How to address fish aquatic toxicity with alternative approaches? – Possibilities, gaps and challenges to be addressed

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Purpose of animals used

Of the 8,624,692 animals used in research in 2020, 7,938,064 animals (92%) were used for experimental purposes while 686,628 animals (8%) were used for the creation and breeding of genetically altered animals. Animals used for experimental purposes decreased by 7.5% compared to 2019, while animals used for the creation and breeding of genetically altered animals increased by 4.1%.

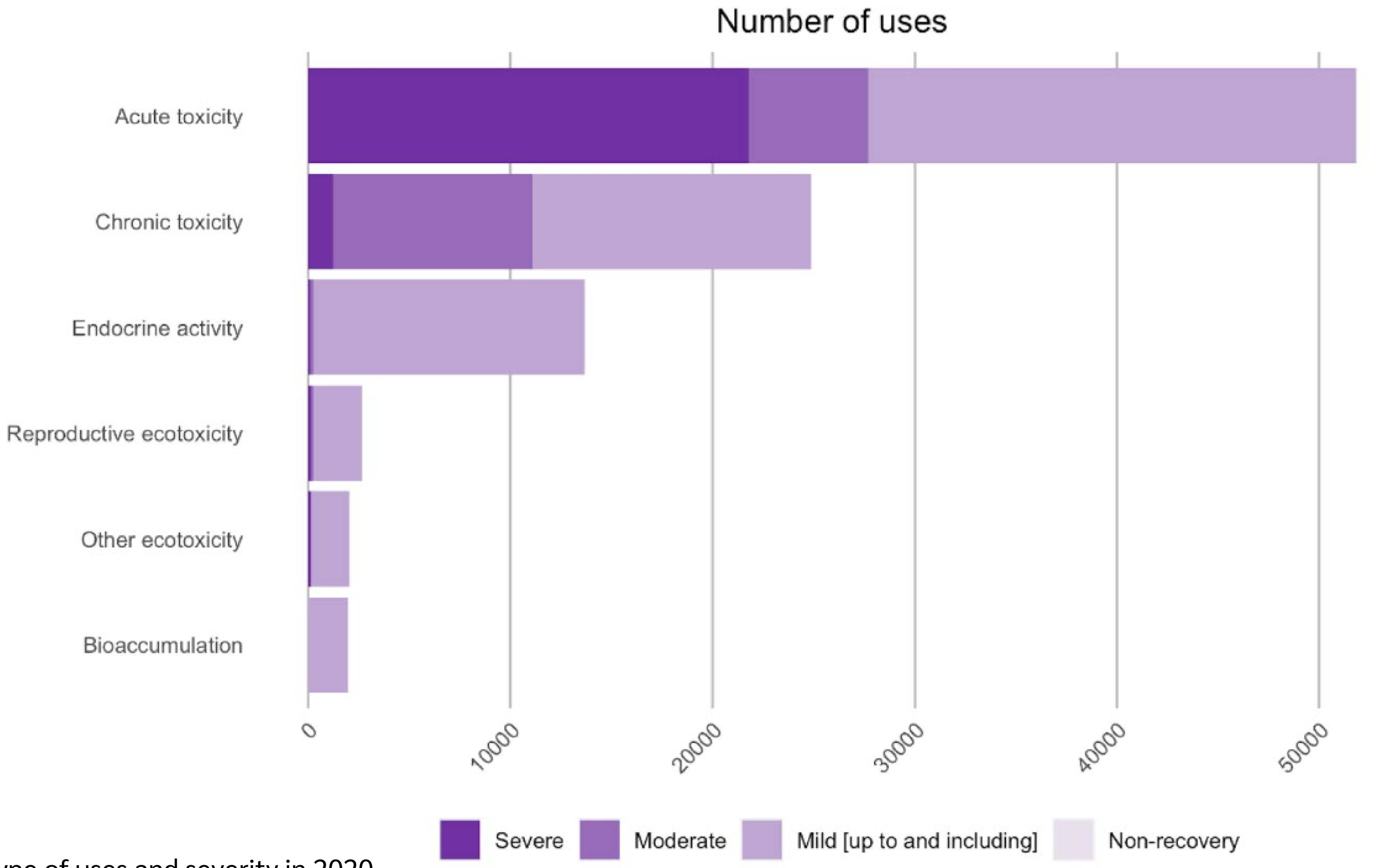
91% of animals used for experimental purposes in 2020 were mice, fish, rats, and birds, whereas cats, dogs, and primates accounted for 0.2%.

Number of Animals Used for Experimental Purposes in the EU and Norway in 2020

Species	Number of animals used for experimental purposes (2020)	% of total	% change from 2019
Mice	3,879,691	48.87%	-10.17%
Fish	2,191,367	27.61%	-3.37%
Rats	665,155	8.38%	-16.09%
Birds	510,108	6.43%	2.39%
Other mammals	634,253	7.99%	2.05%
Reptiles	2,072	0.03%	5.07%
Amphibians	37,821	0.48%	-13.05%
Primates	4,784	0.06%	-10.06%
Cats	2,464	0.03%	15.14%
Dogs	8,716	0.11%	-16.10%
Cephalopods	1,633	0.02%	-90.38%
Total	**7,938,064**		**-7.48%**

EU wide animal research statistics, 2020 :: Understanding Animal Research

Ecotoxicity



Ecotoxicity by type of uses and severity in 2020

Commission Staff Working Document - Summary Report on the statistics on the use of animals for scientific purposes in the EU and Norway (2020) Part 1 (1).pdf

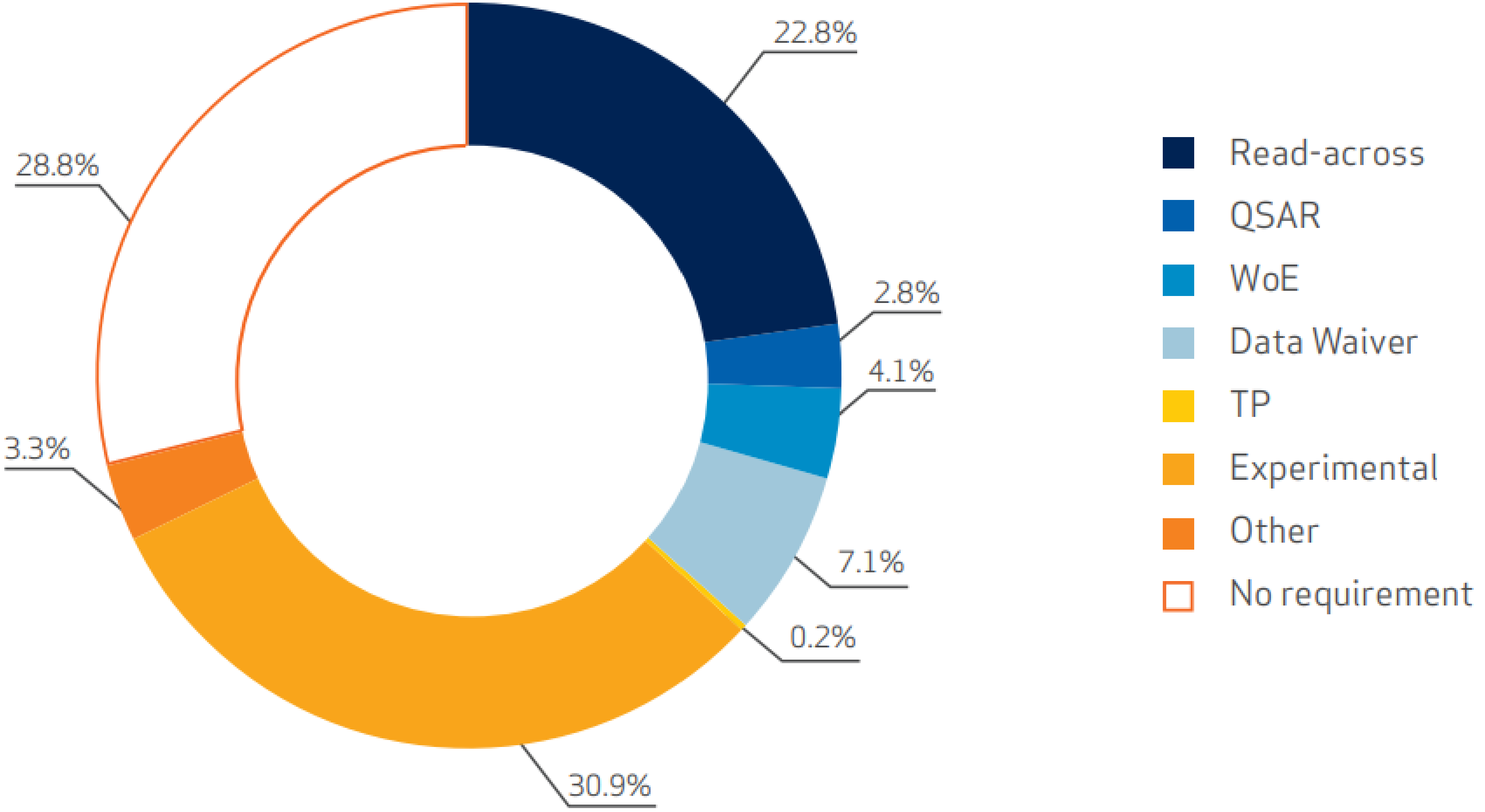


FIGURE 1: Options used to fulfil the information requirements

The use of alternatives to testing on animals for the REACH Regulation Fifth report under Article 117(3) of the REACH Regulation June 2023 [9cfc291e-9baf-ffa2-466c-2bc2c6f06b8e (europa.eu)]

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Fish acute toxicity test

14/
42/
49

480/
560

The need for alternatives

- The 3Rs-

Reduction, Refinement and Replacement

- Additional Rs- **Reproducibility, Relevance, and Regulatory acceptance**

The Rs

RISK based approach,
Robustness,
Repeatability,
Requirements,
Realism,
Reviewed,
Reassess,
Rules,
Redundant…………

- The 3Rs- Reduction, Refinement and Replacement
- Additional Rs- **Reproducibility, Relevance, and Regulatory acceptance**

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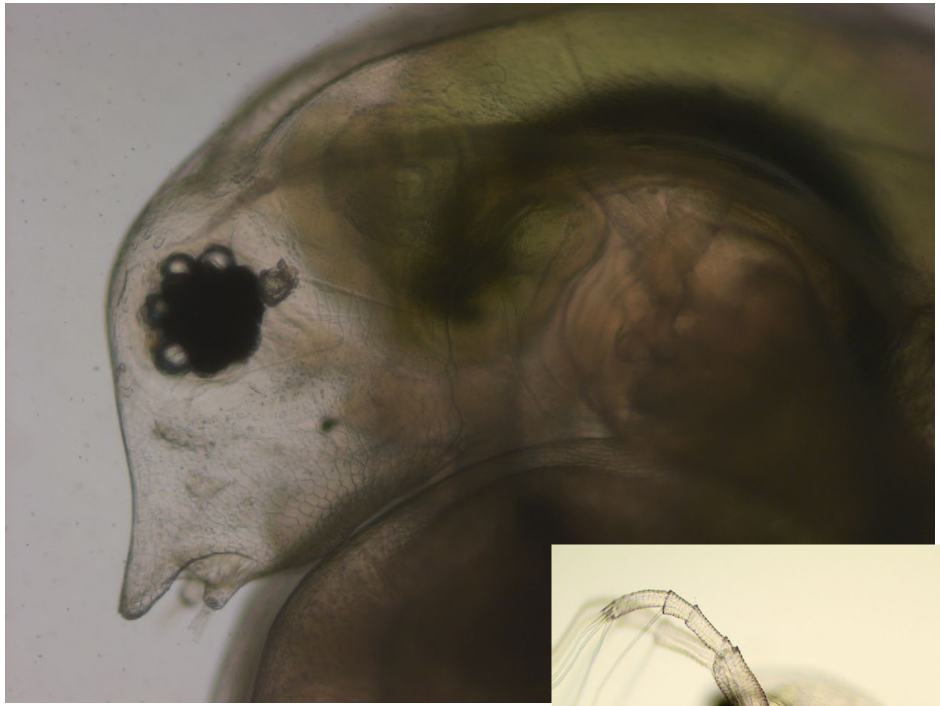
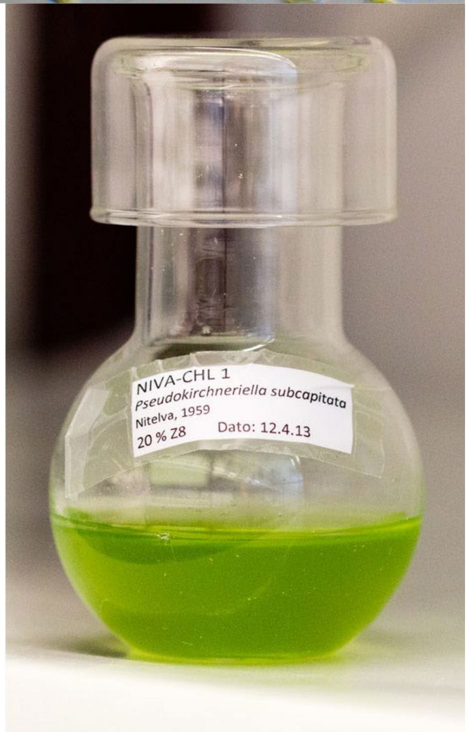
QSARs and exposure based triggers



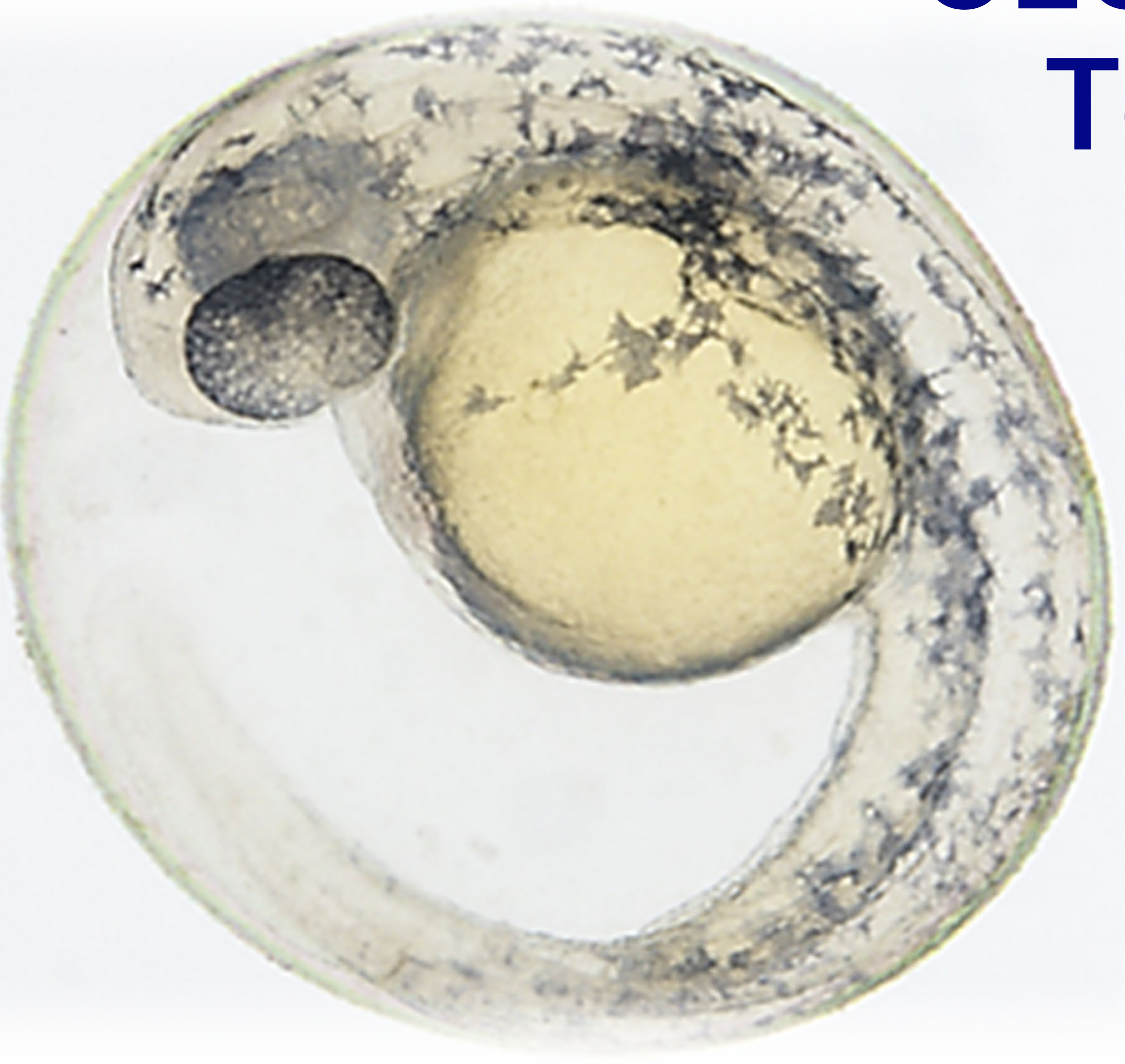

100 µm


CCAP 278/4 - Raphidocelis subcapitata
Culture Collection
of algae & protozoa


NIVA-CHL 1
Pseudokirchneriella subcapitata
Nitelva, 1959
20 % Z8
Dato: 12.4.13

OECD 249 RT Gill cytotoxicity assay

OECD 236 Fish Embryo Toxicity (FET) test



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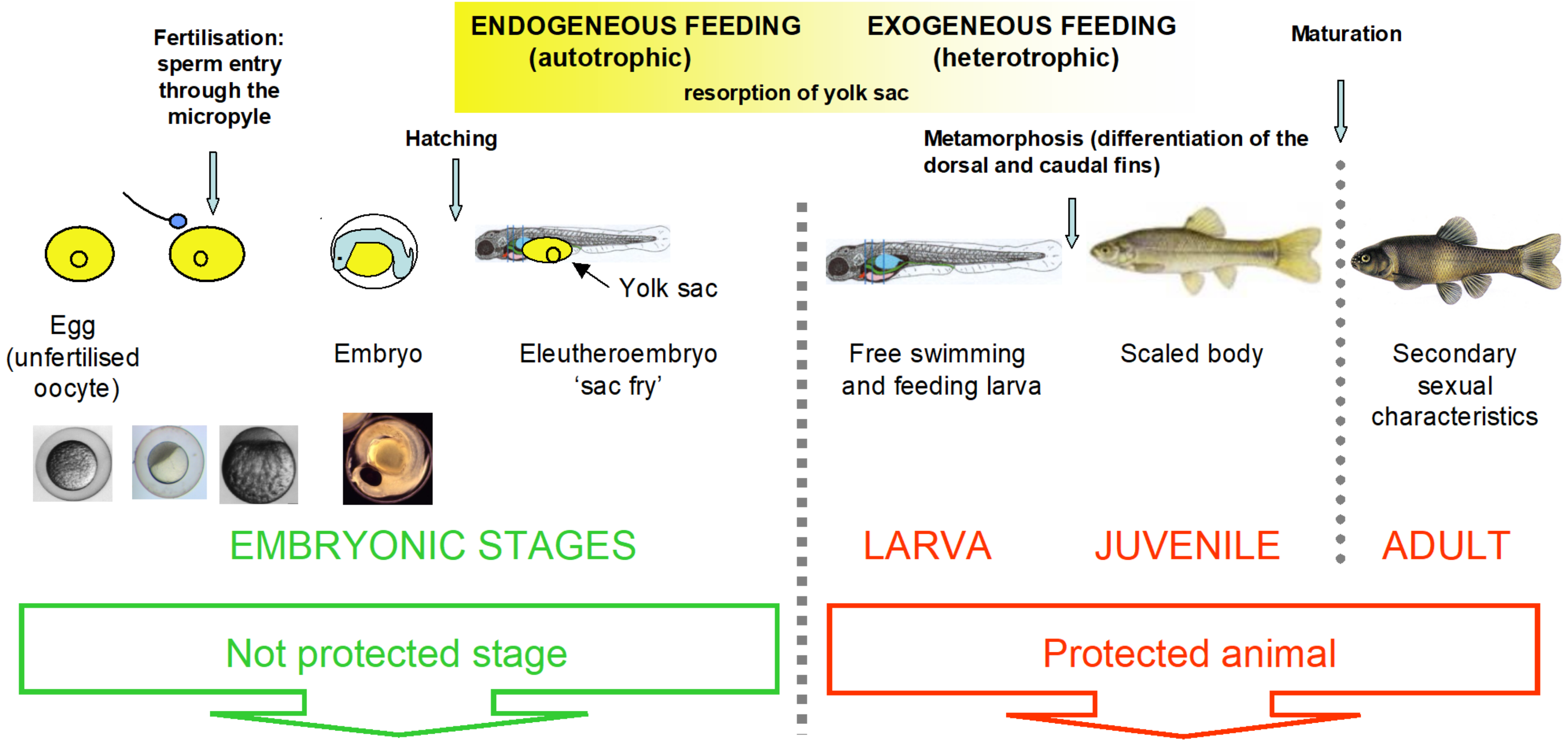
Taken from ECETOC 2005 Technical report 97



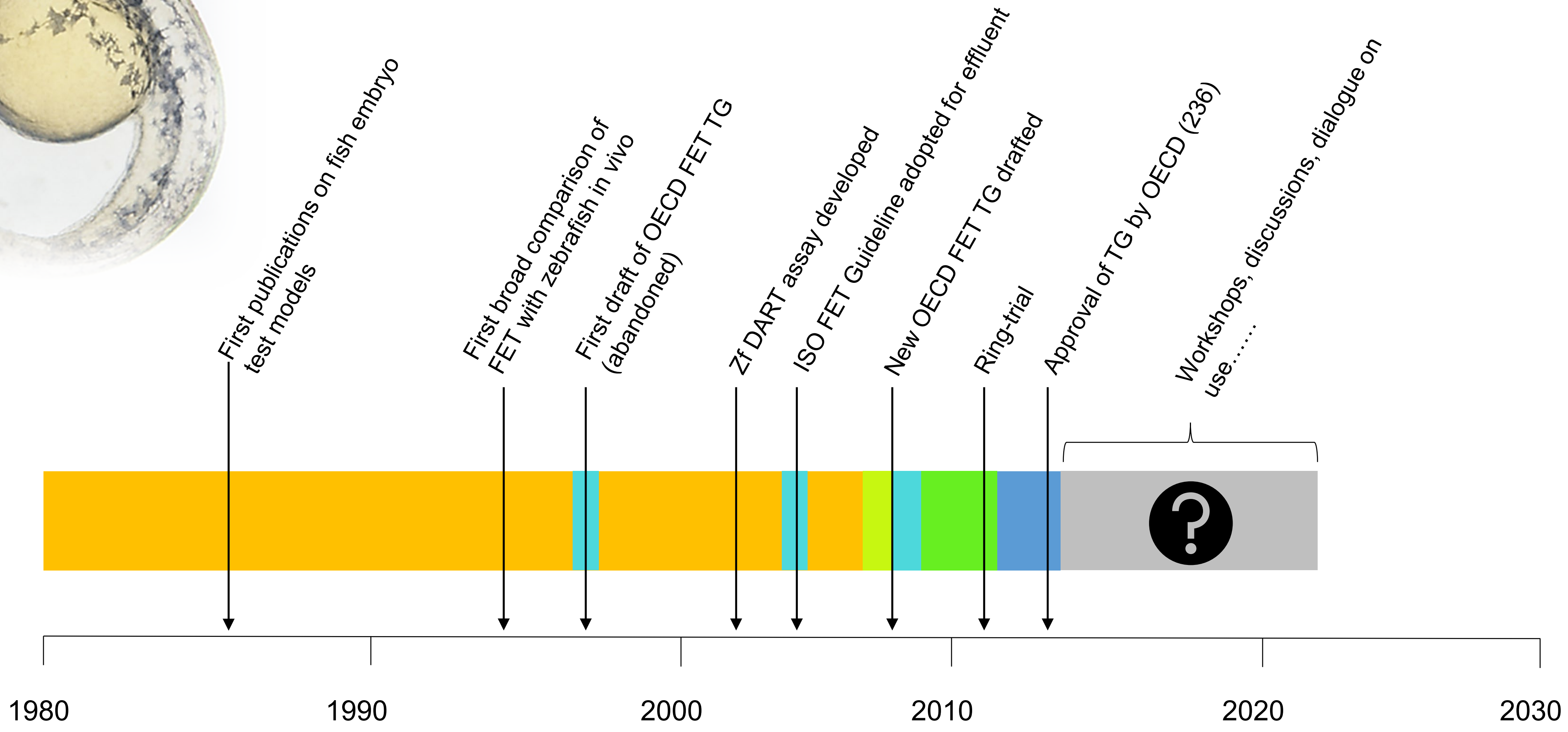
Dir 86/609/EEC: animal means 'any live non-human vertebrate, including free-living larval and/or reproducing larval forms, but excluding foetal or embryonic forms'.

ECVAM (2002): Experiments on lower organisms and vertebrates at early stages of development not classed as 'protected animals'.

UK Animals (scientific procedures) act, 1986: For fish, amphibia and *Octopus vulgaris*, protection extends to certain immature stages of development from the time at which they become capable of independent feeding.

To be replaced/reduced/refined

Some NAM timelines: FET OECD 236



- WoE?

https://swift.hugin.com/models/FET/

HUGIN SWiFT Resources Documentation About SWiFT

A Bayesian network model to predict fish acute toxicity from multiple lines of evidence

By: Jannicke Moe, Adam Lillicrap (Norwegian Institute for Water Research), and Raoul Wolf (Norwegian Geotechnical Institute)
WWW: Anders L Madsen, Mark Christiansen (HUGIN EXPERT)

Latest update: January 2023

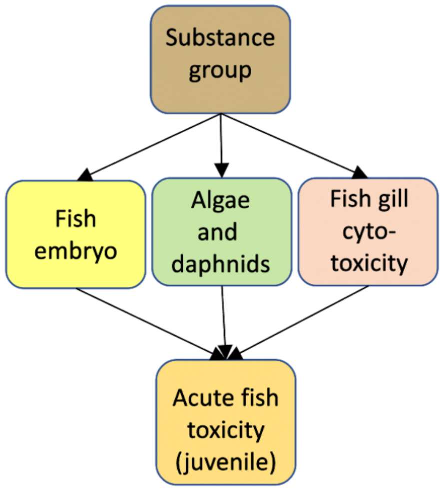
June 2023: under revision

Introduction page | Upload data | Show entered data | Results | Additional information

Bayesian networks (BNs) are gaining popularity in ecotoxicology and ecological risk assessment, because of their ability to integrate different types of data and other information, and to predict the probability of specified states. This example demonstrates the use of a Bayesian network to provide scientific support for decisions on animal testing in ecotoxicology. European legislations require Reduction, Replacement or Refinement of animal testing wherever possible. The use of fish embryos for toxicity testing is considered a promising alternative to the use of juvenile or adult fish. However, fish embryos are not yet accepted as an alternative for regulatory purposes. The European Chemicals Agency (ECHA) has therefore recommended the development of a weight-of-evidence (WoE) approach to evaluate Fish Embyo Toxicity (FET) data in combination with other types of information as a replacement for juvenile fish toxicity data.

We have developed a probabilistic WoE model: a BN to predict the acute toxicity of a substance to juvenile fish based on four lines of evidence (Figure 1). The purposes of this online demonstration version are:

- To demonstrate the functionality of the model by the example substances given below
- To let users predict juvenile fish toxicity for new substances by entering their own data.
- To get feedback from users for improvement of the tool.



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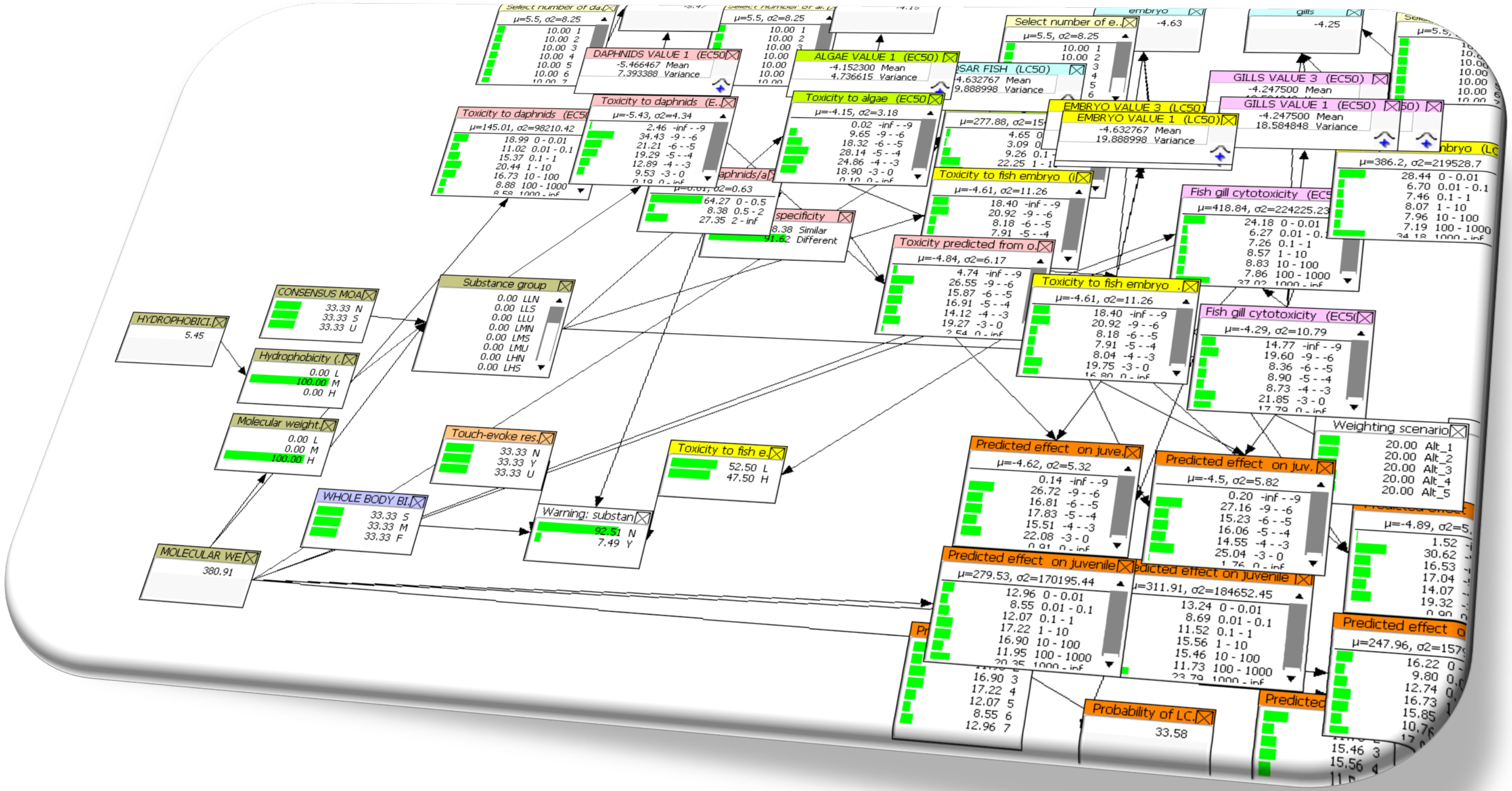
Conditional probability distributions



Actute Fish Toxicity Prediction Report

The results presented below are the output from the SWiFT Bayesian Network model for predicting Acute Fish Toxicity based on multiple lines of evidence. The input data presented in the report were provided by Enter author name from Enter organisation name on 26-10-2021. The producers of the SWiFT model cannot be held responsible for the quality of these data. More information regarding the SWiFT model and guidelines for how to interpret the data can be found at the following website SWiFT - NIVA (https://www.niva.no/en/projectweb/swift) .

Conclusions

The toxicity level of --> Enter substance name<-- to juvenile fish is most likely - (-% probability).

The probability that fish acute toxicity of --> Enter substance name<-- will be medium or higher (LC50 < 5 mg/L) is -%.

The measured endpoint most sensitive to --> Enter substance name<-- is algae.

1. General information

1.1 Date

26-10-2021

1.2 Author

Enter author name

2. Lines of Evidence

2.1 Chemical category of the substance

Node	Orginal value	Orginal unit	Transformed value	Transformed unit	Refrence
MOLECULAR WEIGHT(g/mol)			300	g/mol	
HYDROPHOBICITY(log Kow)			5	g/mol	
CONSENSUS MOA(N/S/U)			-		

2.2 QSAR-predicted toxicity to fish

Node	Orginal value	Orginal unit	Transformed value	Transformed unit	Refrence
QSAR(LC50)			-	mol/L, log10	

2.3 Toxicity to algae

Node	Orginal value	Orginal unit	Transformed value	Transformed unit	Refrence
ALGAE VALUE 1 (EC50)			-	mol/L, log10	

2.4 Toxicity to Daphnia

Node	Orginal value	Orginal unit	Transformed value	Transformed unit	Refrence
DAPHNIDS VALUE 1 (EC50)			-	mol/L, log10	

2.5 Toxicity to fish embryo

Node	Orginal value	Orginal unit	Transformed value	Transformed unit	Refrence
EMBRYO VALUE 1 (LC50)			-	mol/L, log10	

2.6 Fish gill cytotoxicity

Node	Orginal value	Orginal unit	Transformed value	Transformed unit	Refrence
GILLS VALUE 1 (EC50)			-	mol/L, log10	

2.7 Warning

Node	Orginal value	Orginal unit	Transformed value	Transformed unit	Refrence
WHOLE BODYBIOTRANSFORMATION RATE(Km; S/M/F)			-		
Touch-evoke responseof embryo (N/Y/U)			-		
Toxicity to fish embryo (L/H)			-	mg/L	
Warning: substance flagged asoutside of applicability domain			-		

3. Output values

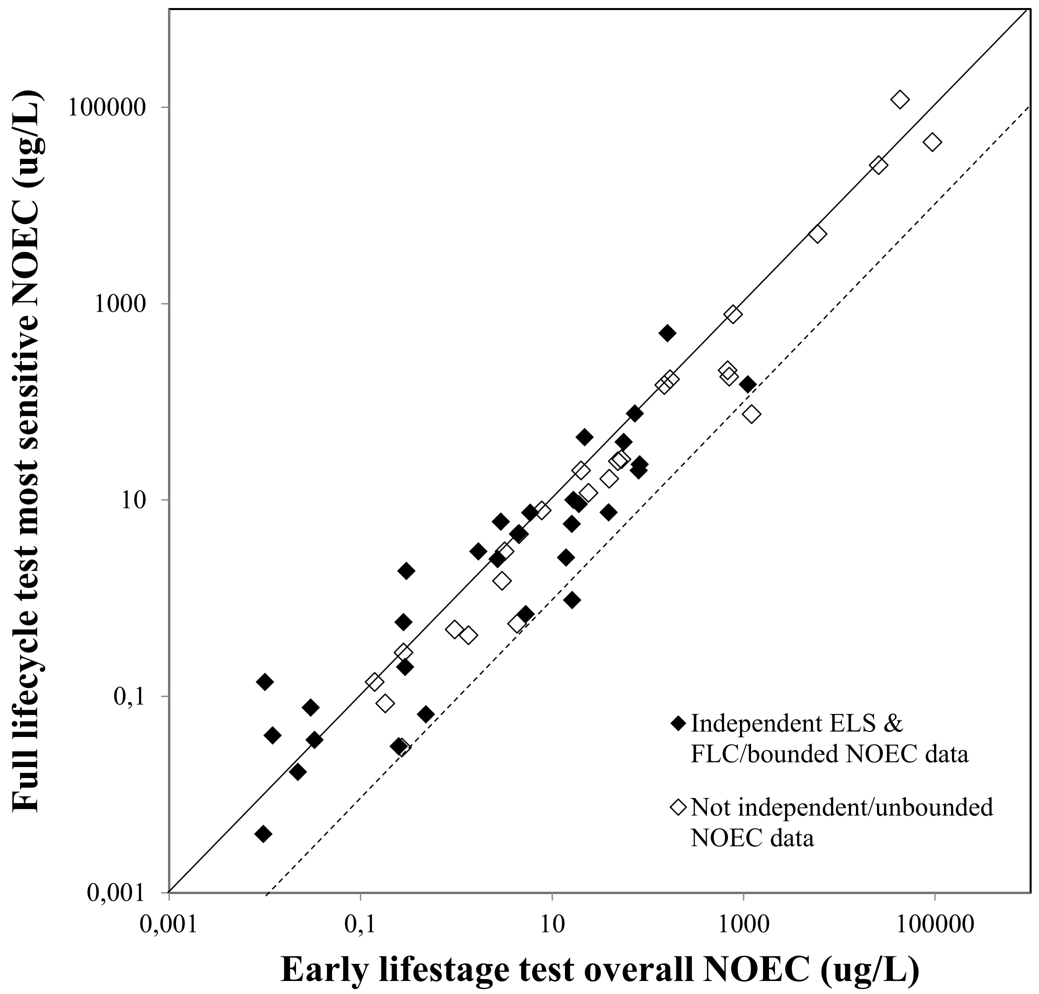
3.1 Physical and chemical properties of the substance

State	Toxicity Level	Probability	Unit
Predicted effect on juvenile fish(LC50, mg/L)	0 - 0.01	0.2	mg/L
Predicted effect on juvenile fish(LC50, mg/L)	0.01 - 0.1	0.12	mg/L
Predicted effect on juvenile fish(LC50, mg/L)	0.1 - 1	0.19	mg/L
Predicted effect on juvenile fish(LC50, mg/L)	1 - 10	0.23	mg/L
Predicted effect on juvenile fish(LC50, mg/L)	10 - 100	0.16	mg/L
Predicted effect on juvenile fish(LC50, mg/L)	100 - 1000	0.06	mg/L
Predicted effect on juvenile fish(LC50, mg/L)	1000 - inf	0.03	mg/L

3.2 Toxicity of the substance's chemical cateogory

Current alternatives

- OECD 210 Fish Early Life stage test is already an alternative to full life cycle designs
    - Macek and Sleight 1977
    - McKim 1977
    - Wheeler et al 2014



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Year	Milestone
2005	ECETOC task force report (97) Alternative Testing Approaches in Environmental Safety Assessment
2007	Animal Alternatives Global Interest Group at SETAC established
2008	HESI-ECETOC International Workshop on the Application of the Fish Embryo Test as an Animal Alternative Method in Hazard and Risk Assessment and Scientific Research
2010	European network for alternative testing strategies in ecotoxicology (Euroecotox, FP7
2016	"Concepts, Tools, and Strategies for Effluent Testing" HESI workshop on alternatives for effluents
2022	ONE conference- ONE – Health, Environment, Society
2023	HESI-NC3Rs Not another NAMS meeting- joint ECO and human safety
2023	ECHA EFSA NAMs workshop- mainly human safety limited ECO
2023	World Congress on Alternatives- first time for an ECO session
2023	HESI workshop on alternatives to chronic fish tests
2023	EFSA Knowledge and Innovation Communities (KIC) on Environmental Risk Assessment (ERA) and New Approach Methodologies (NAMs) workshop (NAMs4ERA)
2023	EPAA workshop on NAMs for ecotoxicity
2023	European Commision workshop on developing a roadmap for alternative approaches
2023	OECD stakeholder workshop on operational and financial aspects of validation of test guideline

Alternatives to Chronic Fish Toxicity

- Aimed at developing a roadmap to guide alternative strategies for the assessment of chronic toxicity to fish.
- New lines of thought:
  - ***Shift from replacement → integrated approaches***
  - *NAMs for chronic fish toxicity are available; need to map, evaluate, and link*
  - *Need for an approach to prevent chronic fish testing (e.g., waiver type approach)*

Alternatives to Chronic

- *NAMs can be used to refine / reduce uncertainty*
- *NAMs can help with grouping approaches to identify specific concerns for chronic fish toxicity*
- *Efforts should be made to link with the population modeling community*
- *Must integrate existing and NAM approaches to create links across methods, endpoints, and species / taxa*

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- REPLACEMENT not a short term fix
- NAMs may aid strengthen waiving tests
- Need harmonization between different initiatives
- Other Taxa under represented
- Intelegant Testing Stratgies

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ecoNAM

Home

ecological Network for Alternative Methods

ecoNAM is a platform to faciliate international information exchange about animal alternatives for ecological safety assessment of chemicals. Our aim is to foster cooperation between government, academic, industry, NGOs, research centers, and other stakeholders involved in the research, development, and application of alternative ecological hazard and risk assessment tools and methods.

The goal of ecoNAM is to facilitate the exchange of knowledge, data, and expertise to promote synergies and collaboration related to advances in ecological alternative methods on a global scale. If you would like to join the ecoNAM platform, please fill in the membership form below.

As of November 2023, we are trying to gauge interest in and resources and need for such a network and will determine next steps and a feasible path forward in the coming months.

Your personal data provided in the registration form will be treated in accordance with the EU general data protection regulation 2016/679 (GDPR).

Please complete the form below to note your interest and add yourself to our distribution list.

Norwegian institute for water research

Thank you for listening