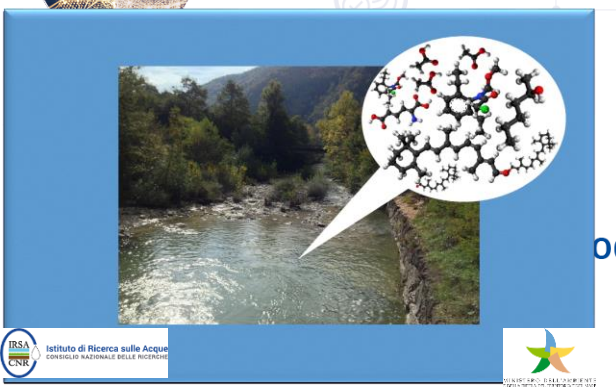
# A Food and feed Safety Perspective for harmonised risk assessment of combined exposure to multiple chemicals in the human health, animal health and ecological areas

**Jean Lou C M Dorne,**
**EFSA, SCER Unit, Parma, Italy**



**VALUTAZIONE DEGLI EFFETTI COMBINATI DELLE MISCELE DI SOSTANZE CHIMICHE**

***Digital Workshop***

25 giugno 2020

od

efsa
European Food Safety Authority

# Four pillars of Chemical Risk Assessment

## Risk assessment

– Fit for purpose

– Uses tiered approaches depending on data available, time and resources


**Identify toxic effects**

**Quantify toxic effects:**

- Dose response
- Reference Point
- Reference value : Safe levels

**Occurrence x Consumption**

**Hazard vs Exposure: Risk**

# The "Cocktail Effect"

Limoncello

\+

Bargnolino

\+

Nocino

Hazardous mixture

Be Careful With This One !

=

# MIXTOX GD

GUIDANCE

EFSA Journal

ADOPTED: 20 February 2019

doi: 10.2903/j.efsa.2019.5634

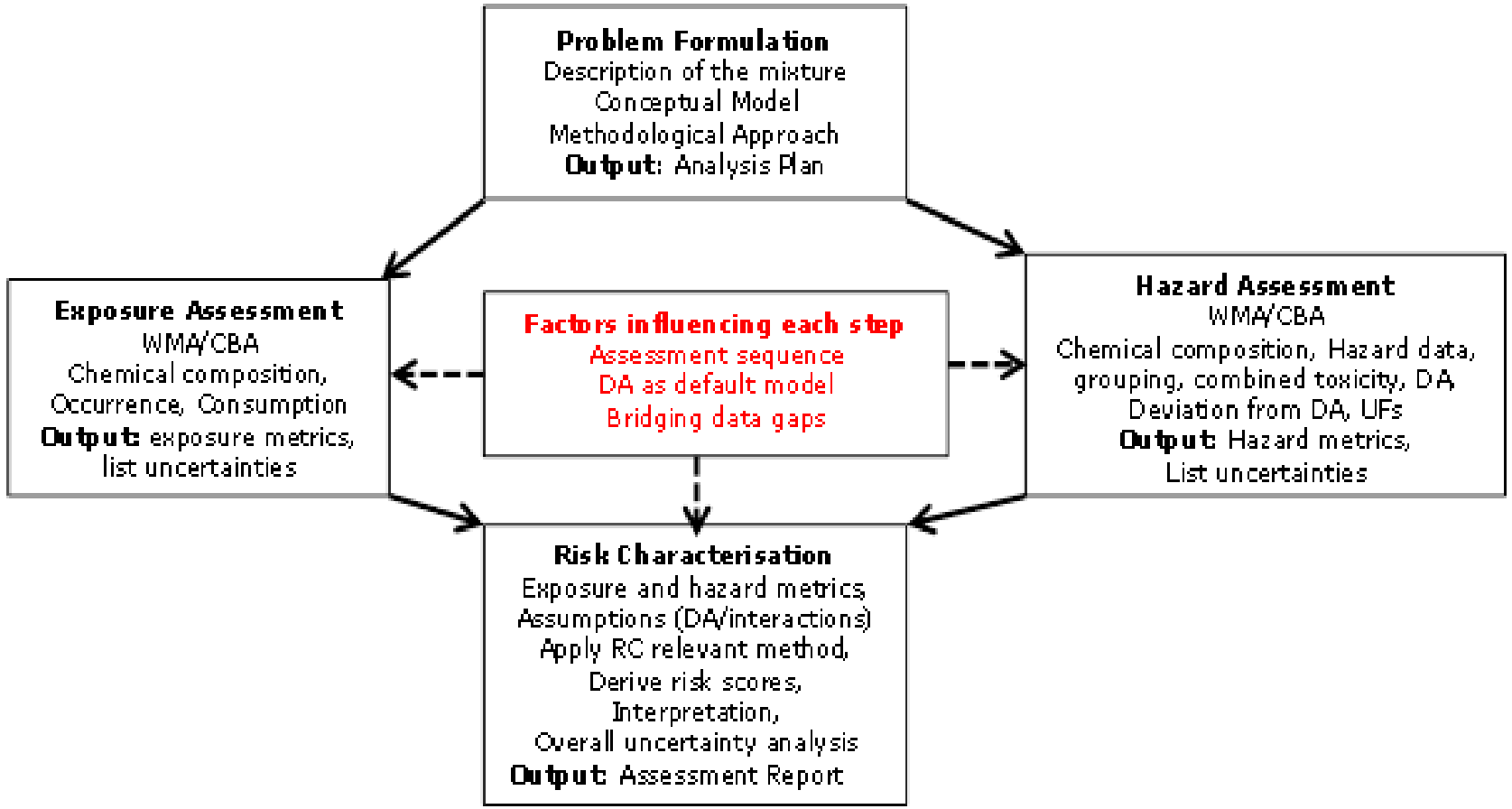
## Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee,
Simon John More, Vasileios Bampidis, Diane Benford, Susanne Hougaard Bennekou, Claude Bragard, Thorhallur Ingi Halldorsson, Antonio F Hernández-Jerez, Konstantinos Koutsoumanis, Hanspeter Naegeli, Josef R Schlatter, Vittorio Silano, Søren Saxmose Nielsen, Dieter Schrenk, Dominique Turck, Maged Younes, Emilio Benfenati, Laurence Castle, Nina Cedergreen, Anthony Hardy, Ryszard Laskowski, Jean Charles Leblanc, Andreas Kortenkamp, Ad Ragas, Leo Posthuma, Claus Svendsen, Roland Solecki, Emanuela Testai, Bruno Dujardin, George EN Kass, Paola Manini, Maryam Zare Jeddi, Jean-Lou CM Dorne and Christer Hogstrand

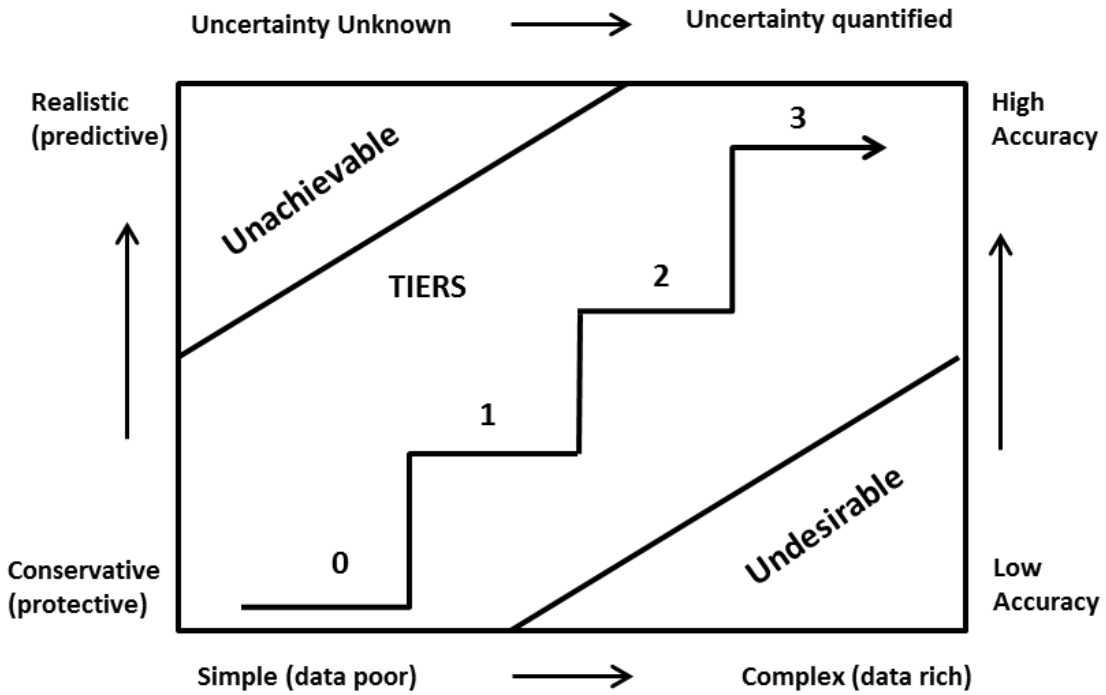
# Content of Guidance

| Chapter | General Considerations | Whole mixture | Component-based | Tiering Principles | Stepwise Guidance |
|---|---|---|---|---|---|
| Problem Formulation | ✓ | ✓ | ✓ | ✓ | ✓ |
| Exposure Assessment | ✓ | ✓ | ✓ | ✓ | ✓ |
| Hazard Assessment | ✓ | ✓ | ✓ | ✓ | ✓ |
| Risk Characterisation Uncertainty | ✓ | ✓ | ✓ | ✓ | ✓ |
| Reporting table | | ✓ | ✓ | ✓ | ✓ |
| Case studies<br>Humans ( RA contaminants)<br>Animals ( RA essential oils)<br>Bees (Hazard Synergy Pesticides) | | | ✓ | ✓ | ✓ |

# Harmonised Framework



# Tiering Principles




**Relationships between tiers, data availability, uncertainty, accuracy and outcome of a risk assessment.**
**From: Solomon et al. (2006).**

# PROBLEM FORMULATION

**Risk Assessment Question**
Human/Sub-population(s)
Farm/Companion Animals
Environmental Specie(s)
Ecosystem(s)

**Step 1 : Description of the mixture**
Characterisation of the composition
Data availability for components or whole mixture
Is co-exposure and/or co-effect Likely ?
(if No then stop)

**Step 2 : Conceptual Model**
Question/Terms of reference
Source of the chemicals, exposure pathways
Species/sub-population
Regulatory framework
Other ?

**Step 3 : Methodological Approach**
Overview of available data
Whole mixture approach, component based approach or both
Assessment group
Other ?

**Step 4 : Analysis Plan**

**Proceed with Risk Assessment**

**Update/ Modify : Iterative manner**

# EXPOSURE ASSESSMENT

| | Occurrence data | Exposure estimate | Consumption data |
|---|---|---|---|
| Tier 0 | Default values, permitted levels | Semi-quantitative point estimates | Default values, portion sizes |
| Tier 1 | Modelled and experimental data | Deterministic | Food balance sheet food basket |
| Tier 2 | Monitoring Surveys | Semi-probabilistic | Summary statistics |
| Tier 3 | Individual co-occurrence data | Probabilistic | Individual data |

Note: Occurrence and consumption tiers often do not match. The resulting exposure tier will be determined by the available data including for the occurrence of different components of a mixture

# HAZARD ASSESSMENT-COMPONENT-BASED APPROACH

**Hazard Identification**
**Hazard characterisation**
Human/Sub-population(s)
Farm/Companion Animals
Environmental Specie(s)
Ecosystem(s)

**Step 1: Confirm components of Assessment Group**
Criteria for grouping (hazard, exposure etc.)

**Step 2: Collect available hazard information**
e.g. Toxicity data, reference points, reference values, mode of action, toxicokinetic information, potency information. Identify the relevant entry tier for the assessment.

**Step 3: Evidence for combined toxicity**
Assess evidence for combined toxicity, including potential deviation from dose addition. Identify the most appropriate tool for risk characterisation

**Step 4: Hazard characterisation**
Derive reference points f and reference values for each component of the assessment group, identify appropriate uncertainty factors using relevant tier.

**Step 5: Summarise Hazard characterisation for each component of AG**
List assumptions (potency, dose addition, interaction)
List uncertainties

**Go to risk characterisation**

# RISK CHARACTERISATION

**Risk characterisation**
Human/Sub-population(s)
Farm/Companion Animals
Environmental Specie(s)
Ecosystem(s)

**Step 1. Summary Exposure and Hazard metrics**
Exposure and Hazard information: WMA/CBA
Decision points from analysis plan
Assumptions (dose addition, interaction)

**Step 2. Confirm/Revise approach for risk characterisation**
Start with a fit for purpose methodology based on problem formulation and available data and (hazard index, margin of exposure etc).

**Step 3. Summarise risk characterisation results**
Associated assumptions (exposure, potency, DA, Interaction)
List uncertainties.

**Step 4. Interpretation of risk characterisation metric**
Combined risk is acceptable or not (e.g. hazard index >1)

**Discuss with risk managers**
**Stop or refine to higher tier**

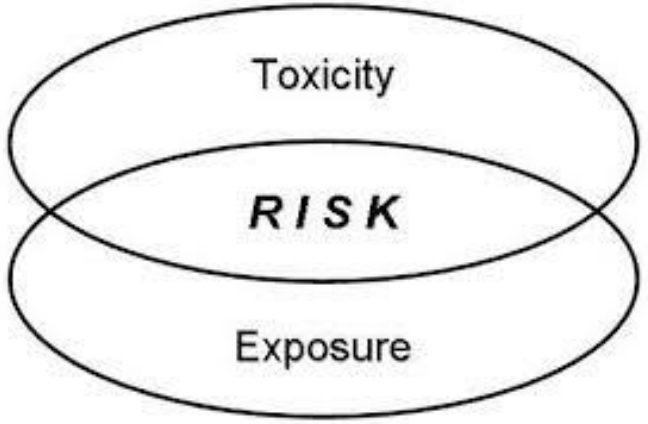
**Refine**

# REPORTING SUMMARY

| | | |
|---|---|---|
| **Problem formulation** | ***Description of the mixture*** | Simple or complex mixture, Composition, Data availability for components or whole mixture |
| | ***Conceptual Model*** | Question/Terms of reference, Source, exposure pathways, Species/sub-population, Regulatory framework, Other ? |
| | ***Methodology*** | Overview of available data whole mixture or component based approach or a mixture of the two.<br>Assessment group, Other ? |
| | ***Analysis Plan*** | |
| **Exposure assessment** | ***Characterisation Whole Mixture Components Assessment group*** | |
| | ***Summary Occurrence (concentration) data*** | |
| | ***Summary exposure*** | Assumptions, Exposure metrics |
| **Hazard Assessment** | ***Mixture Composition WMA/CBA*** | |
| | ***Reference points Reference values*** | |
| | ***Summary Hazard metrics*** | Assumptions combined toxicity (DA, RA), hazard metrics |
| | | Uncertainties |
| **Risk Characterisation** | ***Summary Exposure and hazard metrics*** | |
| | ***Risk characterisation Approach*** | |
| | ***Summary Risk Metrics*** | Associated Assumptions (DA, RA, interactions), Risk metrics |
| | | Uncertainties |

# Relevant questions: Current examples@EFSA

**Human Health**
**Pesticides**
**Contaminants**




**Animal Health**
**Essential oils in feed**

**Ecological Risk Assessment**
**MUST-B : Multiple Stressors in bees**

# IMPLEMENTATION OF MIXTOX IN PRACTICE

TECHNICAL REPORT

APPROVED: 10 December 2019

doi:10.2903/sp.efsa.2020.EN-1759

## Human risk assessment of multiple chemicals using component-based approaches: A horizontal perspective

European Food Safety Authority (EFSA),

Jean Lou CM Dorne, Amélie Crépet, Jan Dirk te Biesebeek, Kyriaki Machera, and Christer Hogstrand

TECHNICAL REPORT

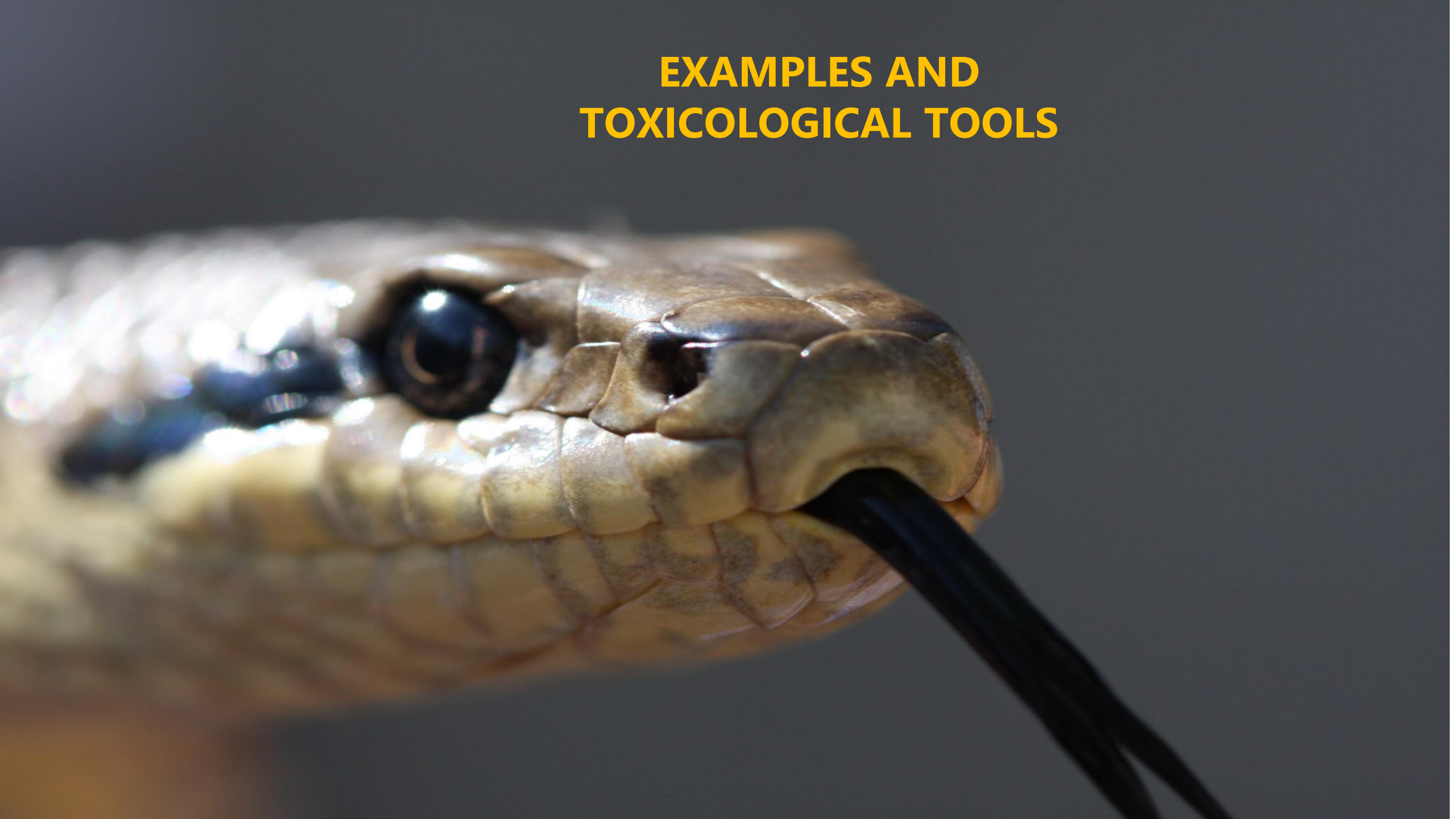
APPROVED: 10 December 2019

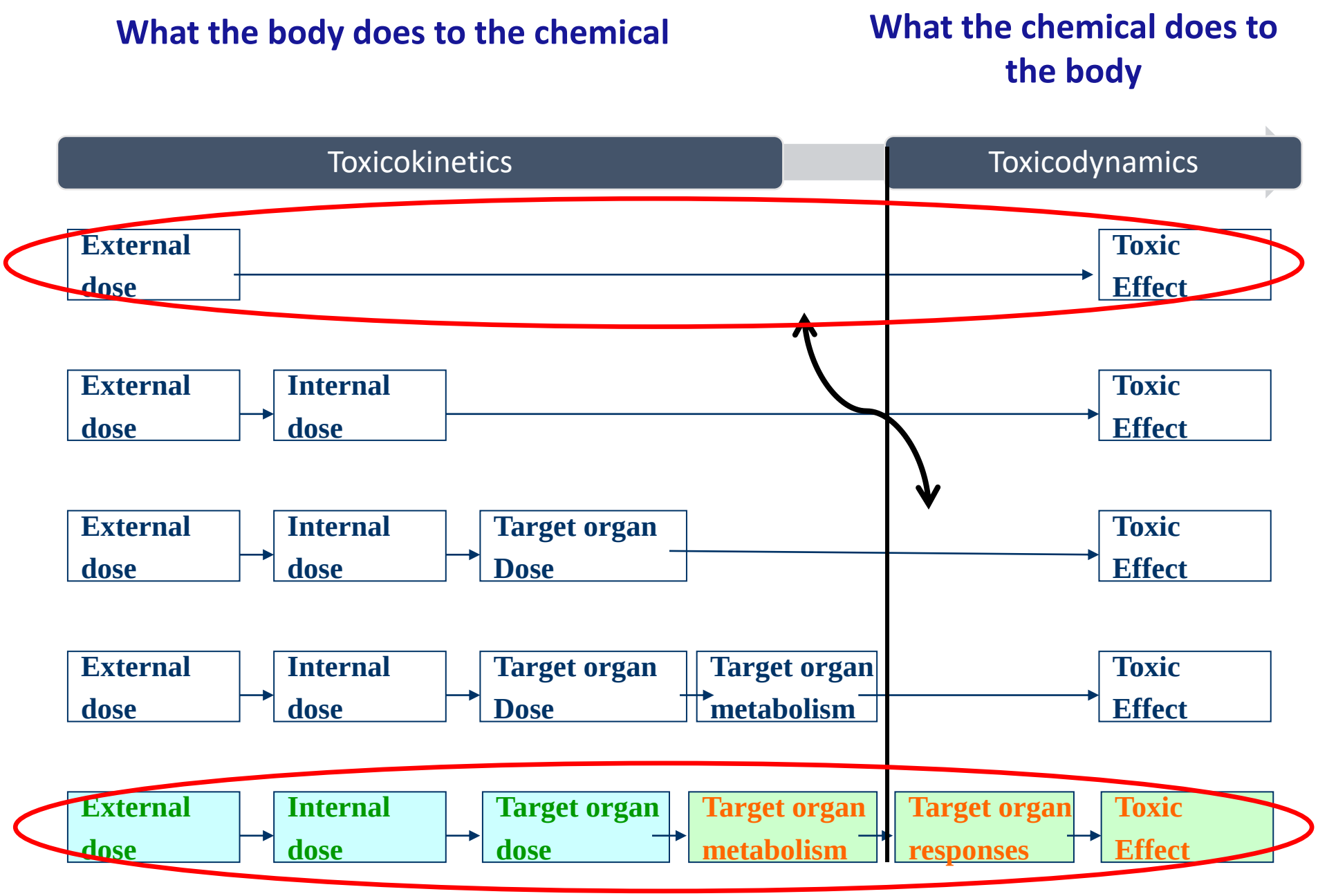
doi:10.2903/sp.efsa.2020.EN-1760

## Animal Health Risk assessment of multiple chemicals in essential oils for farm animals

European Food Safety Authority (EFSA),
Jean Lou CM Dorne, Paola Manini and Christer Hogstrand

# EXAMPLES AND TOXICOLOGICAL TOOLS




What the body does to the chemical
What the chemical does to the body
Toxicokinetics
Toxicodynamics
External dose
Toxic Effect
External dose
Internal dose
Toxic Effect
External dose
Internal dose
Target organ Dose
Toxic Effect
External dose
Internal dose
Target organ Dose
Target organ metabolism
Toxic Effect
External dose
Internal dose
Target organ dose
Target organ metabolism
Target organ responses
Toxic Effect

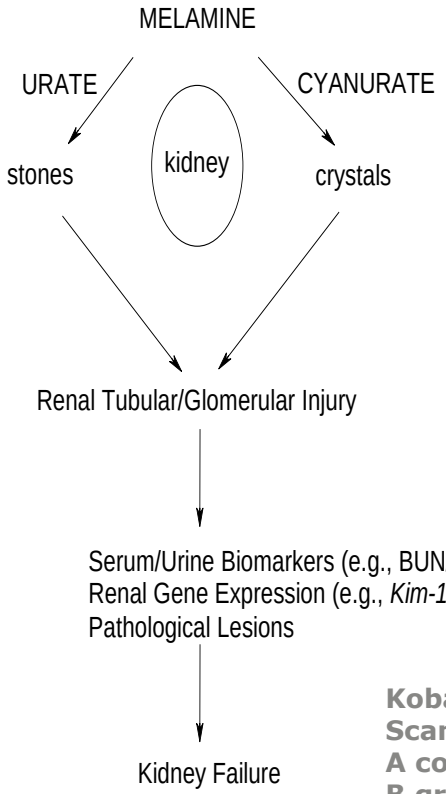
# Food Fraud: The melamine case study

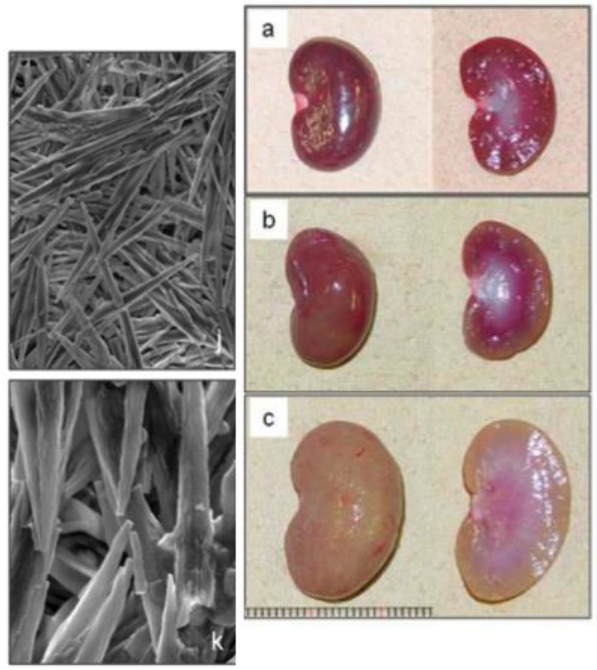
# MELAMINE: WHAT HAPPENED ?

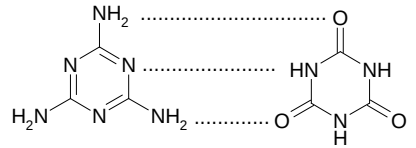
- Melamine acutely toxic: **complex with uric acid** kidney toxicity
- Used for manufacture of plastics/ approved as food contact material- Deliberate addition to food not allowed. Used illegally to increase apparent content of protein.
- 2007 US crisis: pet food imported from China adulterated with scrap melamine (melamine/cyanuric acid) 1000s of pets/animals with severe health effects.
- September 2008: app. 500,000 cases of infants/children hospitalised with kidney stones, reported deaths from adulterated milk powder used for infant formula.
- 2009-2010 RA: WHO and EFSA Tolerable Daily Intake **0.2 mg/kg b.w per day** melamine alone for humans

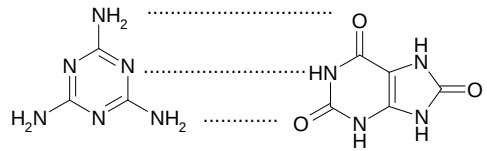
# Mode of action of Melamine-Cyanuric Acid toxicity

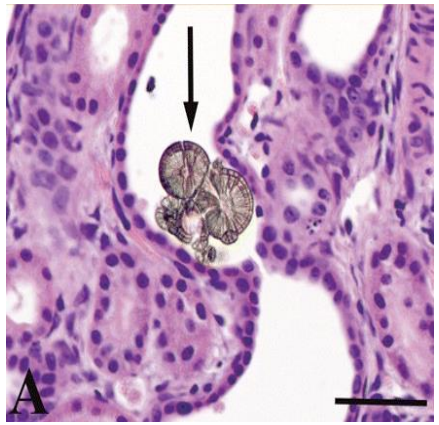
## Melamine and cyanuric acid Synergistic toxicity : Covalent complex




**Kobayashi et al. (2010)**
**Scanning electron microscope x 7000 and x 14000**
**A control group day 7**
**B group m 24 mg/kg/day at 7 reddish brown, smooth surfaces**
**C small irregularities on surface yellow - significant changes in weight**

Toxicology and Applied Pharmacology 370 (2019) 184–195

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

# Investigating the interaction between melamine and cyanuric acid using a Physiologically-Based Toxicokinetic model in rainbow trout

Cleo Tebby[a], Céline Brochot[a], Jean-Lou Dorne[b], Rémy Beaudouin[a,c,*]

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[b] European Food Safety Authority (EFSA), Scientific Committee and Emerging Risks Unit, Via Carlo Magno 1A, 43126 Parma, Italy

[c] Institut National de l'Environnement Industriel et des Risques (INERIS), UMR-I 02 SEBIO, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France

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***Digital Workshop***

25 Giugno 2020

Archives of Toxicology
https://doi.org/10.1007/s00204-018-2325-6

TOXICOGENOMICS

# The Yin–Yang of CYP3A4: a Bayesian meta-analysis to quantify inhibition and induction of CYP3A4 metabolism in humans and refine uncertainty factors for mixture risk assessment

Nadia Quignot[1] · Witold Wiecek[2] · Billy Amzal[1] · Jean-Lou Dorne[3]

**VALUTAZIONE DEGLI EFFETTI COMBINATI DELLE MISCELE DI SOSTANZE CHIMICHE**

***Digital Workshop***

25 Giugno 2020

# MYCOTOXIN MIXTURES AND CLIMATE CHANGE – 2017-2019


APPROVED: 4 December 2019
doi: 10.2903/sp.efsa.2020.EN-1757

**Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach:**

**MYCHIF**

Author(s)

Paola Battilani, Roberta Palumbo, Paola Giorni, Chiara Dall'Asta, Luca Dellafiora, Athanasios Gkrillas, Piero Toscano, Alfonso Crisci, Carlo Brera, Barbara De Santis, Rosaria Rosanna Cammarano, Maurella Della Seta, Katrina Campbell, Chris Elliot, Armando Venancio, Nelson Lima, Ana Gonçalves, Chloe Terciolo, Isabelle P Oswald

- Environmental variables and their impact on mycotoxin production
- Influence of climate change, temperature, season, pests, nutrient Comparative toxicity and kinetics in farm animals and humans
- Occurrence data in Cereals (Maize, rice, wheat etc…)
- Risk characterisation using Relative potency factors and Margin of exposure

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

# Investigating combined toxicity of binary mixtures in bees: Meta-analysis of laboratory tests, modelling, mechanistic basis and implications for risk assessment

Edoardo Carnesecchi[a,b], Claus Svendsen[c], Stefano Lasagni[d], Audrey Grech[e], Nadia Quignot[f], Billy Amzal[f], Cosimo Toma[b], Simone Tosi[g], Agnes Rortais[h], Jose Cortinas-Abrahantes[h], Ettore Capri[i], Nynke Kramer[a], Emilio Benfenati[b], David Spurgeon[c], Gilles Guillot[j], Jean Lou Christian Michel Dorne[h,k,*]

## Acute contact Toxicity

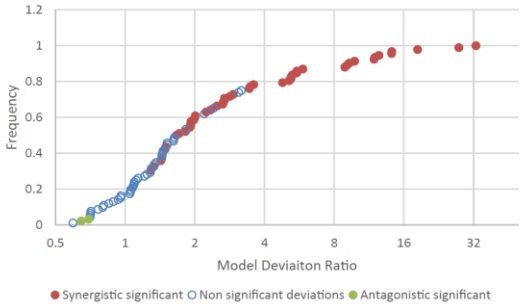
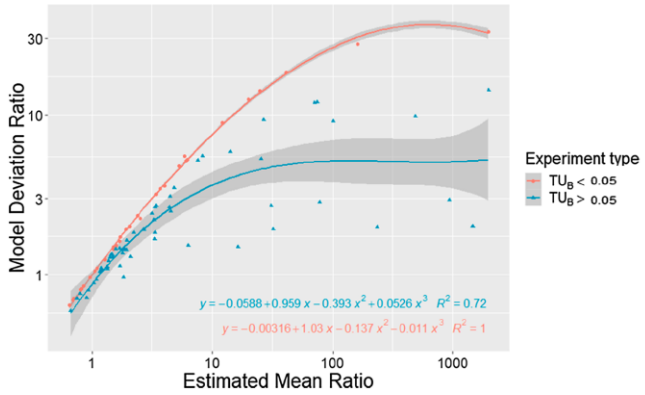
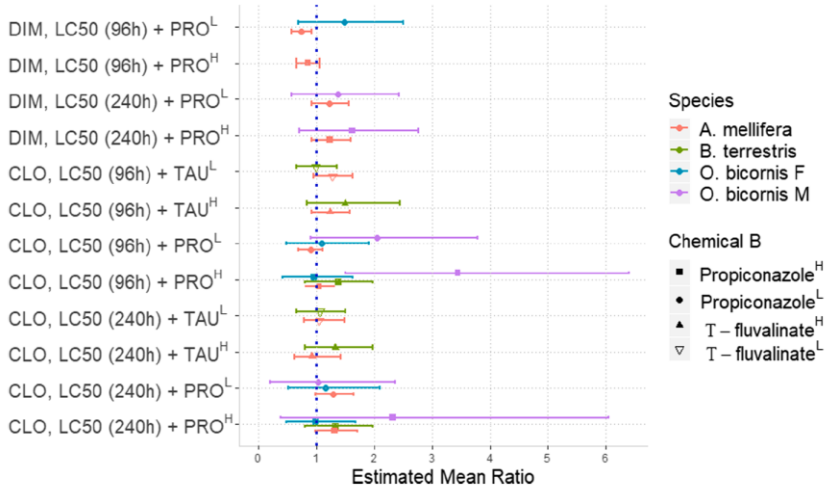



**Fig. 11.** Cumulated frequency of Model Deviation Ratio. MDR for statistically significant studies resulting from the meta-analysis of acute contact toxicity studies on honey bees (Iwasa et al., 2004; Johnson et al., 2013, 2006, 2009; Ellis et al., 1997). MDR > 1.25 represents "synergistic" interactions, 0.83 < MDR < 1.25 represents "additive" effects; MDR < 0.83 represents "antagonistic" interactions.




## Chronic oral Toxicity



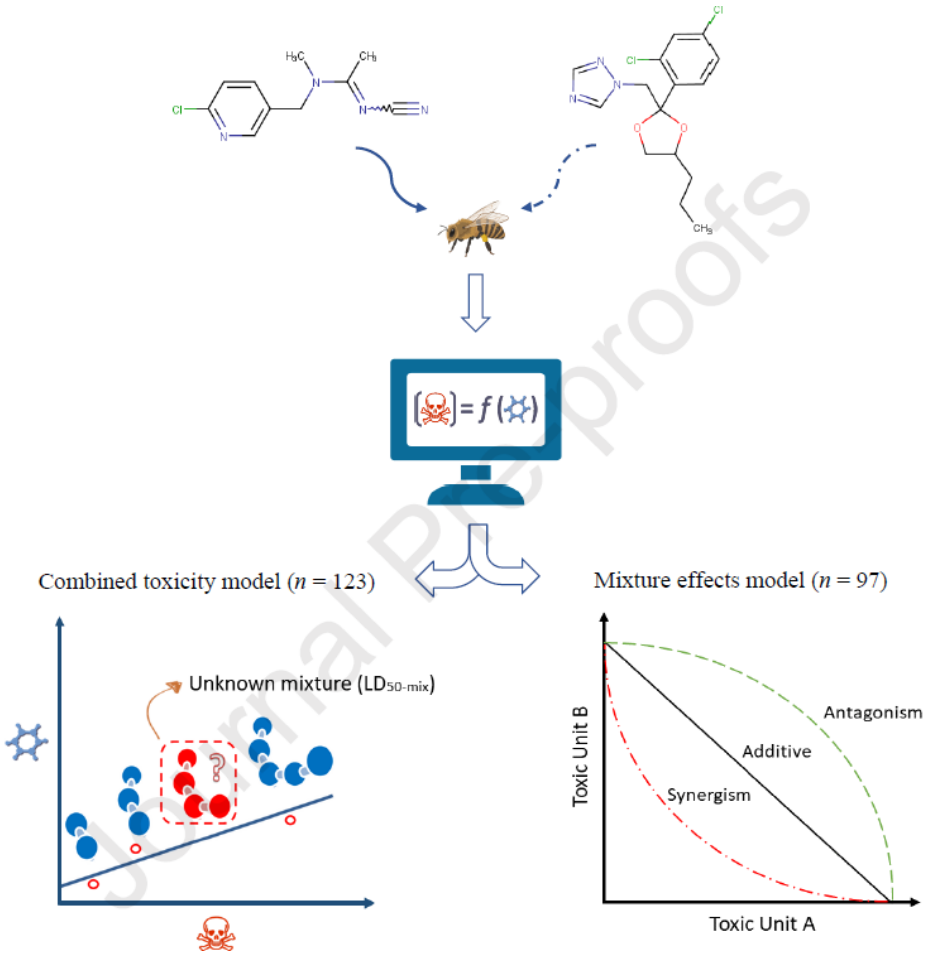

- **Interactions/Synergism in bees**

Mostly due to inhibition of CYP metabolism

- **From limited data (oral) magnitude interactions acute contact toxicity > acute oral/chronic oral**
- **Few acute oral and chronic oral tox data**
- **Addressing (co)-exposure dimension**
- **Mortality as common metrics for risk characterisation (starting point)**

- **QSAR models predicting the nature of combined toxicity and binary mixture toxicity**

**(Acute contact toxicity)**






**Predicting acute contact toxicity of organic binary mixtures in honey bees (*A. mellifera*) through innovative QSAR models**

Edoardo Carnesecchi [a,b,*], Andrey A. Toropov [a], Alla P. Toropova [a], Nynke Kramer [b], Claus Svendsen [c], Jean Lou Dorne [d], Emilio Benfenati [a]

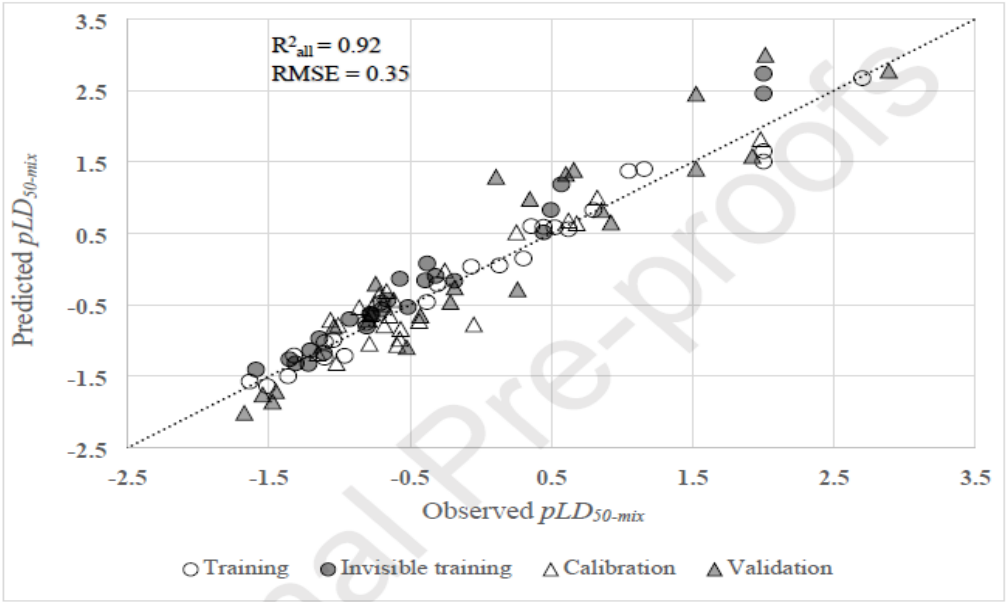



*Figure 3. Observed versus predicted log [1/$LD_{50\text{-}mix}$] of binary mixtures for the regression-based model (Approach B), split 1 "best split". $R^2_{all}$ (determination coefficient) and RMSE (root mean squared error) are provided for all compounds (i.e. compounds from training, invisible training, calibration, and validation sets).*

# Other models to combine toxicity data for RA

- **Response addition**

✓ Not used very often in human RA since evidence limited
✓ Sometimes used in ecological RA for mortality data
✓ Dose addition is the consensus model around the world

- **Interactions**

✓ MIXTOX GD set basis for assessing TK/TD interactions
✓ **Synergy**: **very limited evidence in human RA** : low levels in food. **Evidence in eco RA** for mortality data (bees) most often TK (inhibition metabolism)
✓ Models for binary mixtures

✓ **Antagonism ?** Lots of evidence from food detoxifying chemicals (e.g. cabbage, garlic etc.)

Risk Benefit ? NDA Remit (novel food/health claims)

# Tools for TK TD MODELLING

- **Models in development at EFSA**
  - ➢ Generic models for humans, farm animals and Eco submitted to OECD PBPK GD
  - ➢ Platform under construction for models: TKplate (2020)
  - ➢ Link with NOAEL/BMDL based on internal dose
  - ➢ Future link AOP/MoA: *in vitro in vivo* extrapolation
  - ➢ TKTD Modelling for mortality in ecotox (DEB)
  - ➢ Further models + update launch June 2020
- **Refining mixture RA using internal dose** for each compound and applying dose addition is feasible
- **EUROMIX Toolbox: set of useful models**

but tox only liver and neurodevelopmental

# MIXTOX2 : Scientific criteria for grouping chemicals

- **Focus on Human Health**
- On going mandate on **scientific criteria for setting assessment groups** (due Dec 2020 for public consultation )

➢ Consider

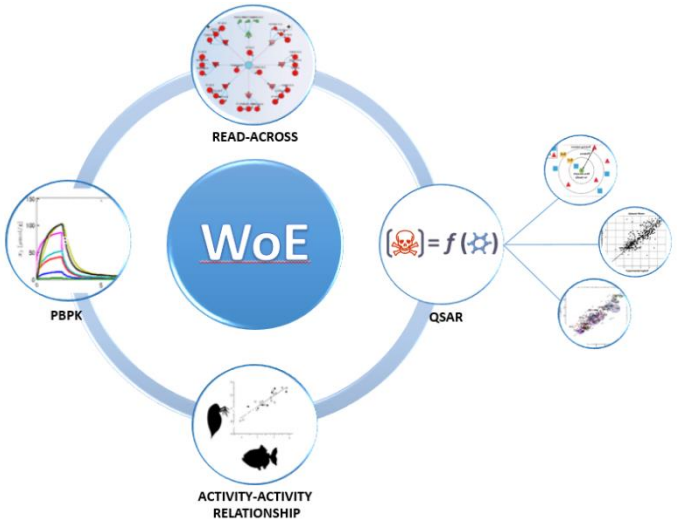
- **Toxicological effects (MoA/AOP/Target organ etc..)**
- **Mechanistic data AOP MoA** (human relevance)
- **TK aspects** (persistence, correct for body burden, CONTAM)
- **Exposure** and **risk-based tools** for prioritisation
- **WoE Methodology**
- **International activities** (WHO, OECD, US-EPA etc)

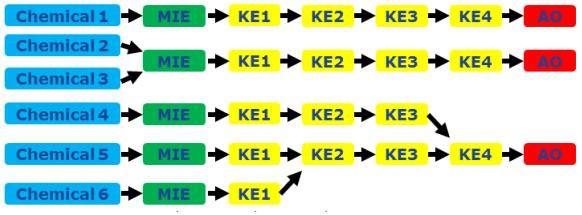
# Conclusion :Integrating State Of The Art Methods And Data Streams

Exposure → Dosimetry → Reference Point → Risk

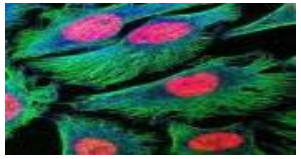
*OpenFoodTox and MATRIX*

*Epidemiological Data*

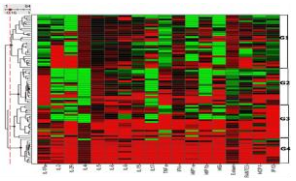


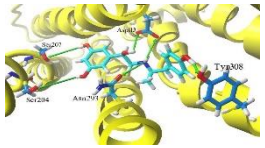

*AOP/MoA*



*In vitro*



*OMICs*



*In Silico*

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**Do you have questions?**

**Jean-Lou.DORNE@efsa.europa.eu**