Strategies to reduce the number of fish used in ecotoxicity tests

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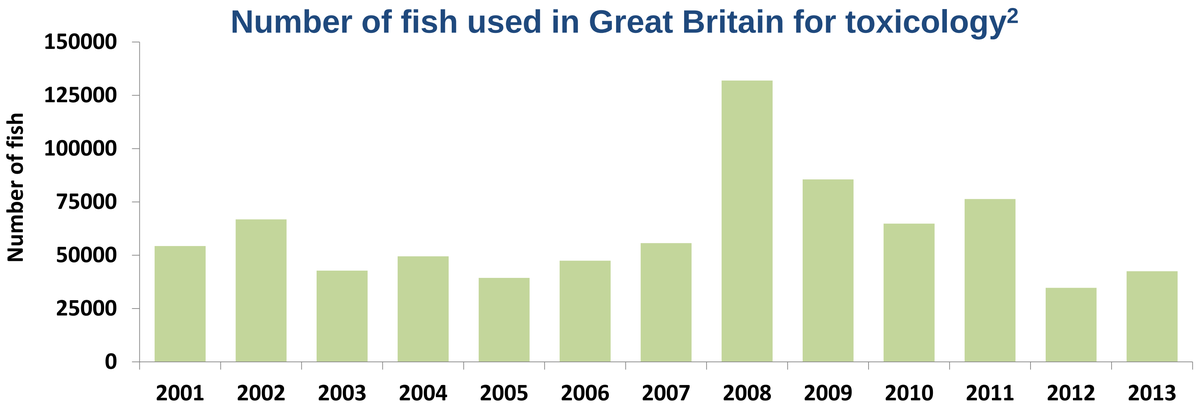
Introduction

- In 2011, nearly 180,000 fish were used for toxicological and other safety assessments in Europe.[1] This number is likely to rise in advance of the 2018 REACH deadline.

Number of fish used in Great Britain for toxicology[2]



- As few nonanimal methods are available to assess the ecotoxicity of chemicals, strategies that reduce the number of animals used in existing tests are urgently needed.

Number of fish used in OECD Test Guidelines for REACH

OECD Test Guideline (TG)	# fish per control	# test concentrations	# fish per test concentration replicate	# of replicates	Total # fish per test if solvent used	# fish saved if no solvent used (%)
TG 203: Fish, Acute Toxicity[3]	7	5	7	1	49	7 (14)
TG 215: Fish, Juvenile Growth[4]	16	5	16	1	112	16 (14)
TG 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages[5]	30	5	10	3	210	30 (14)
TG 210: Fish, Early-life Stage Toxicity[6]	20	5	20	4	560	80 (14)
TG 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure (e.g., aq exposure)[7]	36	2	4	9 time points	144	36 (25)

Experimental design

- In ecotoxicity tests, the test chemical is usually added to the tank water. To overcome practical issues associated with testing poorly soluble chemicals, a small volume of solvent is often used. As the solvent can influence the outcome of the study, two controls - one in the presence of and one in the absence of solvent - are currently required, doubling the number of control fish and having significant animal welfare implications.

Aims

1. Statistical evaluation of historical and simulated data to determine if one control - either the solvent or water control - can be eliminated from ecotoxicity studies when a solvent is used
2. Promote use of alternative methods to solvents

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Use one control when a solvent is used

Possible solvent effects:

- None
- Additive
- Subtractive
- Synergistic
- Antagonistic

- Use of solvent and water controls is often cited as necessary to spot interactions between the solvent and test chemical. However, the very fact that the solvent is being used speaks to a poorly soluble chemical, which calls into question the validity of the approach.

- If only one control is used when a solvent is necessary, the solvent control is favourable and the water control should be eliminated from the study.

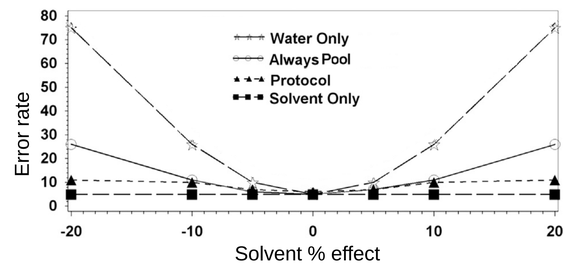
Control used for statistical analysis varies according to region,[8] guideline and test results:

- Water control
- Solvent control (e.g., OECD 2006[9])
- Always pool water and solvent controls
- Protocol: pool controls unless controls are significantly different in which case only use the solvent control (or vice versa, e.g., United States Environmental Protection Agency[10])

Statistical Simulations[11]

Power to detect a 10% treatment effect[11]							
Solvent effect	Control used	Power (%) Two- sided	Power (%) One-sided	Solvent effect	Control used	Power (%) Two-sided	Power (%) One-sided
0	Water	26	38				
	Solvent	26	38				
	Always pool	35	47				
	Protocol	35	47				
5	Water	51	65	- 5	Water	10	17
	Solvent	26	38		Solvent	26	38
	Always pool	49	62		Always pool	21	32
	Protocol	45	58		Protocol	25	36
10	Water	75	85	- 10	Water	5	5
	Solvent	26	38		Solvent	26	38
	Always pool	62	75		Always pool	11	18
	Protocol	49	60		Protocol	21	30

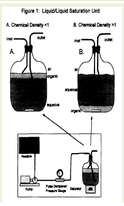
False Positive Error Rates of Control Choices shows the probability (%) of indicating a significant treatment effect as a function of the choice of control, when there is actually no treatment[11]



Future work

- Determine extent that simulations fit historical data and frequency of each scenario.
- Assess regulatory impact by recalculating effect levels assuming only solvent control is used.
- If using only one control is feasible, revise test guidelines and regulatory requirements.

Alternatives to using solvents

Traditional methods[12]	Saturation columns	Liquid-liquid saturation units[16]
• Prolonged stirring /high-shear mixing • Solvent evaporation	• Temperature adjustment • Large water volumes • Ultrasonification • pH adjustment	• Passive or flow through dosing • Excess chemical added to inert carrier (e.g., silica gel, glass wool) moves passively into water[13,14]  15	• Used to test poorly soluble liquids • Generates saturated solutions without undissolved or emulsified material 

Summary

- Need for water control is scientifically questionable when a solvent is used.

Advantages of using single control	Disadvantages of using a single control
• ~ 14-25% fewer animals • All animals included in analysis • Fewer resources (e.g., time/money) • Single approach adopted • Lower false-positive rates	• Decrease in power in some instances • Revisions to TGs and regulatory requirements needed

- Review of historical and simulated data is needed to determine which controls are required.
- Regulatory harmonisation and mutual acceptance of data is required to implement changes to controls used in regulatory testing.
- Methods that avoid solvents are available and dramatically reduce fish use.
- Animal welfare must be considered when determining control requirements.

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