Bayesian network model for risk assessment based on fish embryo testing: a probabilistic weight-of-evidence approach

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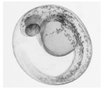

Introduction

Reduction, Replacement or Refinement of animal testing is required by legislations such as the EU Directive 2010/63/EU wherever possible. **Fish Embryo Toxicity** (FET) testing could be an **alternative to juvenile fish** in acute toxicity testing. However, FET data are not yet accepted as a replacement for regulatory purposes such as REACH. The European Chemicals Agency (ECHA) has therefore recommended the development of a **weight-of-evidence (WoE)** approach for FET data.

We propose a **Bayesian network (BN)** model, which is increasingly used in ecological risk assessment. A BN can integrate large amounts of data and other information sources by discrete probability distributions, and predict the probability of specified states.

Objectives

1. To develop and evaluate a BN model for predicting toxicity of substances to juvenile fish from embryo toxicity data in combination with other relevant information.
2. To apply the BN model in a WoE approach which can support replacing juvenile fish toxicity testing with fish embryo toxicity testing.

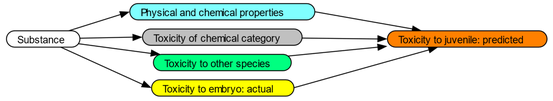

Figure 1. Simplified illustration of the BN model, which predicts levels of toxicity to juvenile fish based on four lines of evidence.

Data and Methods

- The conceptual model (Figure 1) has four pathways for predicting the toxicity of substances to juvenile fish:
  - (1) **Physical and chemical properties** of the substance
  - (2) Toxicity of substances in the same **chemical category** (to juvenile fish)
  - (3) Toxicity of the substance to **algae and Daphnia**
  - (4) Toxicity of the substances to **fish embryos**
- Data for 237 substances are provided by P&G, including QSAR (n=197), LC50 for embryo (n=541) and juvenile (n=1459), and EC50 for algae (n= 264) and Daphnia (n=2328).
- Toxicity values (EC50 and LC50 values) are discretized to **5 intervals**:
  - very low: >100 mg/L
  - low: 5-100 mg/L
  - medium: 0.5-5 mg/L
  - high: 0.01-0. 5 mg/L
  - very high: <0.01 mg/L
- The links between nodes are quantified by **conditional probability tables (CPT),** where the values are obtained by two methods:
  - **Count of observations**: all nodes linked to "Substances" (e.g. Table 1)
  - **Expert judgement**: all subsequent nodes (e.g. Table 2)
- All pathways are assigned the **same weight**.
- A selection of **20 substances with ≥3 observations** for both juveniles and embryos (Table 1) are used for evaluation of the model:
  - comparison of predicted and observed toxicity to juvenile fish (Figure 2)
  - assessment of the model predictions for a WoE approach.

Table 1. Example of a conditional probability table (CPT) based on counts of observations, for the node "Toxicity to juvenile: actual". For each substance, the probability of a toxicity level corresponds to the proportion of counts in that level.

Substance	Chemical category	Count	very low	low	medium	high	very high
1,2-Dichlorobenzene	Neutral organic	7	0 %	57 %	43 %	0 %	0 %
1-Octanol	Neutral organic	10	0 %	100 %	0 %	0 %	0 %
2,4,6-Trichlorophenol	Phenol	13	0 %	23 %	54 %	23 %	0 %
2,4-Dichlorophenol	Phenol	5	0 %	40 %	60 %	0 %	0 %
3,4-Dichloroaniline	Aniline	9	0 %	100 %	0 %	0 %	0 %
4-Chlorophenol	Phenol	6	0 %	33 %	67 %	0 %	0 %
4-Nitrophenol	Phenol	19	0 %	79 %	21 %	0 %	0 %
Carbamazepine	Substituted urea	4	0 %	100 %	0 %	0 %	0 %
Dimethylsulfoxide	Neutral organic	6	100 %	0 %	0 %	0 %	0 %
Ethanol	Neutral organic	5	80 %	20 %	0 %	0 %	0 %
Juglone	Quinone	12	0 %	0 %	0 %	100 %	0 %
Malathion	Esters (dithiophosphates)	36	0 %	0 %	14 %	78 %	8 %
Naphthalene	Neutral organic	15	0 %	40 %	60 %	0 %	0 %
Parathion-ethyl	Esters (monothiphosphates)	37	0 %	0 %	57 %	43 %	0 %
Prochloraz	Imidazole	3	0 %	0 %	100 %	0 %	0 %
Quinoline	Neutral organic	5	0 %	80 %	0 %	20 %	0 %
Tetradecyl sulfate	Anionic surfactant	3	0 %	0 %	100 %	0 %	0 %
Toluene	Neutral organic	5	0 %	100 %	0 %	0 %	0 %
Triclosan	Phenol	6	0 %	0 %	0 %	100 %	0 %
Triethylene glycol	Neutral organic	5	100 %	0 %	0 %	0 %	0 %

Table 2. Example of a conditional probability table based on expert judgement, for the node "Membrane crossing". The numbers are the assumed probability of a substance's ability (low, medium or high) to cross a membrane, conditional on the states of the parent nodes (Hydrophobicity and Molecular weight of the substance).

Hydrophobicity (Kow)	Molecular weight (g/mol)	Membrane crossing		
		low	medium	high
≤ 5	≤ 600	0 %	25 %	75 %
≤ 5	> 600	25 %	50 %	25 %
> 5	≤ 600	25 %	50 %	25 %
> 5	> 600	75 %	25 %	0 %

Results

- Examples of BN model results are shown for 3 substances with different levels of toxicity and from different chemical categories (Figure 2)
- The BN model predicted **correct toxicity level to juveniles for 14 substances**, and **lower toxicity for 6 substances**.
- For the 6 substances with incorrect prediction,
  - 5 substances (2,4-Dichlorophenol, 4-Chlorophenol, Malathion, Naphthalene, Prochloraz) are **less toxic to fish** than to Daphnia or algae, which will drive the risk assessment
  - 1 substance (Juglone) is **less toxic to juveniles** than to embryos, which will drive the risk assessment
- Conclusion: for the 20 substances, the predicted toxicity level for juvenile fish was either **correct** or **not driving the risk assessment**.

Further developments

- Testing of the BN model with fish embryo and juvenile toxicity data from more sources
- Making the model available online (e.g. on http://demo.hugin.com)
- Application of the BN model to assess the risk of contaminants e.g. based on chemicals registered in the European Chemicals Agency database (http://echa.europe.eu)
- Refine the BN model to predict bioaccumulation in fish

Acknowledgements

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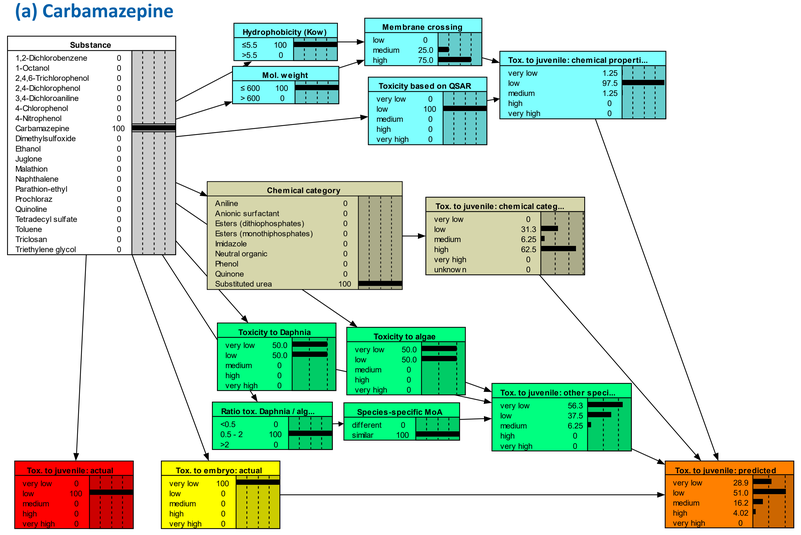
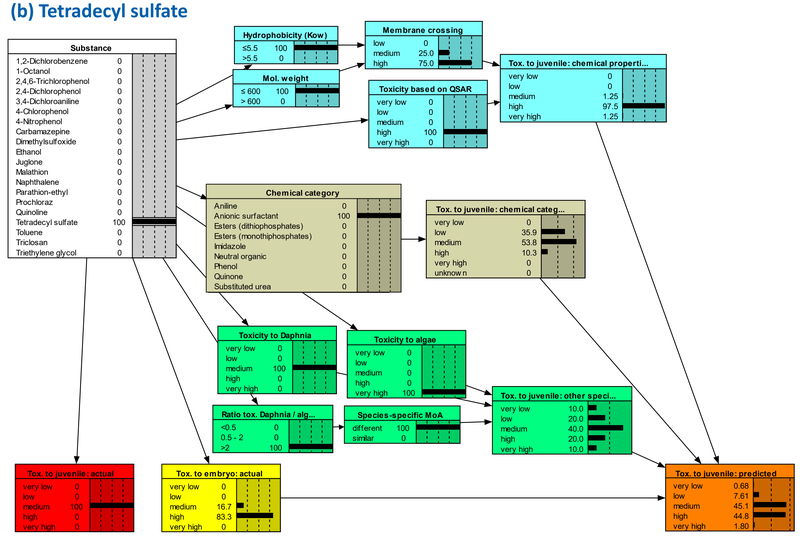
(a) Carbamazepine



(b) Tetradecyl sulfate



(c) Triclosan

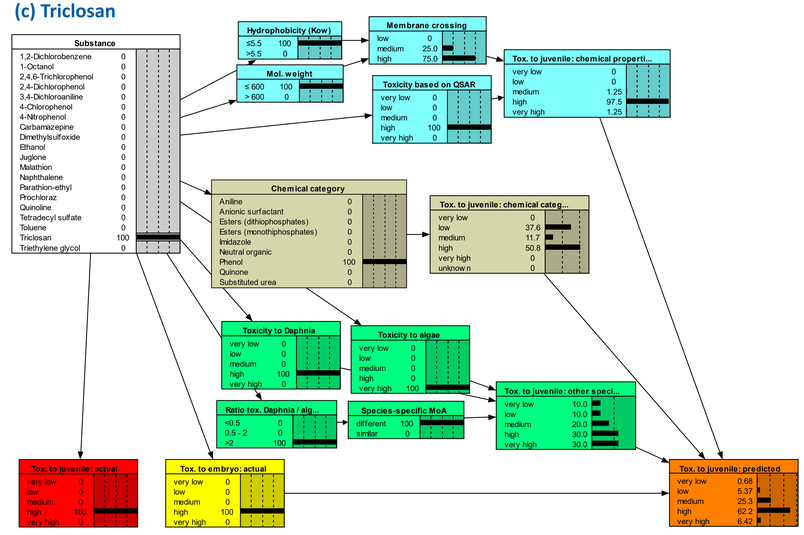


Figure 2. Examples of model predictions for three selected substances: (a) Carbamazepine, (b) Tetradecyl sulfate, (c) Triclosan. The predicted toxicity to juveniles (orange nodes) should be compared to the observed toxicity to juveniles (red nodes).