

Analytical methods and performance criteria

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Contaminants in feed

- Mycotoxins
- Plant toxins
- Heavy metals
- Persistent organic pollutants
- Pesticides
- Antibiotics
- Others

Screening and/or confirmation

- Both screening and confirmatory methods can be applied
- Screening methods are:
 - cheaper, often more rapid and require less equipment
 - Can be used to separate negative samples from suspected samples
 - Proper discrimination between negative and suspected samples essential
 - Can not be used for final confirmation of the result in official control

Confirmation methods

- Required for proving the identity of compound
- Required for establishing level
- Requires use of MS-technologies
- Therefore relatively expensive, low throughput
- Confirms known compounds



Screening methods

- Bioanalytical methods
 - Immunoassays
 - Receptor assays
 - Bioassays
- Chemical analytical methods
 - Non-MS based techniques (UV, fluorescence)
 - Multi-methods (pesticides, mycotoxins, veterinary drugs)
 - Untargeted screening GC- or LC-MS
- Allows broader detection of compounds (novel risks?)

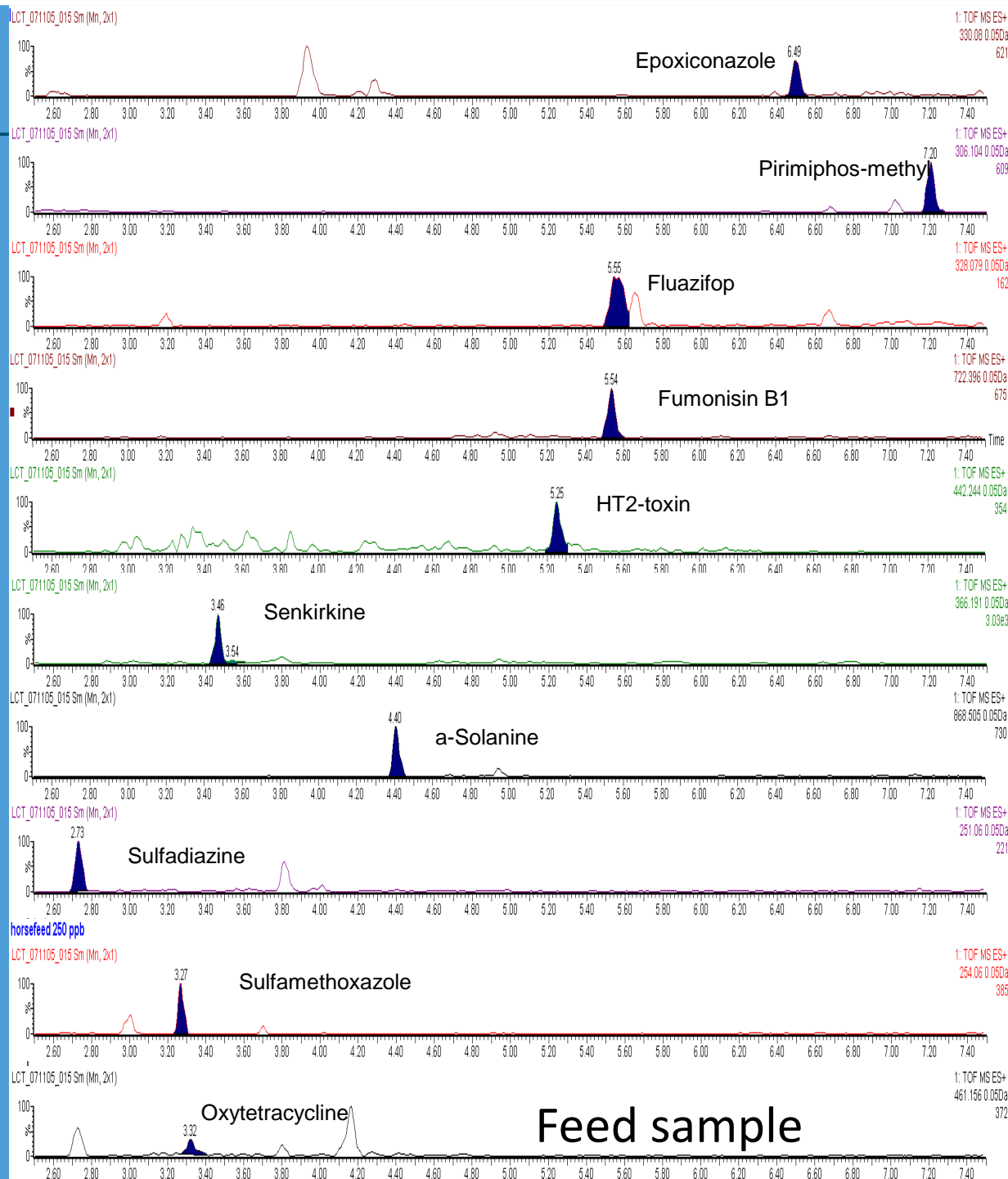
Example

Feed

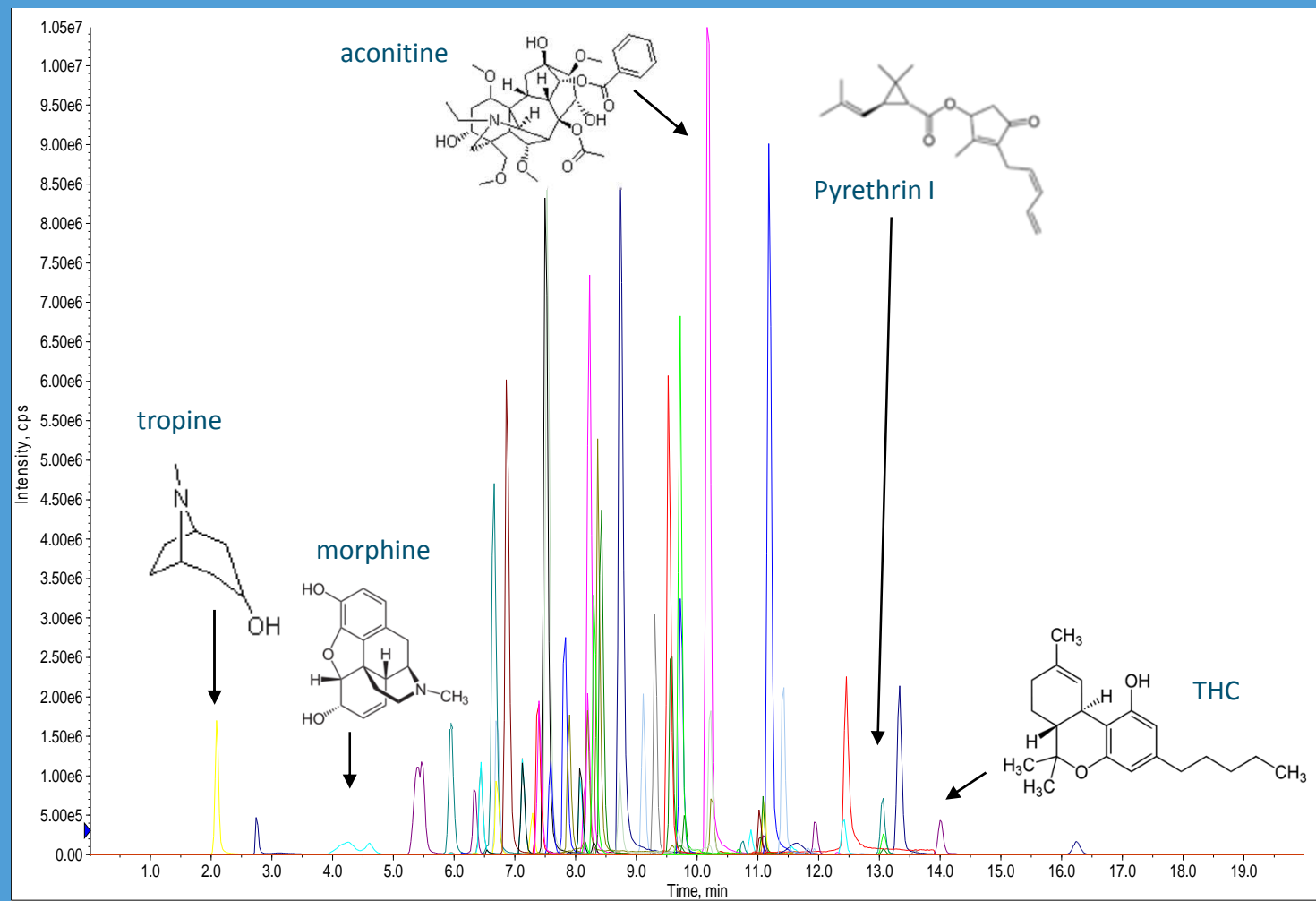
Fortified with 258 pesticides,
mycotoxins, plant toxins
veterinary drugs
at 0.25 mg/kg



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LC-full scan hrMS....

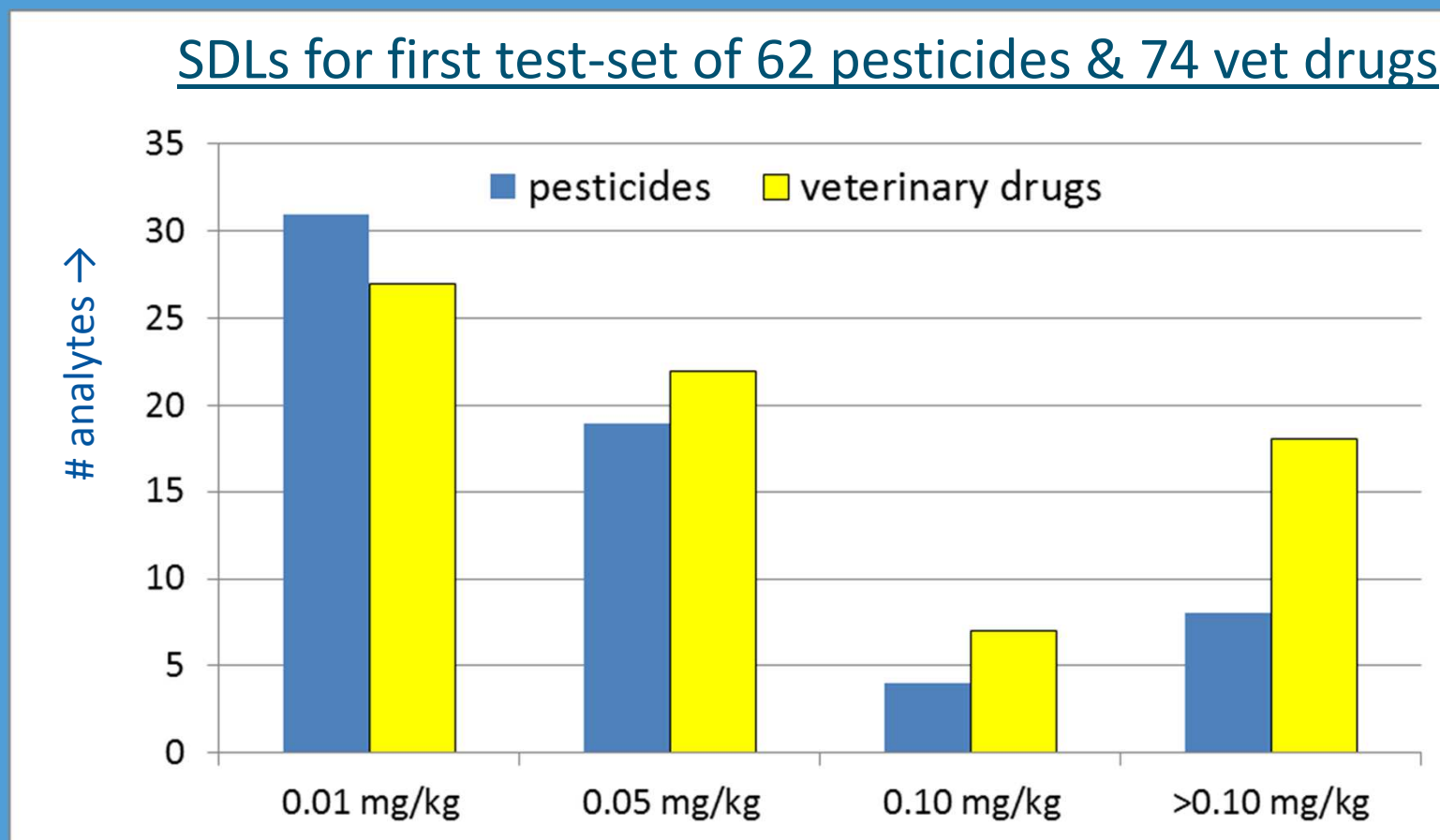


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100 x 3 mm ID, 3 μ m Atlantis T3
Water/MeOH, 5 mM NH_4COOH , 0.1 % formic acid

Validation of chemical screening method

SDLs for first test-set of 62 pesticides & 74 vet drugs



Balance between false-negatives and looking for more compounds

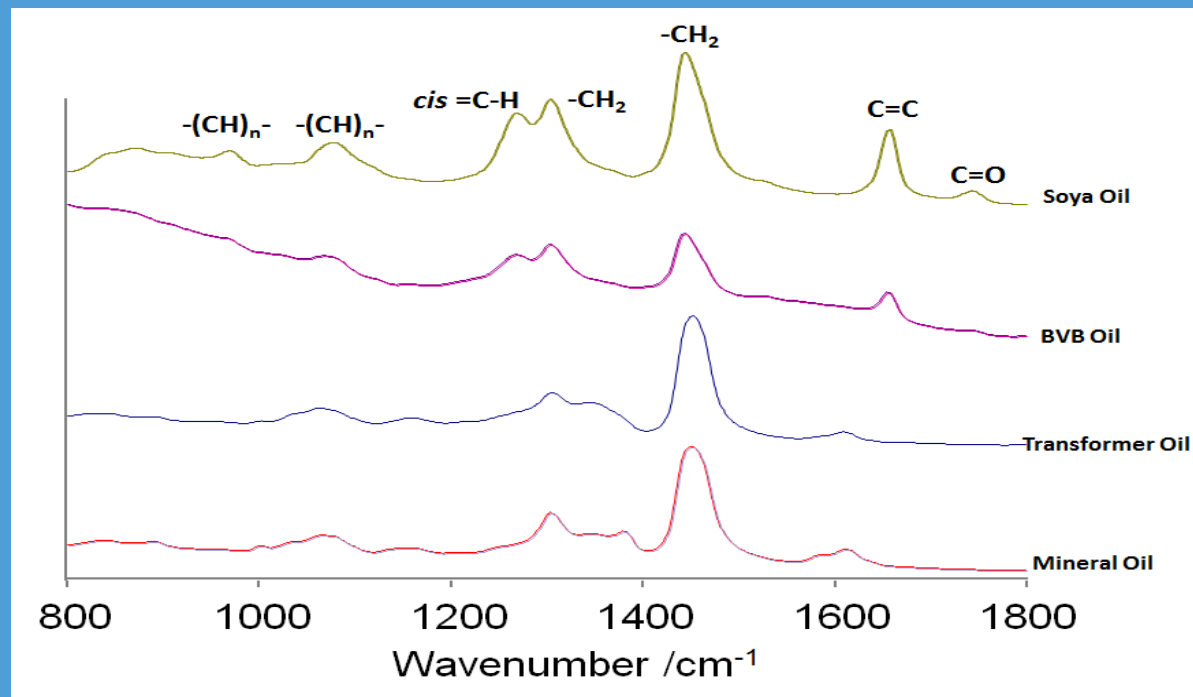
Raman Feasibility Study

Feed Oil Adulteration

- Soya Oil and Basic Veg Blend (BVB)
 - QSAFFE Partners
- Mineral Oil and Transformer Oil
 - Obtained from Sigma-Aldrich

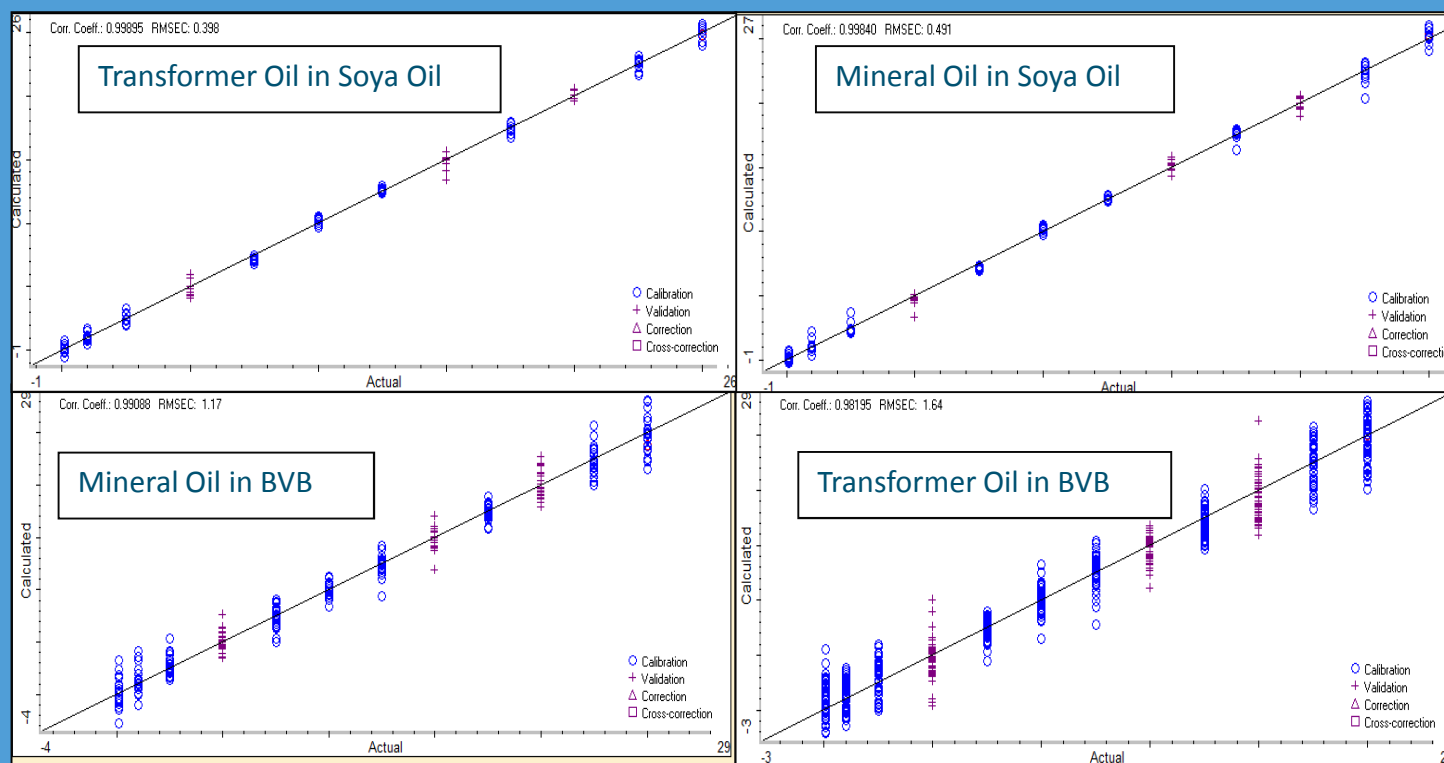
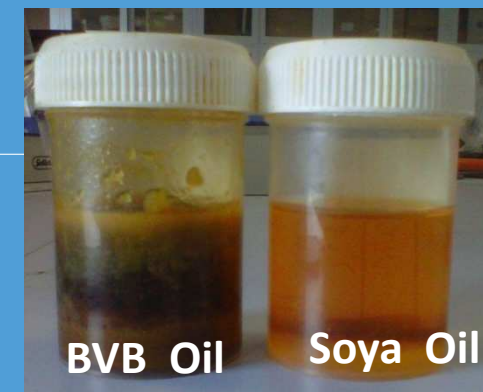


Typical Raman Spectra Obtained



Raman Feasibility Study

Quantitative Data

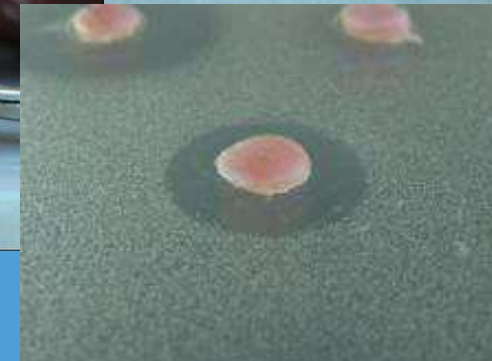
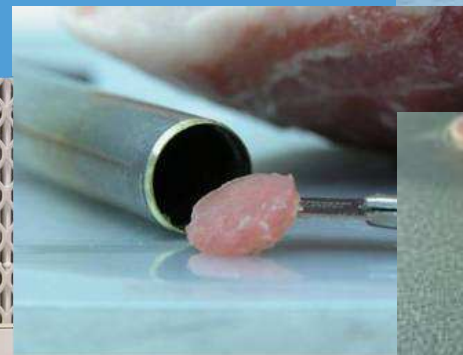
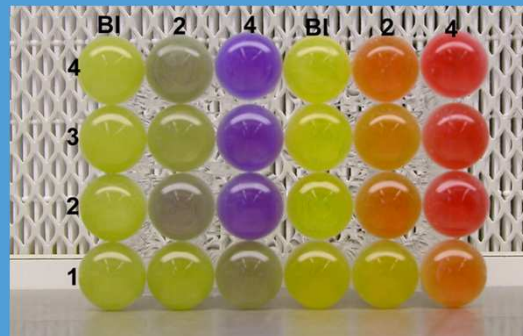


Bioassays

- Bacterial assays for antibiotics
- CALUX-assay for dioxins and dioxin-like PCBs
- Yeast assays for hormonal compounds
 - Estrogens, androgens, corticosteroids
- Rat and mouse bioassays for marine biotoxins
 - Actually used as confirmatory method

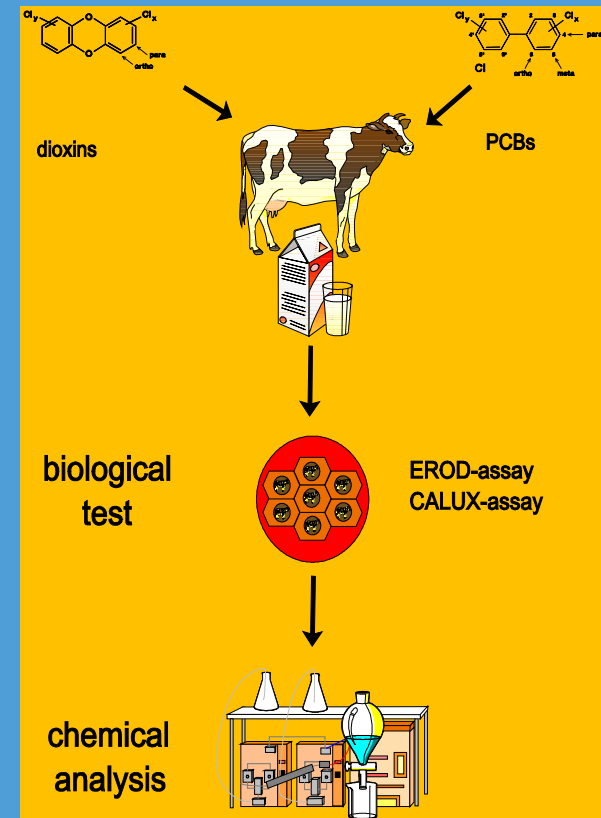
Bacterial growth inhibition assays

- Antibiotic screening
 - Tube test
 - Plate test
- RIKILT: NAT, PoultrySCAN, EggSCAN, FishSCAN etc.
- Extensively used in NMP (slaughter animals, poultry, egg, milk, feed)

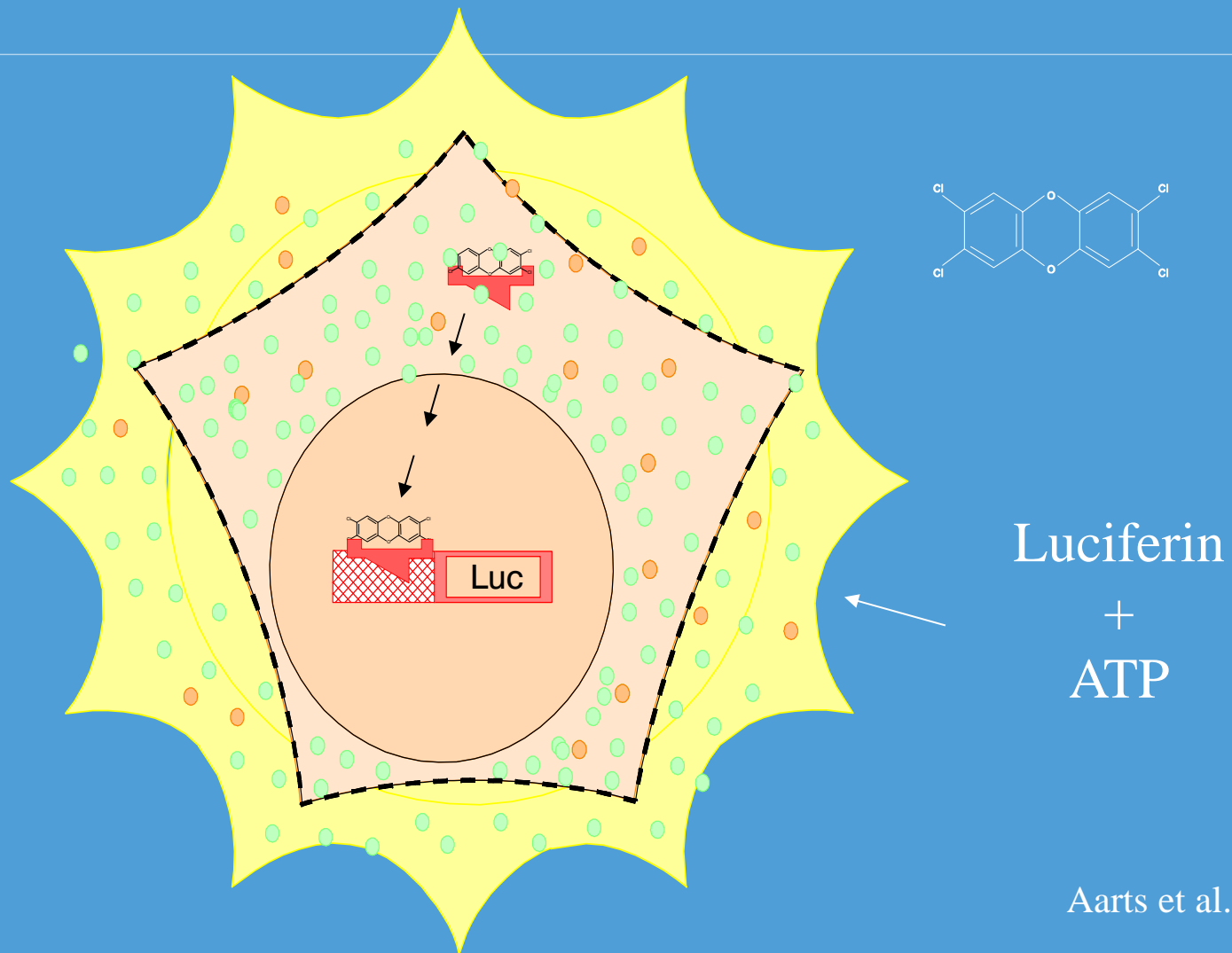


CALUX bioassay

- (DR) CALUX: screening
 - removal negative samples
 - confirmation suspects
- GC/HRMS: confirmation
 - Also GC/MS/MS suitable

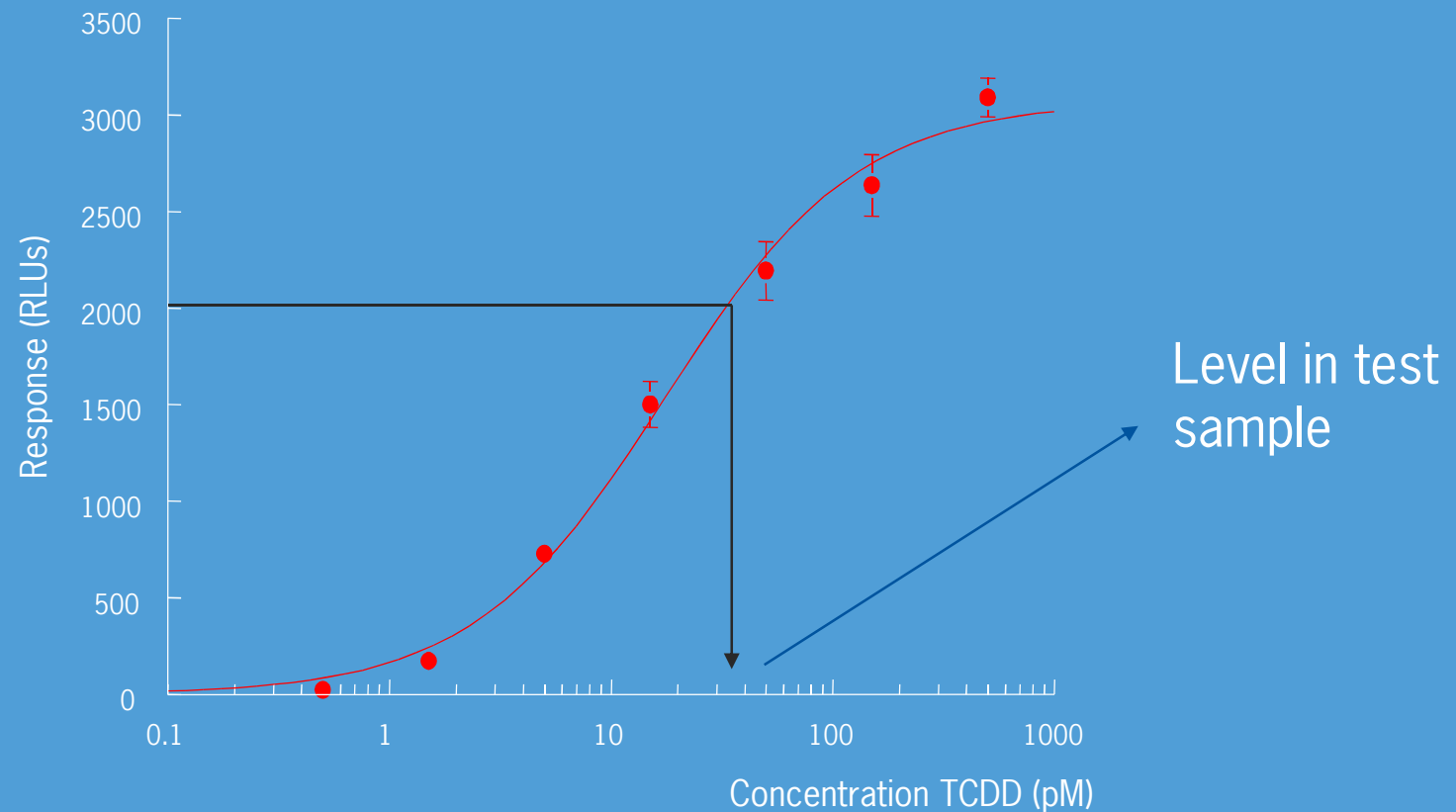


CALUX screening assay



Aarts et al. 1993

Estimation of level in sample



Performance criteria versus prescribed methods

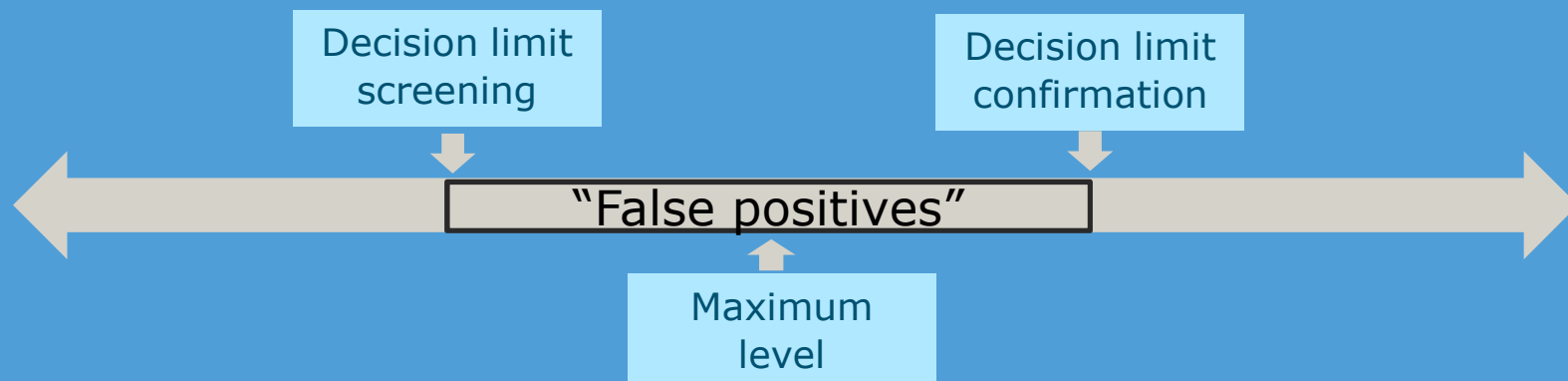
- Criteria give more flexibility for changes
- Dioxins and dl-PCBs
 - Commission Directives 2002/69/EC (food) and 2002/70/EC (feed): also application of bioassays
 - Upgraded to Commission Regulations EC (No) 1883/2006 (food) and 152/2009 (feed)
 - Recently replaced by Commission Regulations EC (No) 252/2012 (food) and 278/2012 (feed)
- Always based on the work of expert groups
 - EURL/NRL network

Screening vs quantitative approach

- CALUX is a screening method (yes/no answer)
 - Estimation may be given; support confirmation analysis
- Should be in BEQs and not TEQs
 - Relative response congeners in test not identical to TEFs
 - Also other compounds (w/o TEF) may show response
 - **Screening result should be recognizable**

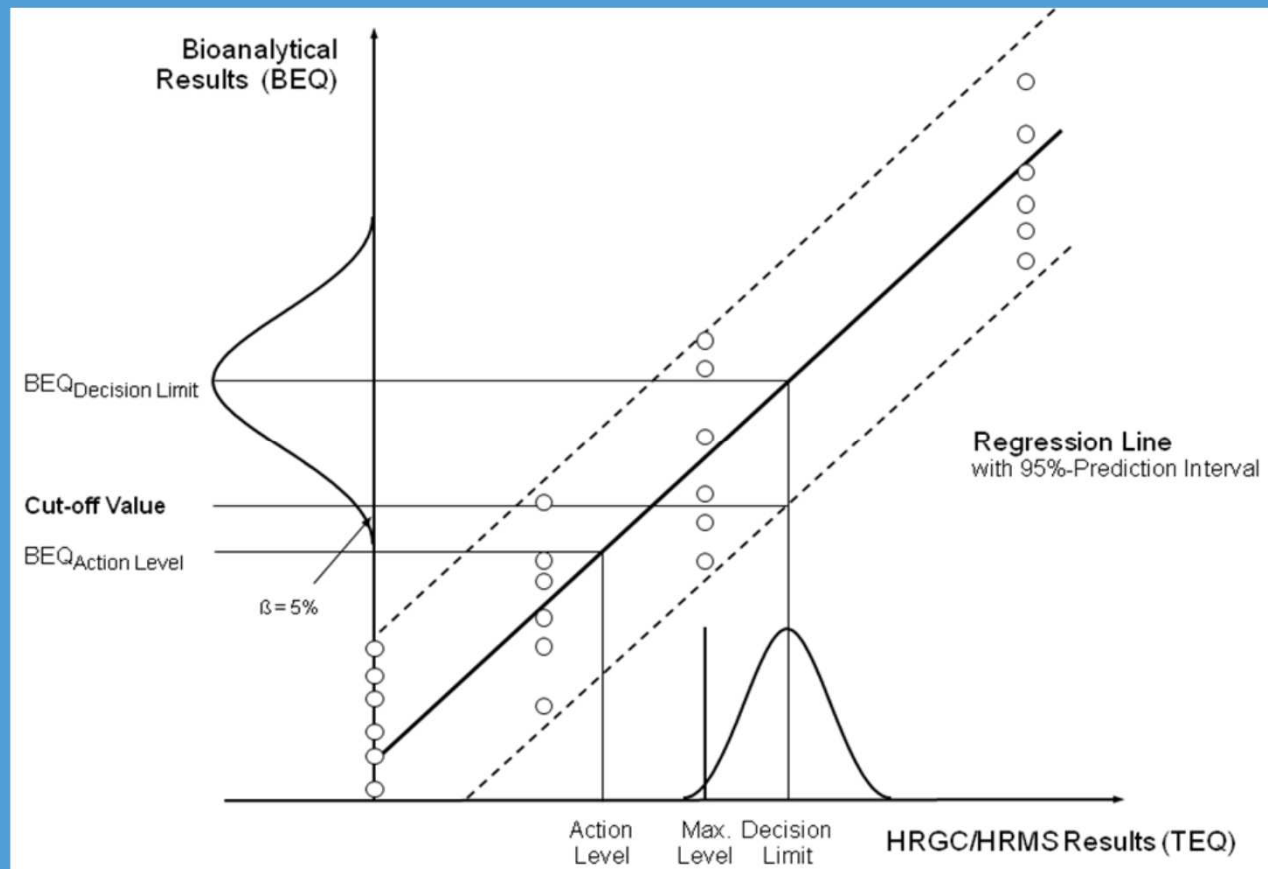
Screening versus confirmation

- Screening should not miss positive samples
 - Chance less than 5%
- Confirmation should not falsely decide on positive result
 - Chance less than 5%
 - Application of measurement uncertainty



Setting of cut-off levels for screening

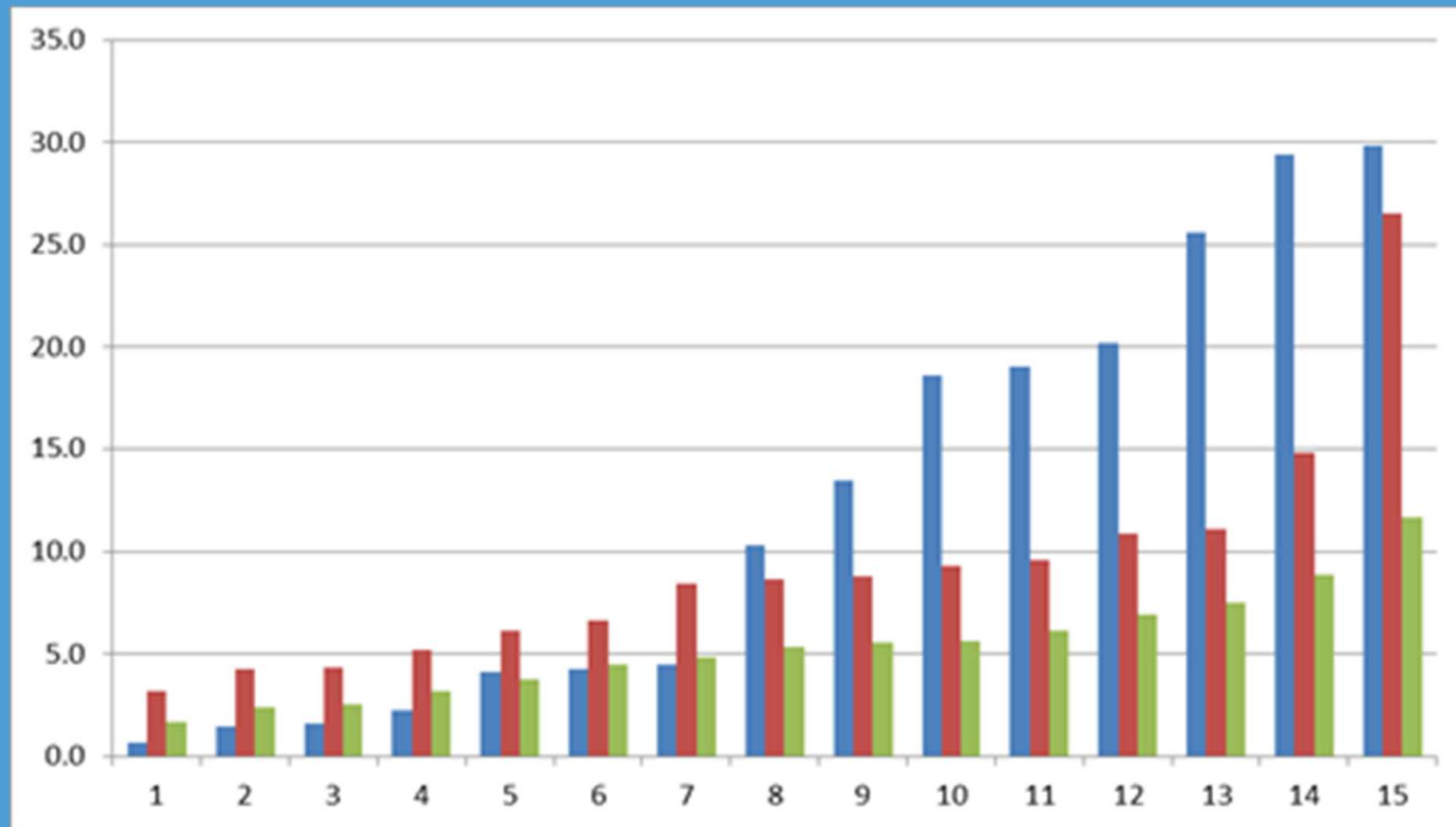
establish relation between screening and confirmatory method



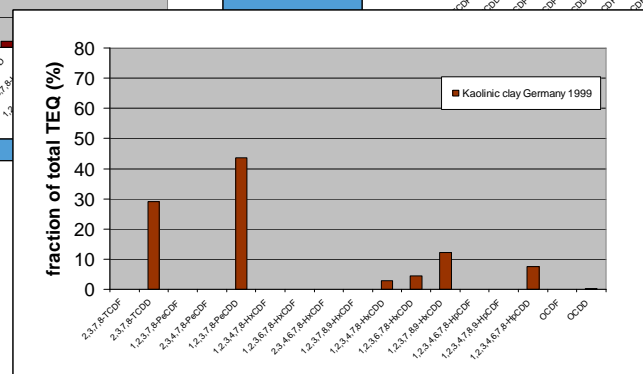
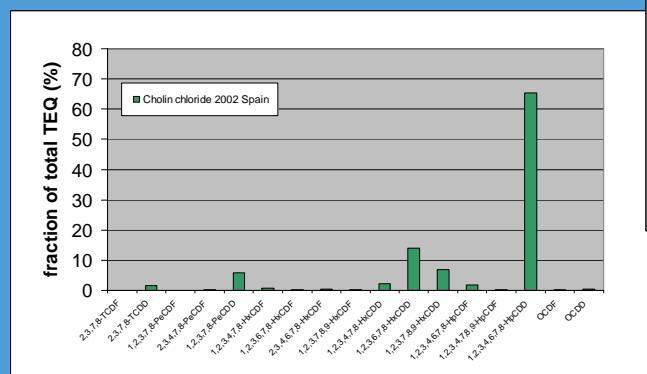
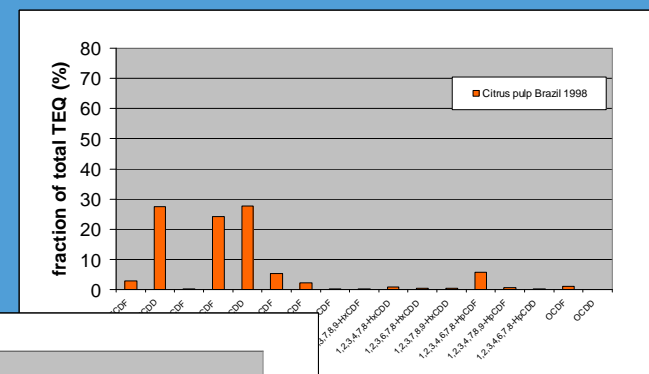
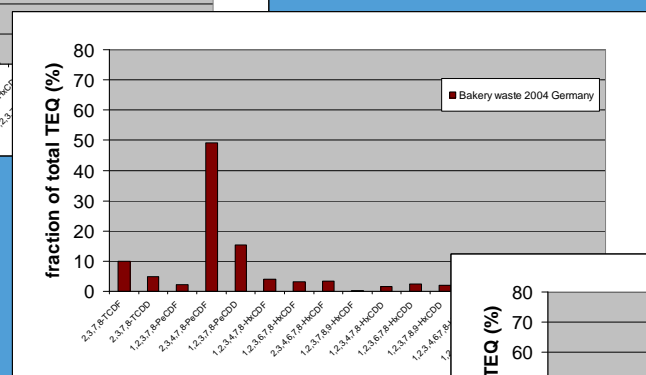
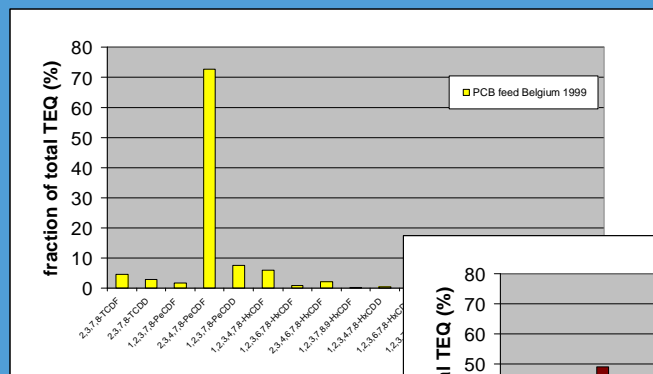
Choice for screening vs confirmation

- Both have advantages/disadvantages
- Choice depends on purpose analysis and number of samples
- Screening allows higher throughput (incidents)
 - Especially if most samples negative
- Confirmation gives a figure
 - But often below LOQ: $<LOQ$ or upperbound level
 - No clear advantage confirmation method

CALUX-analyses individual eggs



GC/HRMS: dioxin patterns typical for source



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Effect-based bioassays for screening



Chemical analysis and bioanalysis are complementary !!!

Detection of novel risks: unknown dioxin-like compounds



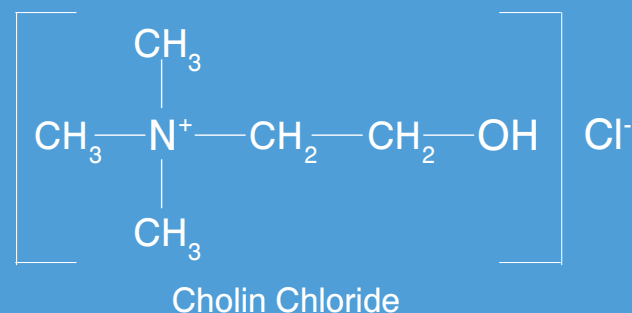
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Novel risks

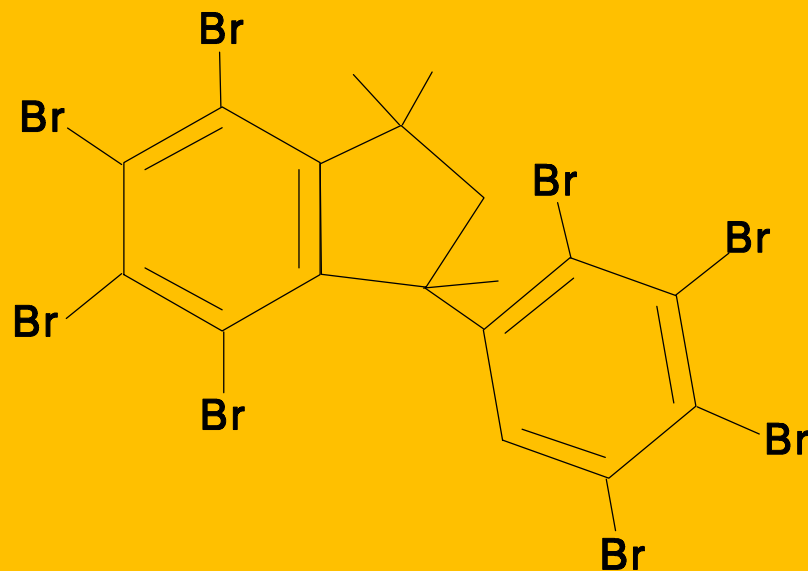
- Bioassays detect compounds based on their effects
- Therefore they may detect novel risks
- Also prevent misuse of unknown compounds with known effects (eg illegal hormones)

Cholin Chloride



- Feed additive (up to 1 g/kg)
- Positive test response in DR CALUX (different samples)
- Indicative level around 5 ng BEQ/kg
- GC/HRMS: dioxins and dioxin-like PCBs below LOQ
- Various flame retardants present, including tribromophenols
- But also **brominated dioxins**, considered equally toxic as chlorinated dioxins (but no limits or TEFs (yet))

FR-1808 (OBIND): new flame retardent



octabromo-1,3,3-trimethyl-1-phenylindan
FR-1808 (Mw 867.52)

How do flame retardants get into a feed additive?

Quality of results?

- Proper validation and accreditation
 - ISO 17025
 - Method is “fit for purpose”
- Demonstration of correct performance
 - Analysis of internal control samples
 - Participation in proficiency tests

EU performance criteria

- EC 2002/657
 - In particular for veterinary drugs and hormones
 - CRL 20/1/2010 specific for screening methods for veterinary drugs and hormones
 - SANCO 12495/2011, specific for pesticides
- EC 152/2009: various other ingredients and contaminants in animal feed
 - Including prescribed methods
 - Specific requirements for dioxins and PCBs in feed
- EC 401/2006: mycotoxins (food)

Performance criteria

- Apply for official control
 - Definition? Analysis for regulatory purposes?
- And for laboratories claiming to perform the method according to the Regulation (scope in accreditation)
- And in the field of fats for feed also for private laboratories (EC 225/2012)

Reference laboratories

- Various classes of residues, bacteria and contaminants
- For each class EURL (European Reference Laboratory) appointed
 - Existing national institutes or JRCs (Joined Research Centre)
 - Based on tenders and application
- Per class in each country at least one NRL (National Reference Laboratory)
- In addition OLs (Official Laboratories)

Some examples

- EURL dioxins and PCBs: CVUA Freiburg
- EURL mycotoxins: JRC Geel
- EURL heavy metals: JRC Geel
- EURL marine biotoxins: ASEAN Vigo
- EURL pesticides: 4 different EURLs
- EURL hormones: RIKILT Wageningen

Task EURL

- Described in EU Regulation 882/2004
- Tasks include
 - Organization workshops for NRLs (at least once a year)
 - Discussion on new developments in legislation
 - Discussion of methods
 - Support of EU authorities (DG SANCO)
 - Organization of PT-tests for NRLs (OLs)
 - Support of NRLs to improve methods
 - Support of NRLs in case of conflicts

Task NRLs

- Participation in EURL workshops
- Participation in PT-tests
- Support national authorities
- Support Official Laboratories
 - Exchange of samples
 - Advice on improving methods
 - Confirmation of conflicting results

LOQs (limit of quantification)

- Should be low enough to check for compliance
 - Often reporting limit just below maximum limit (50%)
 - Sample levels above 50% ML should be reanalyzed
- But would be good if they could detect background levels
 - Exposure assessment
 - Trend analysis

Questions?



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