Connecting Exposure and Toxicokinetics and Toxicity: Towards Open Source Tools in Food Safety

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OUTLINE

- EFSA’s Role in Food Safety
- Principles of Chemical Risk Assessment
- OpenFoodTox: EFSA’s chemical Hazards Database
- Open Source Tools
- Conclusions
ARE PESTICIDES SAFE?

I DUNNO, BUT IT SURE MAKES THE WORMS TASTE FUNNY!
EFSA’s Role in Food Safety

Methodology Codex Alimentarius:
- Hazard identification
- Hazard characterisation
- Exposure assessment
- Risk characterisation

**Scientific Risk Assessment**

**Risk Management**

- Independent Risk Assessment
- Risk communication

**EFSA**

- Risk Manager
  - EC
  - EU Parliament
  - Member States
  - Council
FROM QUESTION TO ANSWER

European Commission
European Parliament
Member States
EFSA ("self mandate")

Question?

Terms of reference
Background
Opinion
Risk Assessment

Consumers
Media
Industry
Professionals

Risk Communication

Basic Principles in Chemical RA
Key Chemicals assessed in EFSA

- Contaminants
- Pesticides
- Vitamins and minerals
- Food additives and nutrient sources
- Feed Additives
- Food contact materials, Enzymes
- Flavourings and processing aids
- Proteins used in GMOs
Principles of chemical risk assessment
STEPS IN CHEMICAL RISK ASSESSMENT

Exposure assessment

Occurrence of chemicals in food, feed, water, environmental media

Food consumption

Deterministic vs probabilistic

Hazard Identification

Hazard Characterisation

Toxicokinetics (ADME)
Toxicity: Genotox, acute/sub-chronic/chronic toxicity, NOELs, BMDL, LOAELs (animal, human)
NOEC, PNEcs (Ecology..)
Health based Guidance Value (ADI, TDI...)

Risk Characterisation

Exposure and Hazard
Humans: Health-based guidance values vs exposure
Margin of Exposure (genotox carcinogens)
Margin of safety (animal)
Environmental standards (ecological),
“All things are toxic and there is nothing without poisonous qualities: it is only the dose which makes something a poison”

PARACELSUS (1493-1541)

**Toxicology**
What the body does to a chemical and what a chemical does to the body

**Toxicokinetics**
What the body does to a chemical
How the chemical is eliminated from the body or activated into a toxic species (ADME)

**Toxicodynamics**
What a chemical does to the body
How the chemical exerts its toxicity target receptor/cell/organ
Margin Of exposure (MOE) developed, by the JECFA and EFSA (2005) Point of reference on the dose-response curve* (based on animal and human data) divided by the estimated human intakes. MOE (animal data) >10,000 as of low concern for public health.

BMD/BMDL: Benchmark dose/ limit or NOAEL: No observed-Adverse-Effect-Level)
Aflatoxins

- AFB1 is one of most potent mutagens known, hence considered here
- AFB1 *genotoxic and carcinogenic* in animals and humans causing principally liver tumors i.e. hepatocellular carcinoma (HCC).
- Other risk factors of HCC: chronic infection e.g. hepatitis B (HBV).
- **CONTAM Panel considered liver carcinogenicity as pivotal effect for risk assessment (EFSA, 2008)**
CONTAM Panel applied dose-response modeling for AFB1 using animal and human data with conservative approach: carcinogenic potency of total AF similar to AFB1.

BMDL10 for AFB1 of 170 ng/kg b.w. per day with Fisher rat data the most sensitive species for liver carcinogenicity.

Benchmark dose modelling (BMD)

![Benchmark dose modelling graph](chart)

- 10% Response = BMR
- BMDL10
- BMD10
- Model fitted to data points
- Lower 95% confidence interval on dose giving a 10% response
• Several epidemiological cohort studies (Africa, China) on the intake of AF and risk related to liver cancer exist.

• CONTAM Panel used the Yeh *et al.* (1989) study, used in quantitative risk assessments (Joint FAO/WHO Expert Committee on Food Additives). Limitations of this study

• CONTAM Panel calculated for AFB1: $\text{BMDL}_{10}$ value of 870 ng/kg b.w. per day based on single study in China and a $\text{BMDL}_1$ value of 78 ng/kg b.w. per day based on three studies from Africa.
GUIDANCE

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Update: use of the benchmark dose approach in risk assessment

EFSA Scientific Committee,
Anthony Hardy, Diane Benford, Thorhallur Halldorsson, Michael Jöbstl,
Katrine Helle Knutsen, Simon More, Alicja Mortensen, Hanspeter Naegeli,
Colin Ockleford, Antonia Ricci, Guido Rychen, Vittorio Silano, Roland Solecky,
Marc Aerts, Laurent Bodin, Allen Davis, Lutz Edler, Ursula Gundert-Remy,
Wout Slob, Bernard Bottex, Jose Cortiñas Abrahantes, Daniele Courcot,
George Kass and Josef R. Schlatter
BMD approach in risk assessment
Figure of fitted model

Averaged response model

`Average model' from model averaging analysis of the observed incidences of an epithelial cell vacuolisation. The average model was constructed via averaging all results at a finite set of points (i.e., doses) in order to generate curve. The EFSA BN (development) was used.

If model averaging software was not available, the plots of the recommended were shown:
IN A NUTSHELL...

Exposure assessment

Occurrence data (Concentration in food)

Food consumption

Probabilistic / Deterministic Exposure estimates

Uncertainty Factor

Risk Characterisation

Hazard Assessment

Toxicity

Chronic

Acute

Toxicokinetics / Toxicodynamics

Benchmark Dose/ NOAEL

Health-based guidance value

MOE

ADI / TDI

ARfD

Dorne et al, 2009- Trends in Analytical Chemistry
OpenFoodTox: EFSA’s Open Source Hazards Database
EDITORIAL

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Editorial: OpenFoodTox: EFSA’s open source toxicological database on chemical hazards in food and feed

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Since its inception in 2002, the European Food safety Authority (EFSA) has produced risk assessments for more than 4,400 substances in over 1,650 Scientific Opinions, Statements and Conclusions. The work of its Scientific Panels, Units and Scientific Committee. For each individual substance, a summary of human health, animal health and ecological hazard assessments has been collected and structured into EFSA’s Chemical Hazards Database: OpenFoodTox. OpenFoodTox provides open data for substance characterisation, links to the relevant EFSA output, background regulatory context of critical toxicological endpoints. According to Scientific Advice, it identifies data deficiencies.

openfoodtox170118.jpg
OPENFOODTOX: EFSA' S CHEMICAL HAZARDS DATABASE

- **Catalogue of EFSA’s chemical toxicity data since creation**
  - Contaminants (Human and Animal health)
  - Vitamins and minerals (Human health) (NDA),
  - Food additives and Nutrient Sources, Food contact materials, Flavourings and processing aids (Human Health)
  - Feed Additives (Human and Animal Health, Ecotoxicology)
  - Pesticides (Human and Animal health, Ecotoxicology)

- **Easy Reference and Crisis**
  - One reference DB Chemical Hazards: Search easily and efficiently
  - Crisis: Quick and Easy access to all EFSA’s Hazard Data

- **International Harmonisation**
  - Use OECD Harmonised Templates (OHT) for data model (ECHA/OECD) compatible with IUCLID/ ECHA-OECD QSAR toolbox
  - Search compounds by name, CAS number on e-chem portal
  - Generate data sheet as summary of hazard id and charact (June 2016)
WHAT DOES OPENFOODTOX CONTAIN?

- **Chemical Information**
  Information on chemical nomenclature (EU nomenclature, IUPAC, CAS…), trade name, chemical group/panel (i.e. pesticide), chemical use (i.e fungicide), chemical structure (i.e triazoles, organophosphates…).

- **Document descriptors**
  Information on EFSA’s opinion for the specific chemical or group of chemicals. Info from EFSA ‘s RAW system (question number, mandate, number), link to the document

- **Toxicity Endpoint/ Hazard identification**
  Information on critical toxicity study using OECD picklists when possible (species, dose, target organ…)

- **Critical study to demonstrate genotoxicity status**
  Providing essential information of critical genotoxicity study when assessed

- **Hazard /Risk characterisation**
  Information for health based guidance values (ADI/TDI) uncertainty factors...
1,650 Scientific outputs (metadata + DOI)

10,000 Toxicological endpoint studies

12,000 risk assessment summaries

4,400 Substances (chemical identifiers including SMILES)

OPENFOODTOX
Explore Case studies to develop *in silico* tools e.g. QSAR

- Explore use of metabolism and TK data and tox

- Combined toxicity mixtures (dose addition assumption/synergistic etc...)

- Tools for RA mixtures with little data (e.g. emerging contaminants – new mycotoxins etc.)

Link to OPENFOODTOX
Predicting acute contact toxicity of pesticides in honeybees (Apis mellifera) through a k-nearest neighbor model

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HIGHLIGHTS

- A model to predict acute contact toxicity for bees was built for screening pesticides.
SCIENTIFIC REPORT OF EFSA

Modern methodologies and tools for human hazard assessment of chemicals

European Food Safety Authority

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 11 July 2014, replaces the earlier version published on 24 April 2014*

ABSTRACT

This scientific report provides a review of modern methodologies and tools to depict toxicokinetic and toxicodynamic processes and their application for the human hazard assessment of chemicals. The application of these methods is illustrated with examples drawn from the literature and international efforts in the field. First, the concepts of mode of action/adverse outcome pathway are discussed together with their associated terminology and recent international developments dealing with human hazard assessment of chemicals. Then modern methodologies and tools are presented including in vitro systems, physiologically-based models, in silico tools and OMICs technologies at the level of DNA/RNA (transcriptomics), proteins (proteomics) and the whole metabolome (metabolomics). Future perspectives for the potential applications of these modern methodologies and tools in the context of prioritisation of chemicals, integrated test strategies and the future of risk assessment are discussed. The report concludes with recommendations for future work and research formulated from consultations of EFSA staff, expert Panels and other international organisations.

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KEY WORDS

mode of action, adverse outcome pathway, integrated testing strategy, physiologically-based models, in silico, OMICS
Levels of Knowledge, Toxicokinetic and Toxicodynamic processes

- Toxicokinetics
  - External dose
  - Internal dose
  - Target organ dose
  - Target organ metabolism
  - Toxic effect

- Toxicodynamics
  - Target organ responses
  - Toxic effect
TK AND MULTIPLE CHEMICALS: DATA AND MODELS

- **Integrating TK in Human, animal, environmental RA**

  ✓ **Objective 1:** Review model/Tools in each area human, animal, Env RA

  ✓ **Objective 2:** Collect physiological/biological parameters
    - Develop TK tools and models for single compounds (from simple tools to generic PB-PK models).
    - Case studies 10 compounds relevant to food and feed safety combining TK and TD

  ✓ **Objective 3:** **Develop TK tools and models for multiple chemicals** (from simple tools to generic PB-PK models).
    - Case studies 10 compounds relevant to food/feed safety
Quantitative theory for metabolic organisation from ‘first principles’
- time, energy and mass balance

Life-cycle of the individual
- links levels of organisation: molecule → ecosystems

Kooijman (2010)
What are DEB MODELS?

- Food → Assimilation → Reserve
- Reserve → Mobilisation
  - Somatic Maintenance: $\kappa$, $1-\kappa$
  - Maturity Maintenance
    - Growth
    - Maturation
    - Reproduction
- Reserve → Feces
- Reserve → Eggs

- System can be scaled to remove dimension ‘energy’
- 3-4 states
- 8-12 parameters
- Chemical affects the **probability** to die
- hazard modelling

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**Diagram:**
- Hazard rate
  - NEC
  - Blank value
  - Killing rate
- Internal concentration

**Flowchart:**
- Toxicokinetics model
  - Hazard rate
  - Survival probability
Elimination rate: 0.73 d\(^{-1}\)
Blank hazard rate: 0.0064 d\(^{-1}\)
NEC: 2.8 (2.1-3.1) µg/L
Killing rate: 0.031 L/(µg d)
DYNAMIC ENERGY BUDGET MODELS
FOR TERRESTRIAL AND AQUATIC ORGANISMS

✓ **Objective 1:** Review DEB models (Dec 2015)

✓ **Objective 2:** Collect physiological/ biological parameters - calibration of models single compounds incl DEB (Jan 2016-April 2017)
- Develop generic/specific models for aquatic and terrestrial organisms for single compounds-Endocrine case study

✓ **Objective 3:** Develop tools and models for multiple chemicals (Jan 2016-Jan 2018).

✓ All tools in R and as Open sources on EFSA website
Objective 1: Extensive literature searches and structured data collection on biochemical, genetic and environmental variables and impact on mycotoxin production

Objective 2: Extensive literature searches and structured data collection on realistic occurrence of mycotoxin mixtures, TK and combined toxicity in animals and humans

Objective 3: An integrated approach to the risk assessment of mycotoxin mixtures using modelling

Combine environmental variables, TK, toxicity data for RA using whole food chain approach (from environment to internal dose incl. carry over in farm animals and toxicity) plus comparative approach to mycotoxin toxicity in vertebrates.
-Building Open source TK and DEB tools-

External dose → Internal dose → Target organ dose → Target organ metabolism → Target organ responses → Toxic Response

ALVEOLAR SPACE

LUNG BLOOD

FAT

C_{vf} = \frac{Q_f}{\nu_f}

POORLY-PERFUSED TISSUES

C_{vs} = \frac{Q_s}{\nu_s}

RICHLY-PERFUSED TISSUES

C_{vr} = \frac{Q_r}{\nu_r}

LIVER

C_{vl} = \frac{Q_l}{\nu_l}

METABOLISM

P_{max}, K_m

PB-TK models

DEB Models

Default UFs to CSAFs: Past, Present and Future in Food Safety

Food

feeding

defecation

assimilation

reserve

somatic maintenance

K

1-K

maturity maintenance

growth

maturity reproduction

structure

maturity

offspring
COMBINING VARIABILITY IN TK AND IN VITRO DATA: OPENSOURCE PLATEFORM

Isoform-specific Variability Distribution: Open Source Tool
Meta-analysis TK studies (acute, chronic)
Phase I (CYP), Phase II (UGT), Transporters etc…

TK

TD

Isoform-specific Metabolism

In Vitro Human cell system

Combine Human TK data and Tox data
Combine Human TK data and human epi data
Combine TK data and in vitro TD data
Modelled CASF
ARTICLE IN PRESS

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Toxicokinetic models and related tools in environmental risk assessment of chemicals

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CONCLUSION AND RECOMMENDATIONS

**Basic TK or TD data**
- Quantify differences between subgroups of human population
- Refine uncertainty factors (categorical- or chemical-specific)

**Variability distributions for biological processes**
- TK and/or TD
  - Integrate human variability in ADME and TD
  - Integrate species differences /taxa differences for ERA

**Future use of Integrated Testing strategies**
- Further improvement *in vitro* methods to measure TK parameters
- Use *in vitro* data and variability distributions for RA in open source
- Open source DB and models: OpenfoodTox, PB-PK, DEB models

**Open source Tools: From simple models to full PB-TK-TD: context**
- Illustrate application tools using Tiered approaches/different contexts
  (data poor: emerging contaminant, data rich : regulated/contaminant, mixtures)
-SUMMARY-
Many Thanks

Questions?
Discussion