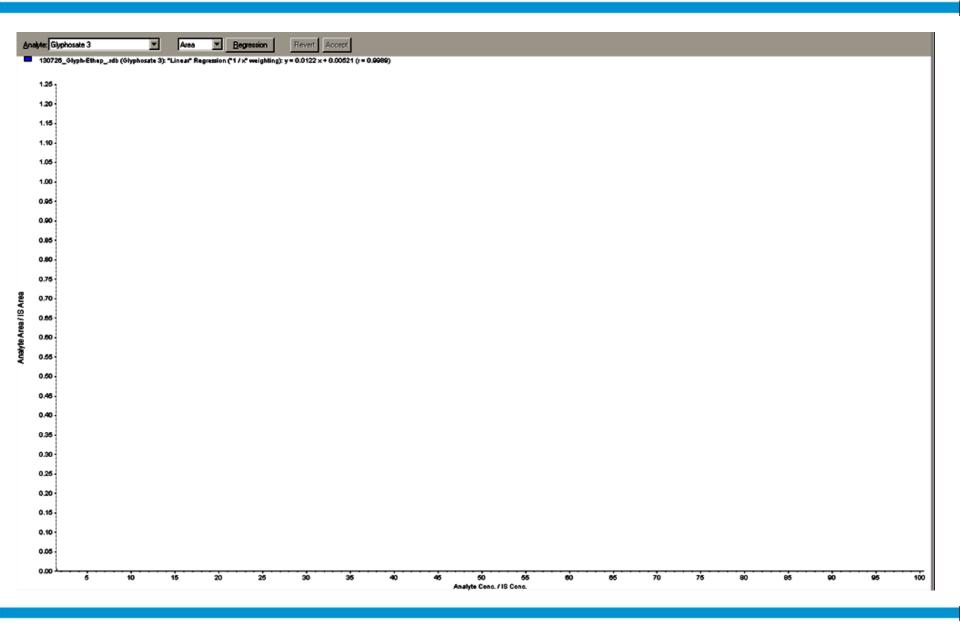
Characteristic mass transition: 168/62 m/z





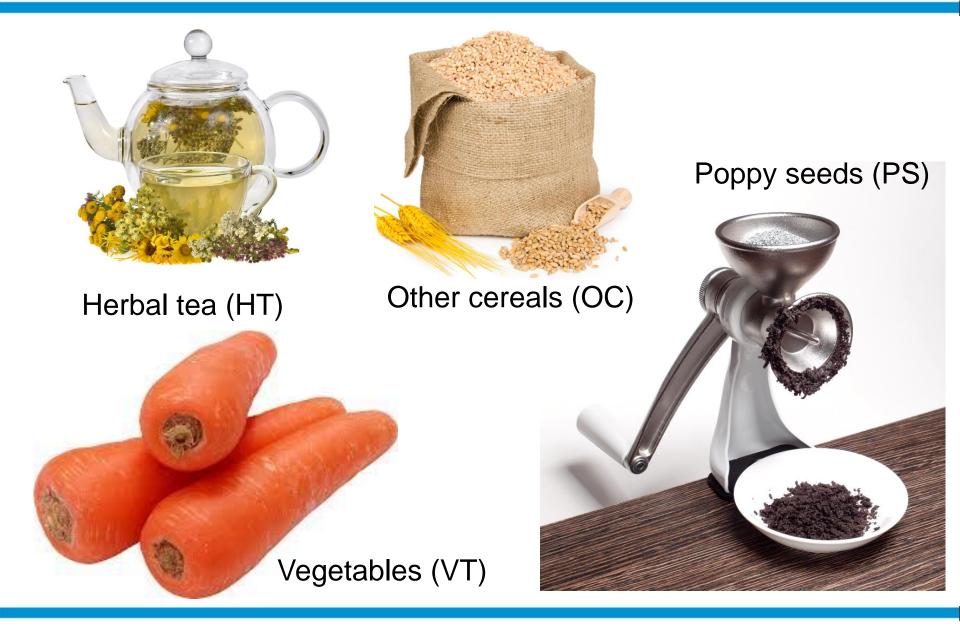
Calibration curve of glyphosate LC-ESP - MS/MS detect.



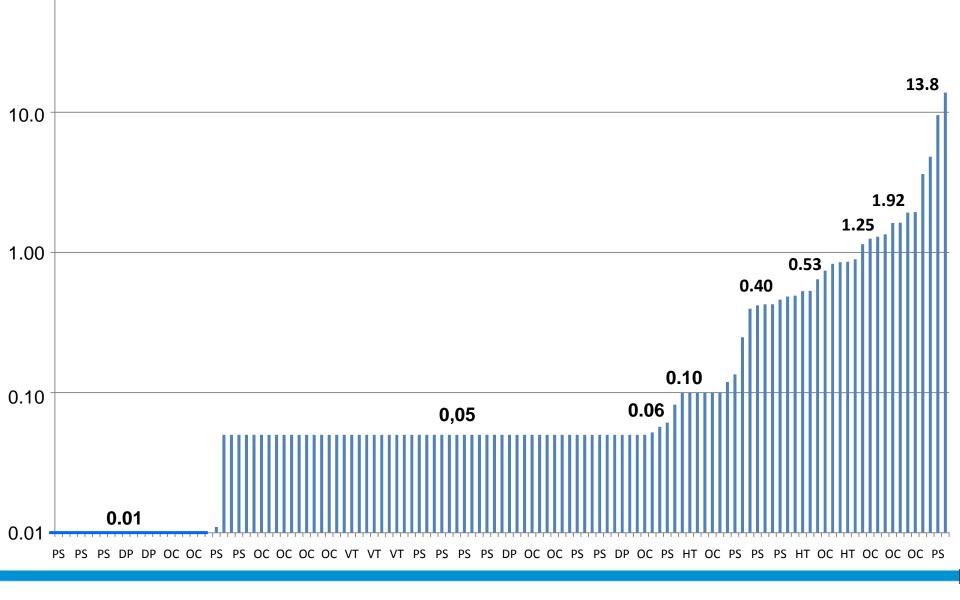


Glyphosate samples in 2015: 172 specimens





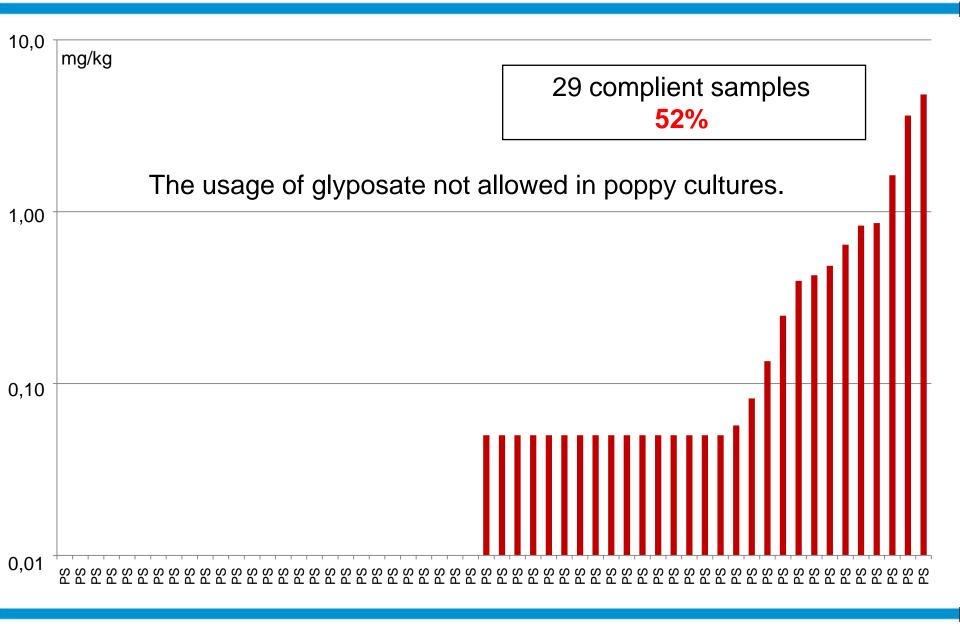
Glyphosat results (mg/kg) in plant origin samples (2015)



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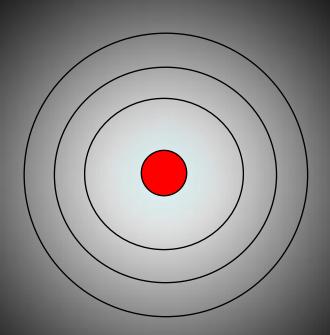
Glyphosate residues in positive poppy seed samples





Measurement uncertainty





The lower limit of the measurement and the measurement uncertainty should have be negotiated between the laboratory and the customer.

Appendix

Food microbiology





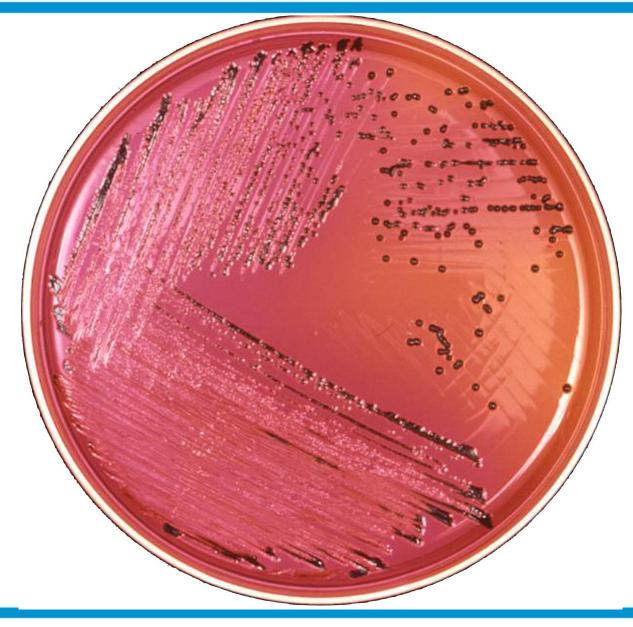
Salmonella sp. Negative sample max. 2 days





Salmonella sp. Positive sample 4 - 5 days





The pathogene confirmation takes more time







Matrix Aided LASER Desorption Ioniosation – Time Of Flight – Mass Spectrometry





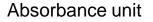
A MALDI-TOF-MS stainless steel plate with 96 places

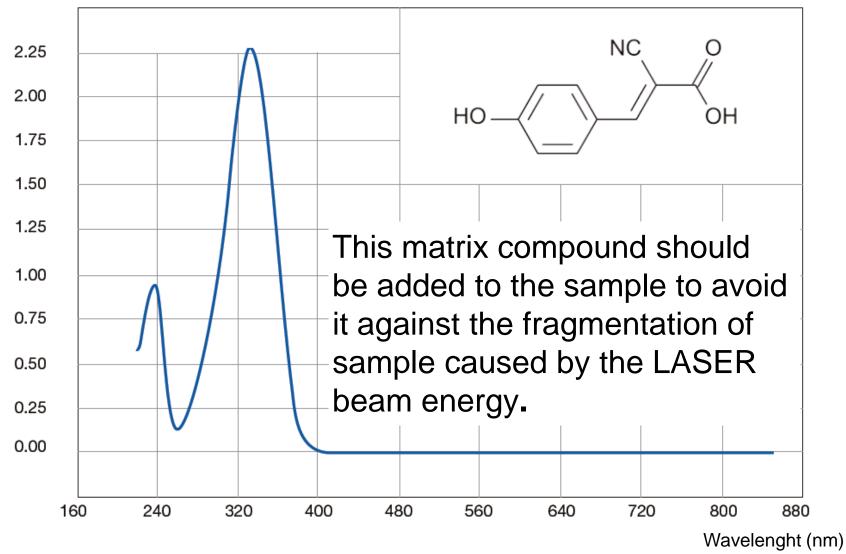




The matrix: α-Cyano-4-hidroxi-cinnamic acid

Quality of Life





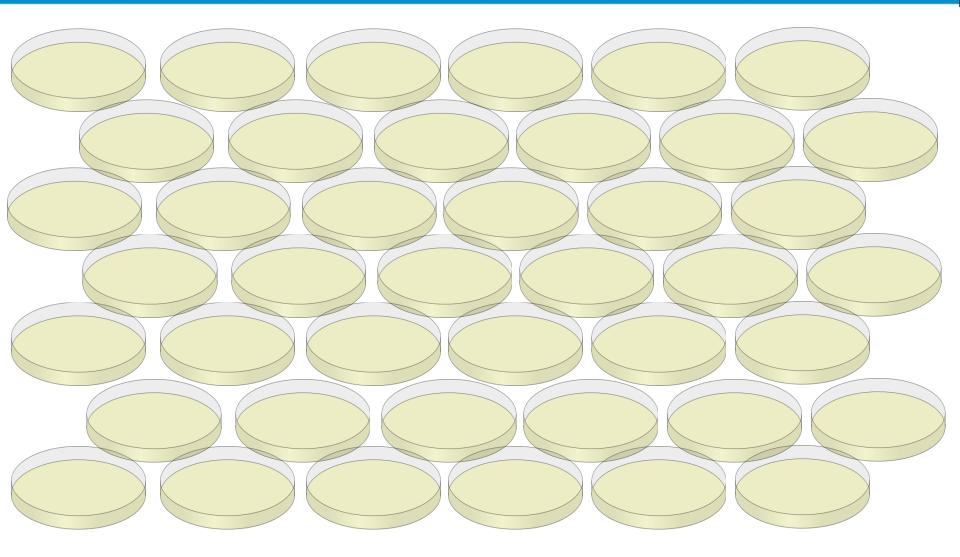
A MALDI-TOF-MS – How to dosage the samples to plate



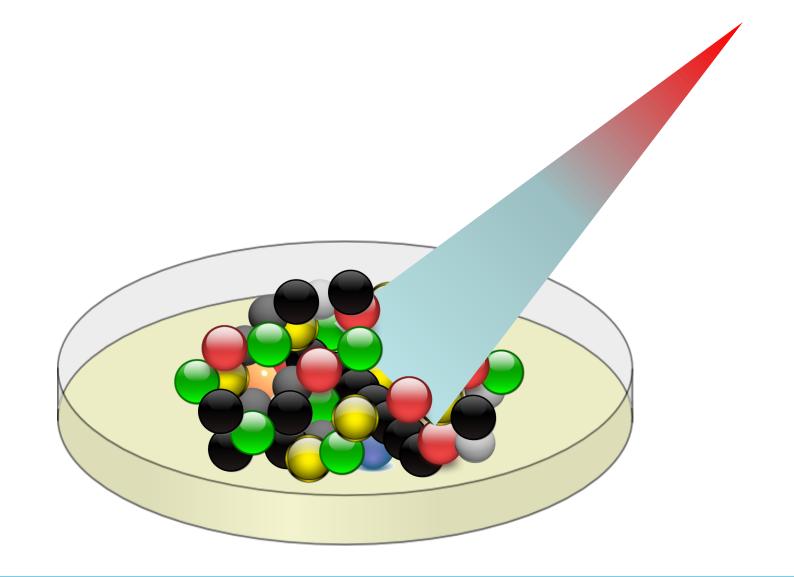


A MALDI-TOF-MS drawing of sample places on the plate













Scematic picture of TOF MS flying tube

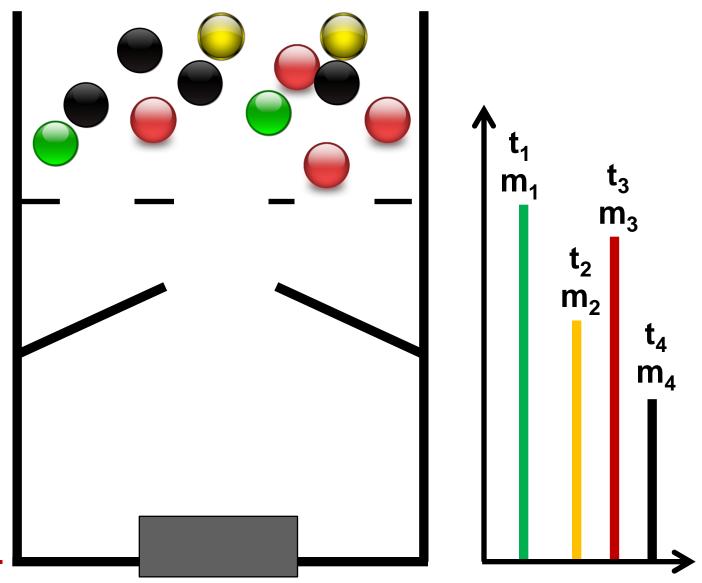


The technic is appropriate to investigate several long chain molecules

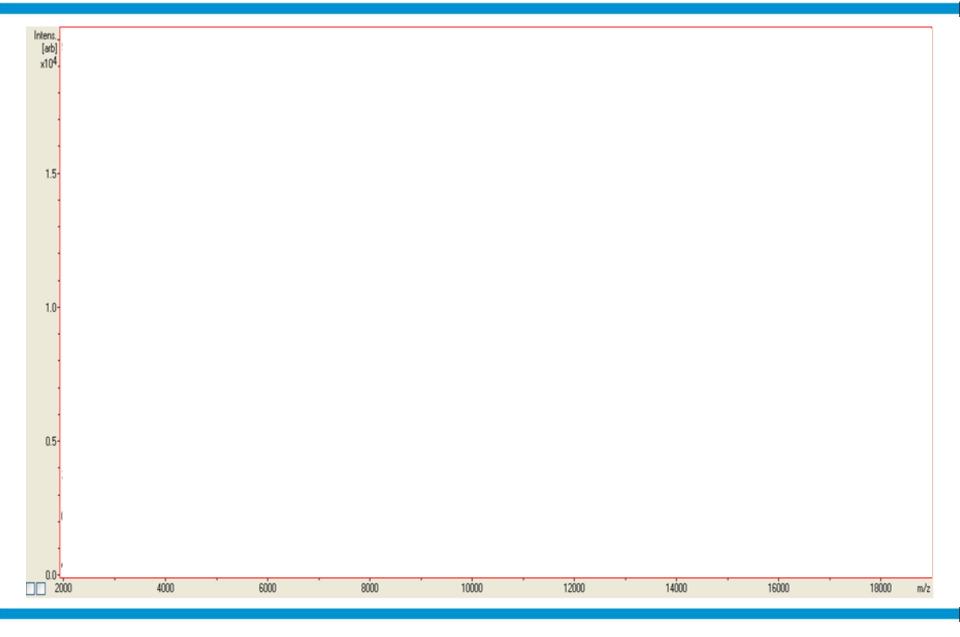
For example:

Hormones, allergenes, Long chain hydrocarbon molecules, protein-Carbonhydrate complexes, **PROTENS OF MICROBES.**

QUALITATIVE ACCREDITED tool.



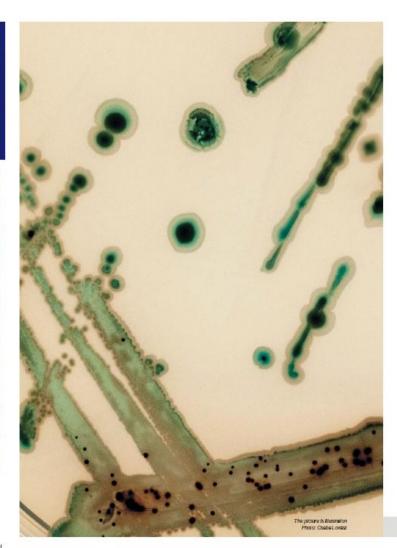




Journal of Food Investigations 2014 Vol 4.



IN FOCUS MASS -SPECTROMETRY Z MICROBIOLOGY



Csaba Lovász¹

Received: 2014. February - Accepted: 2014. July

The Nobel prize in Chemistry and Staphylococcus aureus. The role of modern mass spectrometry in the identification of microorganisms

1. Summary:

Experts and laboratories supervising food safety are faced with new challenges by globalized markets, and rapidly changing food industrial technologies and nutrition trends. One of the most important tasks of microbiological laboratories is the analysis of raw materials and products (feeds and foods) entering the food chain, fast and reliable detection and identification of the presence of pathogenic microorganisms. Detection of pathogenic microorganisms might take 5 to 8 days using standard microbiological culture methods, therefore, the emphasis of modern microbiological methods is shifting towards rapid instrumental analyses. Thanks to research in molecular biology, methodologies that can provide reliable results for a large number of samples in a short time are becoming routine laboratory methods.

One of the latest representatives of alternative microbiological methodologies is mass spectrometry (MALDI-TOF) and related software, with the help of which one can identify microorganisms. The acronym MALDI-TOF stands for a special mass spectrometer: MALDI (Matrix Assisted Laser Desorption Ionization) is a photoionization ion source, while TOF (Time of Flight) denotes a time-of-flight mass analyzer. Identification is based on gentically coded proteins found in microorganisms. Each microorganism has a characteristic protein profile that can be used for identification as a sort of fingerprint. This protein profile is determined during MALDI-TOF analysis, and then it is compared to the protein profiles of microorganisms found in the reference library. In this paper, I would like to present the basis of this method, its applicability for the identification of pathogenic microorganisms, and the place of this new methodology among microbiological tests.

2. Introduction

What does the 2002 Nobel Prize in Chemistry have to do with a microorganism of the genus "wanted criminals", pathogenic microorganisms. Staphylococcus?

becoming blurred by modern science, and a method sciences. The prize was awarded to John B.

for structure determination - mass spectrometry - can be an efficient tool in the hands of microbiologists, it can be helpful in the fast and reliable identification of

The 2002 Nobel Prize in Chemistry was awarded by One might think that the answer is not much", but the Royal Swedish Academy of Sciences to three boundaries between classic scientific disciplines are researchers for their work in the field of the chemical

Wessing Hungary Kit.

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Thanks for my collagues for their contribution!





Tanks for your kind attention!



