Application of the calculation method in regulatory risk assessment

Part 1 – The issue of animal studies

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Are animal studies the ultimate gold standard for classification of plant protection products?

Behavioural tests in mice

Example 1*

- The same set of 6 behavioural tests with ident apparatus and test protocols

- 3 laboratories: Albany (New York, US), Edmonton (Canada), Portland (Orlando, US)

- Highly inbred strains:
  - from the same source
  - shipped in the same way on the same day

- The housing (cages) was same, as well as acclimatisation and the diet…

* Genetics of mouse behaviour: interactions with laboratory environment. Crabbe et al., Science. 1999 Jun 4; 284(5420): 1670-2.

Behavioural tests in mice

Example 1*

- Some of the conclusions of the study:

- „sources of the lab environment effects are unknown, but one viable hypothesis can be proposed: different experimenters at the three labs probably presented idiosyncratic arrays of odour cues and handled the mice somewhat differently“
- “…people make different judgements or ratings of behaviour”
- “the experimenter in Edmonton was highly allergic to mice and performed all tests while wearing a respirator – a laboratory-specific (and uncontrolled) variable. That looks weird to us; it may look strange to a mouse too"

* Genetics of mouse behaviour: interactions with laboratory environment. Crabbe et al., Science. 1999 Jun 4; 284(5420): 1670-2.

Is death a more robust parameter?

Example 2 (Acute oral toxicity studies, rats) – vehicle influence

LD_{50} = 31 mg/kg bw (peanut oil)	Cat 2
LD_{50} = 67 mg/kg bw (polyethylene glycol)	Cat 3
LD_{50} = 87 mg/kg bw (corn oil)	
LD_{50} = 200 mg/kg bw (vegetable oil)	
LD_{50} > 2000 mg/kg bw (suspension in 0.5% methylcellulose)	No Cat
LD_{50} > 5000 mg/kg bw (suspension in 1% methylcellulose)	

Is death a more robust parameter?

Example 3 (Range finding and main developmental toxicity studies, rat)

- Two studies conducted in March/April and April/June 2011
- Animals of the same strain and the same supplier
- Same lab, same technician, same study author
- Same doses, same diet, same vehicle…

- 1 - Range finding rat developmental study:
- 6 tested dams well tolerated the dose of 1000 mg/kg bw/d
- No clinical signs, very slight decrease in bwg, no foetal effects

- 2 - Main developmental rat study:
- All 22 dams badly tolerated the dose of 1000 mg/kg bw/d and were sacrificed at the begin of the study…

Bias in animal studies…

- Biological systems are variable (genetic shift) and are fragile…
- Life cannot be completely controlled and standardised…
- Human factor (technicians, pathologists) is not negligible…
- Evaluation terminology changes over the years…
- Animal studies give us the approximation of reality but they often depend on (unknown) variables…

"We need to understand that every truth is valid only in its place, that something is true only as long as it is claimed under the conditions in which it is originally based"

Steiner, Rudolf (1899). Ernst Haeckel und die `Welträtsel', in *Rudolf Steiner, Methodische Grundlagen der Anthroposophie* (Dornach, Switzerland: Rudolf Steiner Verlag, 1989, pp. 391-402).

Animal studies are not the ultimate gold standard for classification of plant protection products…

One of the alternatives to vertebrate studies…

Calculation method

- Used for acute oral, dermal and inhalation toxicity

- Assumption:

a. Hazardous properties of components contribute to the overall toxicity of the mixture

b. Additivity of acute effects (“1+1 = 2”) can be applied

- Prerequisites:

- Detailed composition of the mixture
- Toxicological information of components in the formulation (LD_{50}, acute toxicity estimates (ATE))

Calculation method…

The principle

Data available for all components:

$$\frac{100}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

Data not available for all components:

$$\frac{100 - \left(\sum C_{\text{unknown if}} > 10\ \%\right)}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

Calculation method…

Sources of information on single components

- MSDS (Regulation (EC) No 1907/2006 (REACH), Art 31)
- Annex VI of Regulation (EC) 1272/2008 (CLP-Regulation)
- REACH Registration Dossiers
- Route-to-route extrapolation
- Literature data, data from closely analogous substances, etc.

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Application of the calculation method in regulatory risk assessment
Part 2 – Calculation method

Korinna Wend

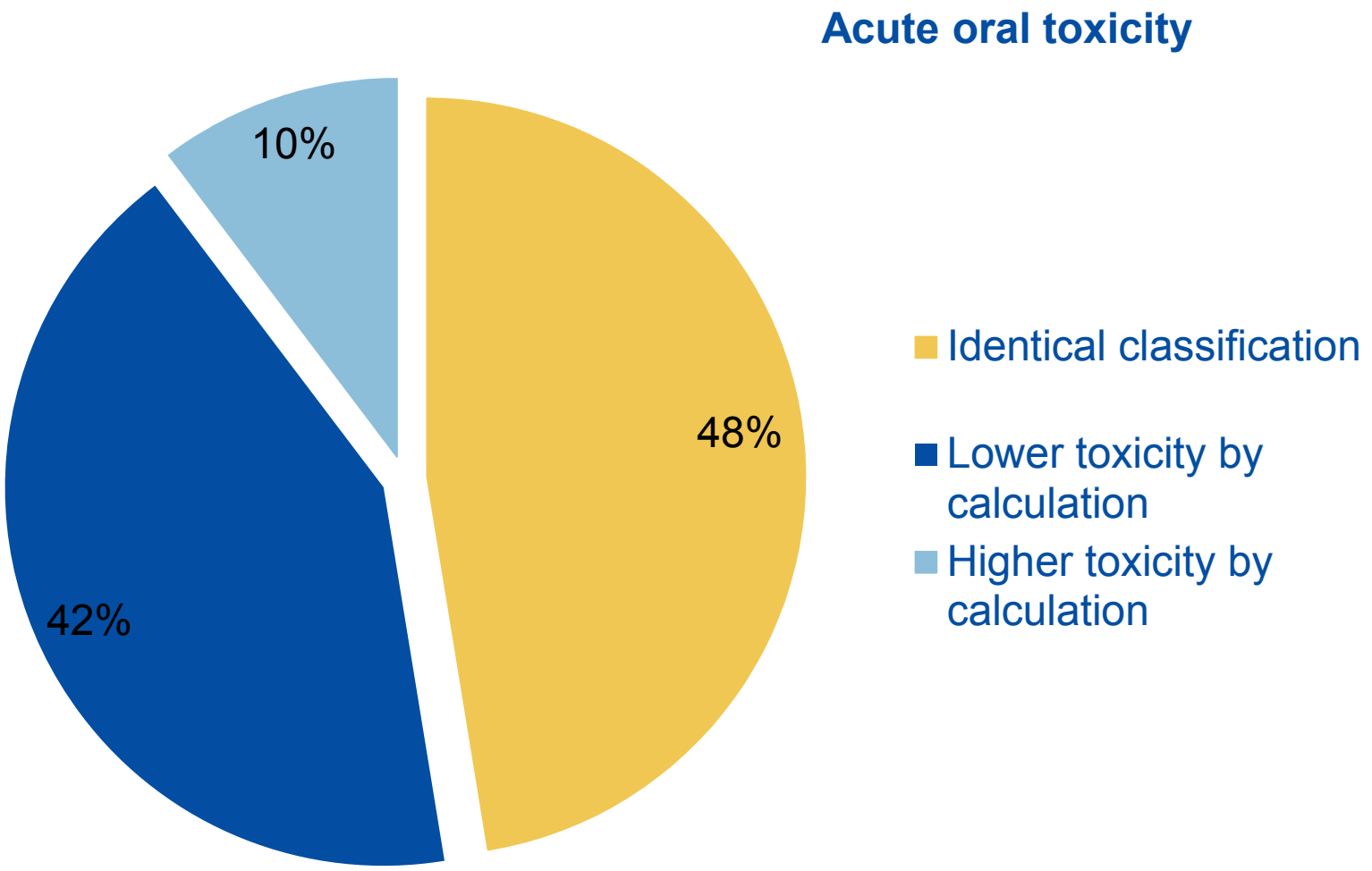
How accurate is the calculation method? BfR pilot study

- **BfR pilot study**
  - Comparison of the results of the calculation method *versus* the results of the corresponding animal studies

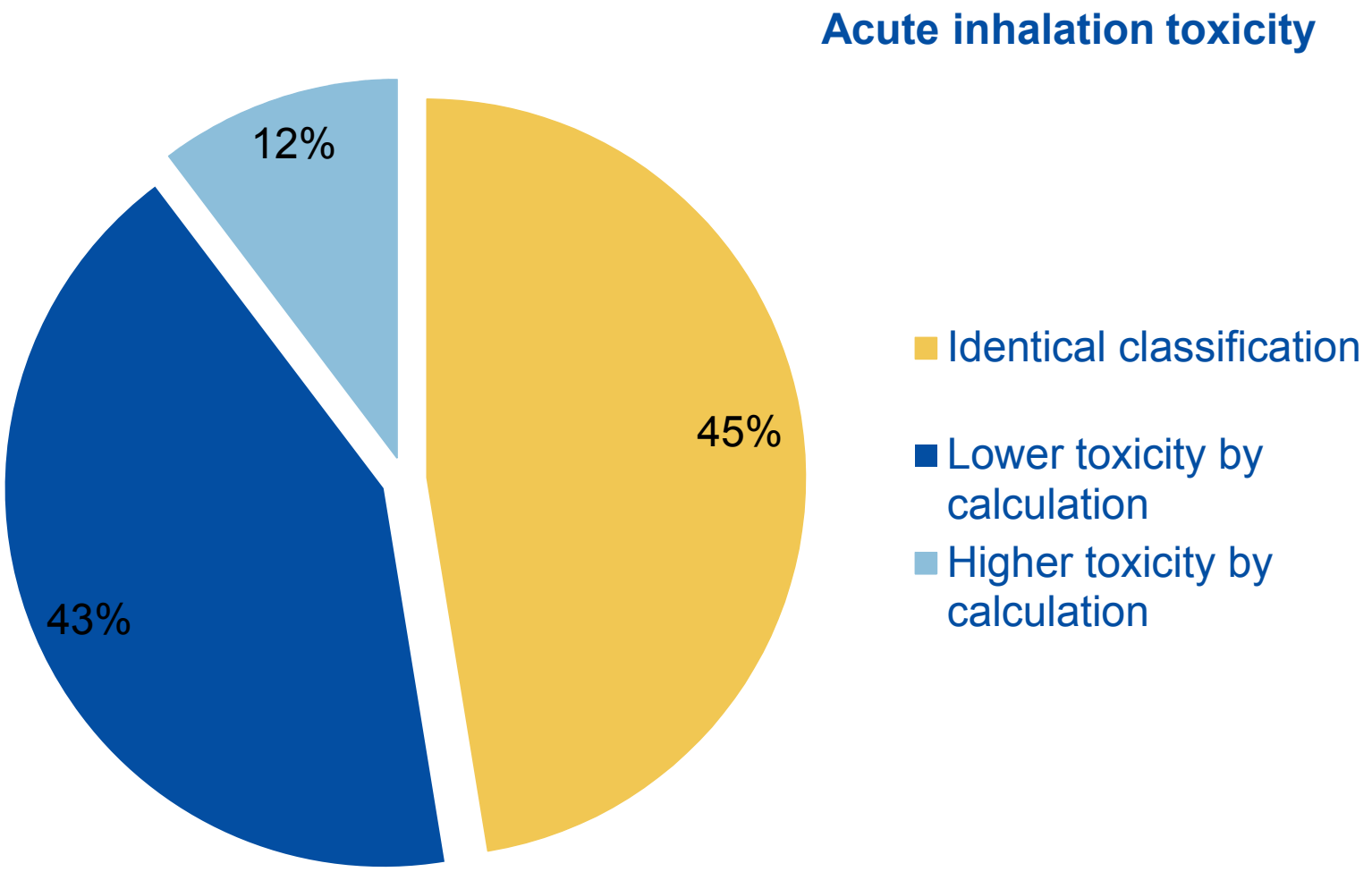
- **Endpoints**
  - Acute oral toxicity
  - Acute inhalation toxicity
  - Acute dermal toxicity

- **Study details**
  - Detailed composition of the mixture
  - Toxicological information of components
  - Animal studies conducted with the formulation according to CLP guidelines
  - Animal studies resulting in classification of the mixture

How accurate is the calculation method? First results



How accurate is the calculation method? First results



How accurate is the calculation method? First results

Acute dermal toxicity

No of studies	Comparison result ATE vs. animal study
0	Identical classification
2	Lower toxicity by calculation
3	Higher toxicity by calculation

How accurate is the calculation method? Case study 1

LD_{50} values close to classification limit

Substance	Amount (%)	LD50 (mg/kg bw)	Source
Active substance 1	4	1369	EFSA
Active substance 2	51	1600	EFSA
Co-formulant A	14	1400	MSDS
Co-formulant B	3	2100	MSDS
Co-formulant C	4	2460	MSDS

Calculated LD_{50}: 2103 mg/kg bw ⟶ no classification

Acute oral tox. animal study result: positive ⟶ Acute Tox. 4, H302

Conclusion of case study 1

- Consideration of the range of acute toxic components in the calculation method
- Consideration of any kind of toxicity, not only 50 percent of dead animals for the derivation of the LD_{50} value

→ Calculation method is of limited applicability regarding the prediction of acute toxicity

How accurate is the calculation method? Case study 2

influence of ingredients on acute oral toxicity

Ingredient	Amount (%)	LD50 (mg/kg bw)	Source	Toxicity of the ingredients
Active substance 1	5	>2000	EFSA	
Active substance 2	13	>2000	EFSA	
Co-formulant A	13	>2000	MSDS	
Co-formulant B	2	>2000	MSDS	
Co-formulant C	29	>2000	MSDS	
Co-formulant D	33	>2000	MSDS	
Co-formulant E	4	>2000	MSDS	
Co-formulant F	**<1%**	**1091**	MSDS	

Calculated LD_{50}: 60.000 mg/kg bw ⟶ no classification

Acute oral tox. animal study result: positive ⟶ Acute Tox. 3, H301

How accurate is the calculation method? Case study 2

influence of ingredients on acute oral toxicity

Ingredient	Amount (%)	LD50 (mg/kg bw)	Source	Toxicity of the ingredients
Active substance 1	5	>2000	EFSA	
Active substance 2	13	>2000	EFSA	Skin Irrit.2, H315
Co-formulant A	13	>2000	MSDS	
Co-formulant B	2	>2000	MSDS	EUH208
Co-formulant C	29	>2000	MSDS	Asp. Tox.1, H304
Co-formulant D	33	>2000	MSDS	
Co-formulant E	4	>2000	MSDS	Eye Irrit.2, H319
Co-formulant F	**<1%**	**1091**	MSDS	Skin Sens.1, H317; Eye Dam.1, H318

Calculated LD_{50}: 60.000 mg/kg bw ⟶ no classification

Acute oral tox. animal study result: positive ⟶ Acute Tox. 3, H301

Conclusion of case study 2

- LD_{50} values of the ingredients and the animal study result do not match.
- Some available data are not reliable.

→ <u>Urgent need</u> to develop alternative test methods for acute toxic endpoints (e.g. validation of *in vitro* tests considering the application domain of the formulation)

One further alternative to vertebrate studies…

Weight of evidence approach

❖ **Definition**

- Evaluation by applying a weight of evidence determination using expert judgement for toxicological assessment of plant protection products (acute toxicity, irritation and sensitisation)

❖ **Legal requirement**

- Regulation (EC) No 1272/2008
- Regulation (EU) No 284/2013

❖ **Weight of evidence approach**

- step 1: All existing data available on the mixture itself
- step 2: All relevant data obtainable on acute endpoints (e.g. *via* bridging principles, *in vitro* methods, calculation method)

→ no methods/guidelines exist for the *in vitro* / *in silico* testing of acute toxicity

→ uncertainties of *in vitro / in silico* tests with the formulation to address mixture effects

Conclusion…

- Gather ALL available information prior to any new data generation:
- Consider the information on comparable mixtures
- Consider the known properties of the single components in the mixture
- Consider if validated *in vitro, in silico* methods are available
- Consider the complexity of the mixture
- Use information from other legal sources (ECHA)
- Combine the information, use integrated - and INTELLIGENT - testing strategy
- Use “weight of evidence” approach …

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Thank you for your attention

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