The fish embryo toxicity test as a screening method to predict the toxicity of biocidal products

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Outline

- Legislative background
- Aim of the presented study
- Method of the fish embryo toxicity test (FET)
- Investigation of five biocides, their active substances and mixtures thereof
- Conclusions

Legislative Background

24.4.98 EN Official Journal of the European Communities L 123/1

DIRECTIVE 98/8/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 16 February 1998

concerning the placing of biocidal products on the market

ANNEX VI

COMMON PRINCIPLES FOR THE EVALUATION OF DOSSIERS FOR BIOCIDAL PRODUCTS

Summary

53. „In each area (…), the Member State shall combine the results for the active substance together with the results for any substance of concern to produce an overall assessment for the biocidal product itself. This should take account of any likely synergistic effects (…).“

54. „For biocidal products containing more than one active substance any adverse effects shall also be combined (…).“

Technical Guidance

- „Competent authority must consider effects arising from the active substance(s) and individual substances of concern (…).”
- „Careful consideration should be given to (…) any additive, synergistic or other effects which can reasonably be foreseen (…).“

TECHNICAL NOTES FOR GUIDANCE

IN SUPPORT OF ANNEX VI OF DIRECTIVE 98/8/EC OF THE EUROPEAN PARLIAMENT AND THE COUNCIL

CONCERNING THE PLACING OF BIOCIDAL PRODUCTS ON THE MARKET

COMMON PRINCIPLES AND PRACTICAL PROCEDURES FOR THE AUTHORISATION AND REGISTRATION OF PRODUCTS

SHORT TITLE: TNsG on Product Evaluation

Two approaches proposed

- Whole-mixture approach

$$(PEC/PNEC)_{product} = PEC_{product}/PNEC_{product}$$

- Component-based approach

$$(PEC/PNEC)_{product} = \Sigma\,(PEC/PNEC)_{components}$$

Proposal of UBA

- Presented on several meetings so far
- Still work in progress
- Input from MS, ongoing research and workshops
- Currently: toxicity test with product required for most sensitive endpoint, followed by component-based approach for risk assessment

Requirements and Open Questions

- Toxicity data for active substances
- Toxicity data for other relevant components (substances of concern, transformation products)

- Component-based approach valid when combining different endpoints and assessment factors ?
- Evidence for absence of synergistic effects required ?

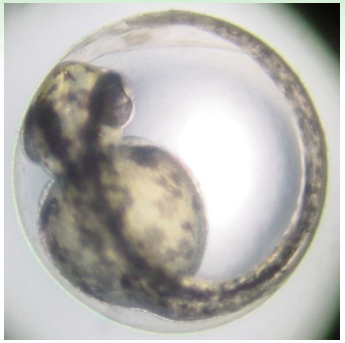
Aim of the Present Study

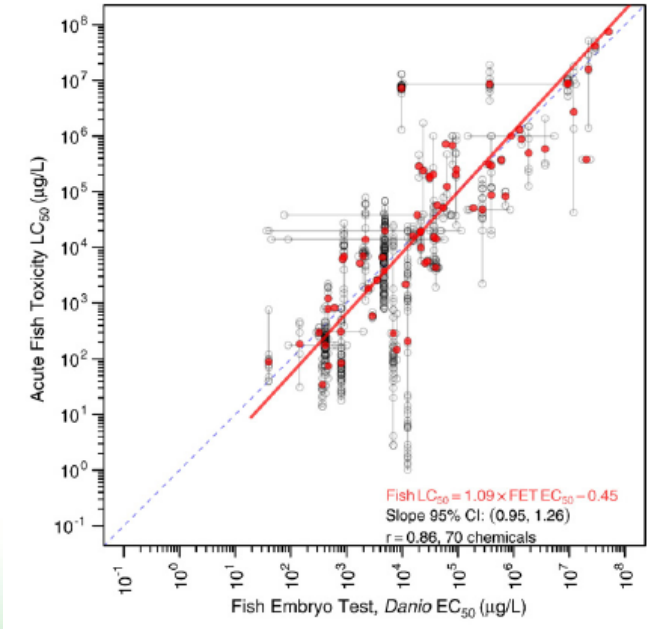
To confirm that the toxicity of wood preservation products can be assessed by a component-based approach

- reliability of prediction
- precision of prediction
- relevance of formulation additives

The Fish Embryo Toxicity (FET) Test

- Non-animal test: proposed alternative to fish acute toxicity test (OECD 203)
- Quick and less expensive
- Draft OECD guideline available
- Good correlation with results of acute toxicity tests (Lammer et al. 2009)

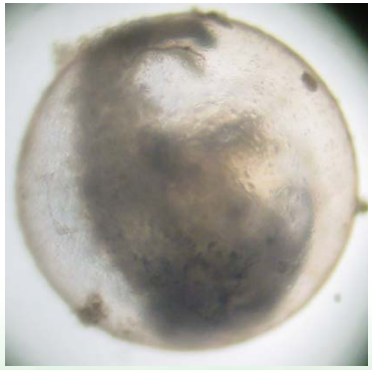




E. Lammer et al / Comparative Biochemistry and Physiology, Part C 149 (2009) 196–209

FET - Method

- Concentration-response curves with 5-8 concentration levels
- 4 replicates with 10 eggs each
- 48 h static exposure
- Assessment of mortality and determination of LC_{50} with 2-parameter log-logistic model
- Verification of test concentrations by chemical analysis
- All LC_{50} values corrected by mean measured initial concentrations

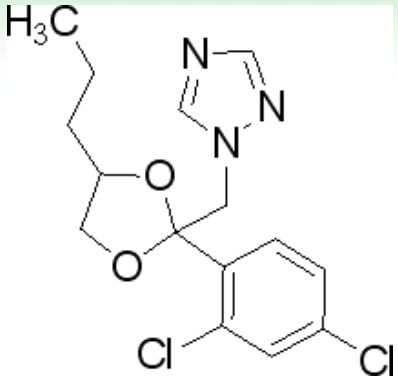


Investigated Wood Preservation Products

Product	Active Substances	Formulation Type	Hazardous Components
A	Propiconazole & IPBC	Water-based	None
B	Propiconazole & IPBC	Oil-based	2
C	Propiconazole & Fenoxycarb	Oil-based	3
D	Propiconazole & Fenoxycarb	Water-based	1
E	Propiconazole & Fenoxycarb	Water-based	None

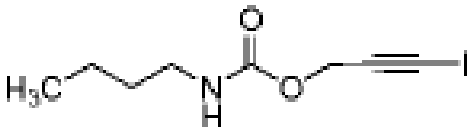
Active Substances

Propiconazole



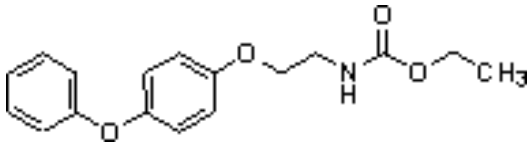
- Triazole fungicide inhibiting C14-demethylase in ergosterol biosynthesis

Iodocarb (IPBC)



- Carbamate fungicide affecting cell membrane permeability (proposed)

Fenoxycarb



- Carbamate insecticide mimicking insect juvenile hormone

Mixture Toxicity Prediction

Dissimilar Mode of Action

- Concept of Independent Action (IA)

Concept of Concentration Addition (CA)

- Small difference to IA prediction: max. twofold for binary mixtures (Junghans et al. 2006)
- Default model according to guidance
- LC_{50} values for single substances as input

Mixture: Prediction and Compliance

$$LC_{50\,mix} = \frac{1}{\sum_i \frac{P_i}{LC_{50\,i}}}$$

Predicted toxicity of the mixture

$$P_i = \frac{C_i}{\sum_i C_i}$$

Relative proportion of each substance in the mixture

$$MDR = \frac{predicted\ LC_{50\,mix}}{observed\ LC_{50\,mix}}$$

Deviation between prediction and observation

Active Substances in the FET

	Corrected 48 h LC_{50} (mg/l)	Mean corrected 48 h LC_{50} (mg/l)
Propiconazole	21.3 / 20.4 / 18.4 / 21.6	20.4
IPBC	0.418 / 0.279	0.349
Fenoxycarb	3.97 / 2.31	3.14

Active Substances in the FET

Reliability of toxicity estimates

- Tight confidence intervals
- Toxicity estimates below limit of solubility

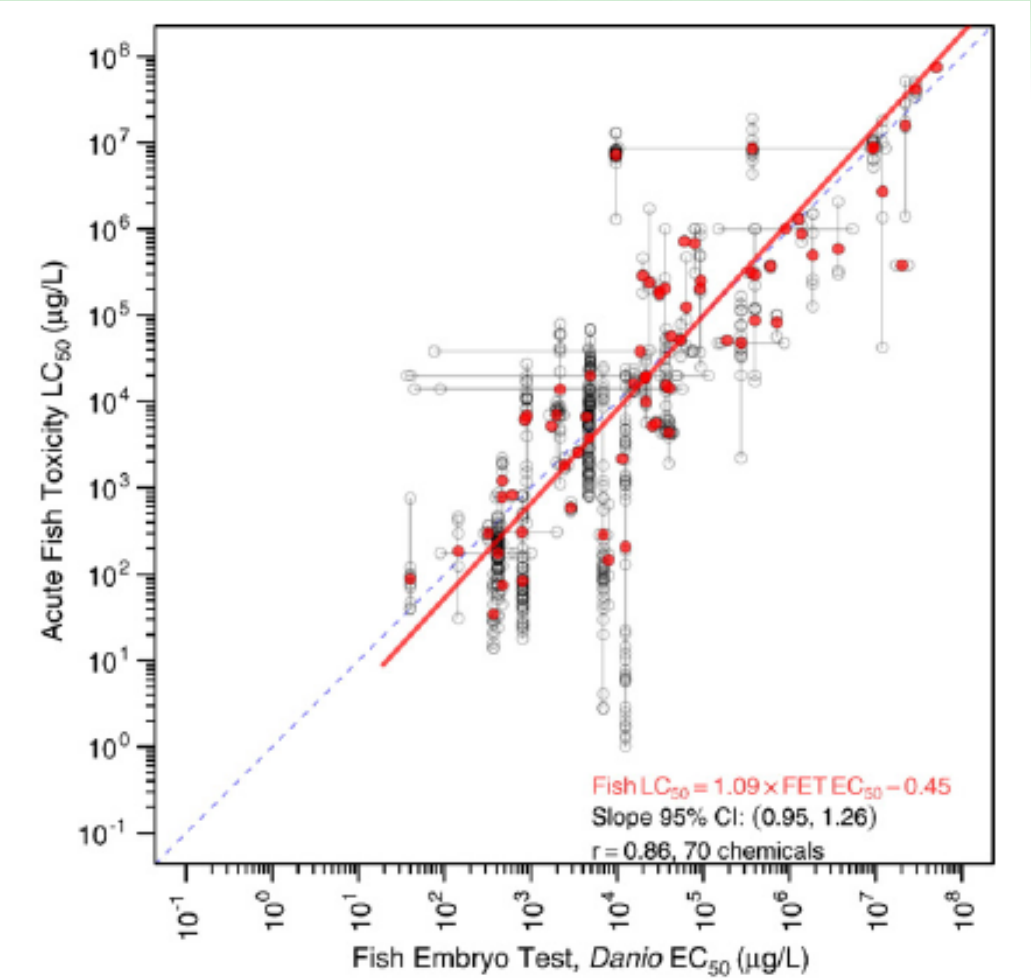
Inter-laboratory reproducibility

- Deviation of less than factor 2 between estimates

Comparability to fish acute toxicity

- Estimates up to 10-fold higher than LC_{50} fish acute toxicity

Active Substances in the FET



Comparability FET and fish acute toxicity

- Deviation of up to three orders of magnitude can occur

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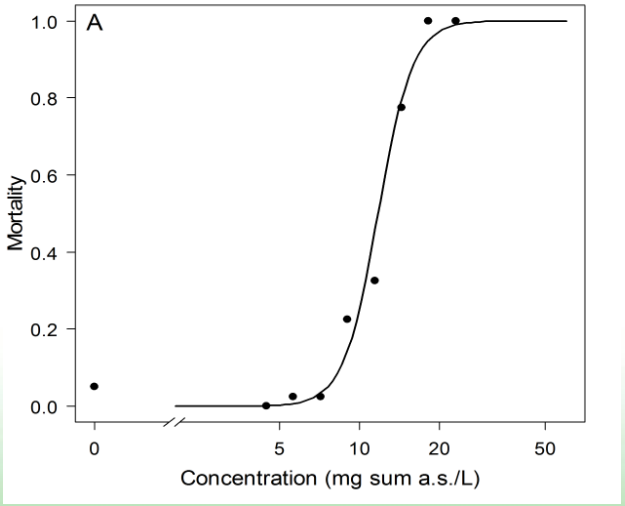
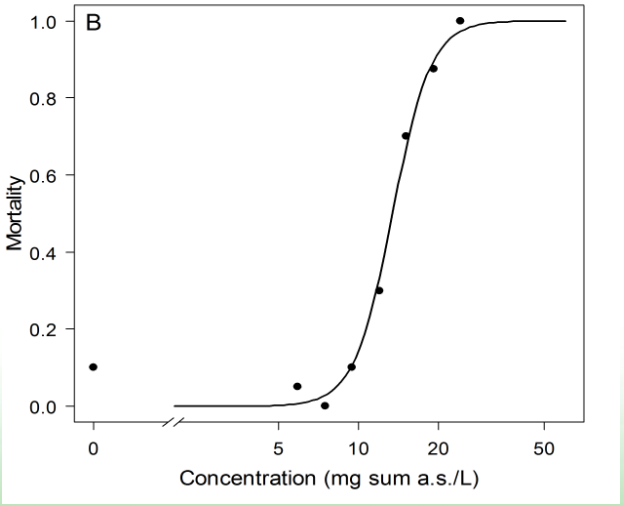
Equipotent Generic Mixtures in the FET

	Predicted LC_{50} (mg/l)	Observed corrected LC_{50} (mg/l)	MDR
Propiconazole & Fenoxycarb (A)	10.21	12.79	0.80
Propiconazole & IPBC (B)	8.92	12.80	0.70





Other Generic Mixtures in the FET

	Predicted LC_{50} (mg/l)	Observed corrected LC_{50} (mg/l)	MDR
Propiconazole & IPBC	1.01	1.03	0.98
Propiconazole & IPBC	0.45	0.47	0.96
Propiconazole & Fenoxycarb	19.7	17.0	1.16
Propiconazole & Fenoxycarb	18.3	19.6	0.94

Generic Mixtures - Predictability

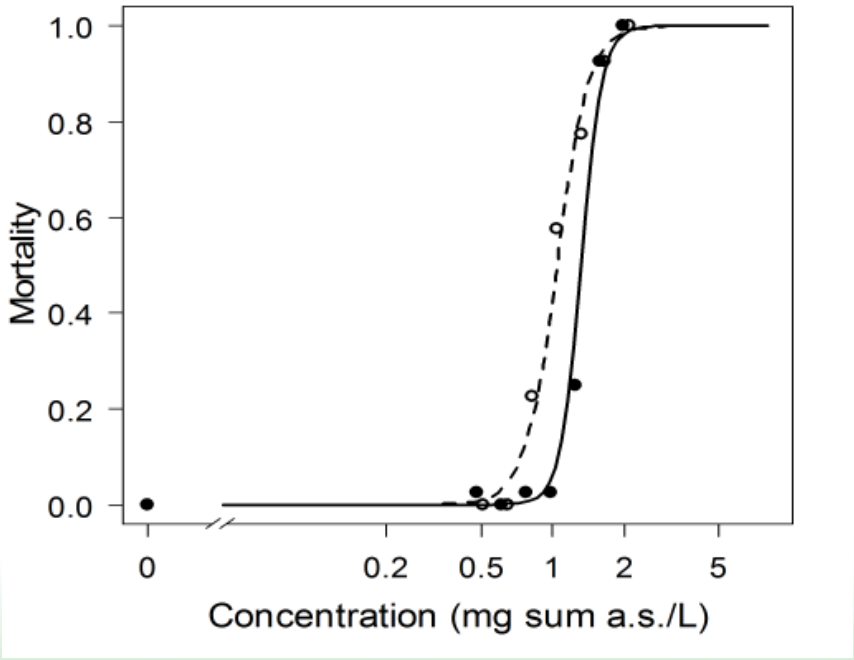
- Toxicity of binary mixtures can be predicted by *CA*
- Observed and predicted toxicity estimates deviate by less than factor 2: in range of reproducibility of single substance estimates
- Predictability independent of relative concentrations

Expectation: toxicity of the products can be predicted by *CA*

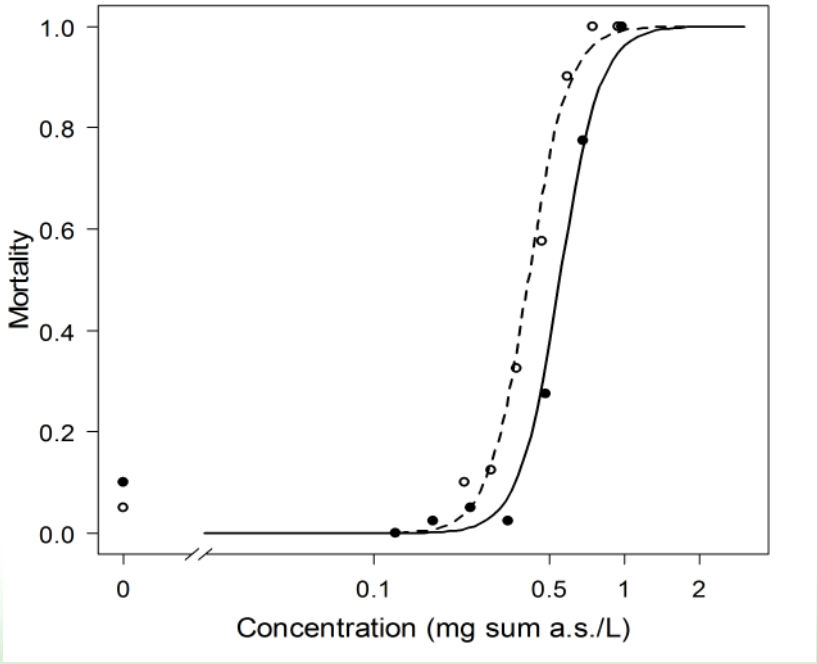
Products with Propiconazole & IPBC

- Toxicity of products as predicted
- Similar to response-curve of generic mixture

MDR = 0.55



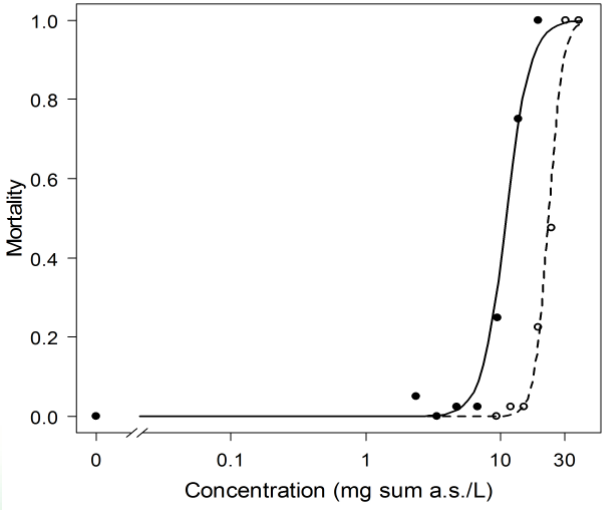
MDR = 1.04



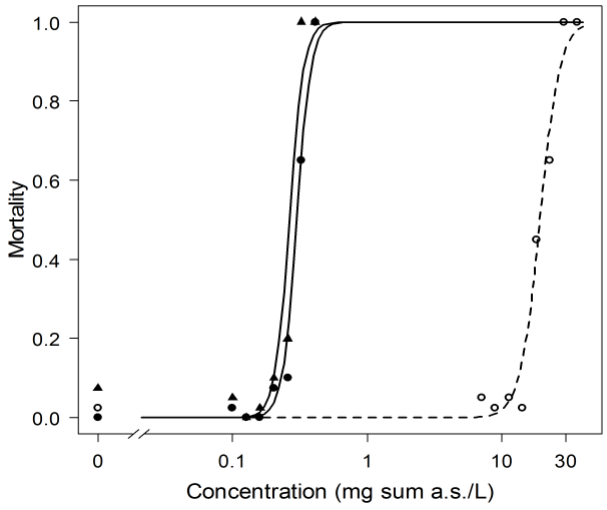
Products with Propiconazole & Fenoxycarb

- Toxicity of products not always as predicted
- Deviation not due to interaction between active substances

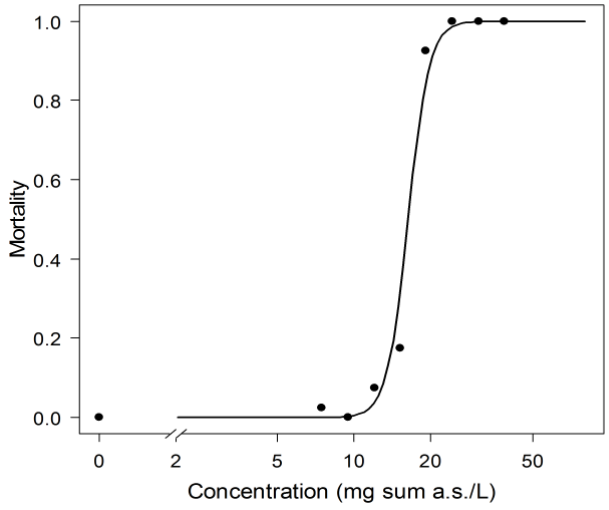
MDR = 4.57



MDR = 64.3 / 76.5



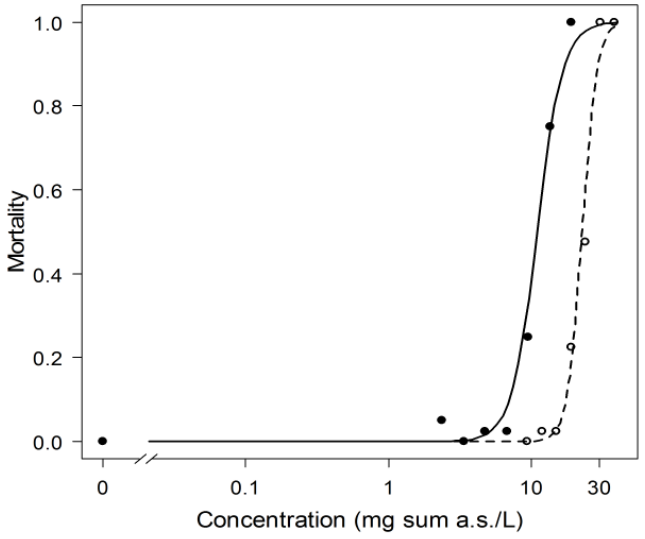
MDR = 1.28



Reasons for Deviation: Product 3

- Tested as water-accommodated fraction (<30% of nominal)
- High content of organic solvent (petroleum)
- Petroleum not toxic in FET
- Other hazardous substances with low reported fish acute toxicity
- Petroleum may have influenced bioavailability and/or interfered with analytical measurement

MDR = 4.57

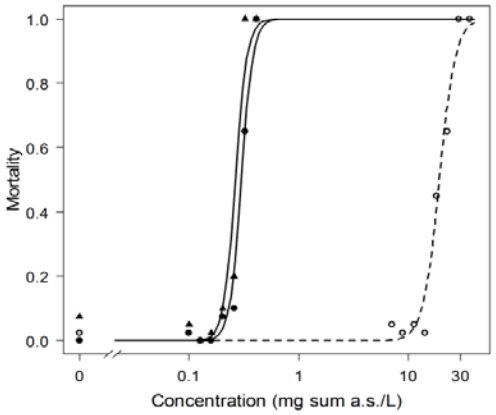


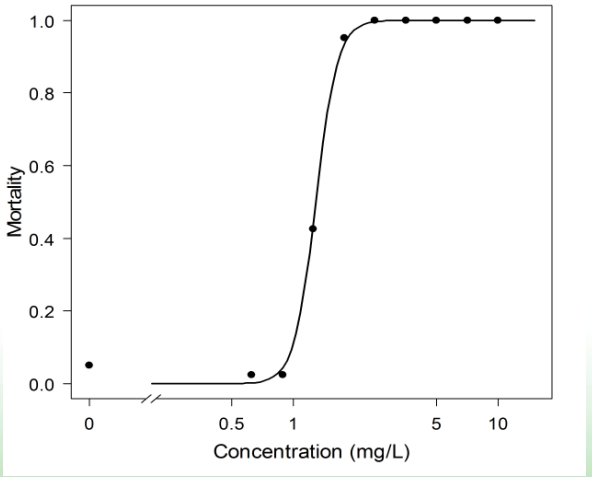
Reasons for Deviation: Product 4

- One hazardous substance: (C12-C16)alkyl-dimethylamine at 10% content
- High toxicity in FET with LC_{50} of 1.28 mg/L
- Toxicity fully explains observed deviation

MDR = 1.07 and 1.21

MDR > 60





Conclusions I

Toxicity of products can be assessed by CA

- Confirmation of technical guidance
- Precision in range of single-substance toxicity estimates
- Reliable if hazardous components are considered within a threshold of 5-fold deviation (n=5)
- FET suitable to provide evidence for absence of synergistic interaction

Conclusions II

Extrapolation to other organisms and products?

- Adult fish, algae, crustaceans
- Other product types among biocides, plant protection products …

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THANK YOU !