STRATEGIES FOR REDUCING THE NUMBER OF FISH USED IN AQUATIC TOXICITY TESTS

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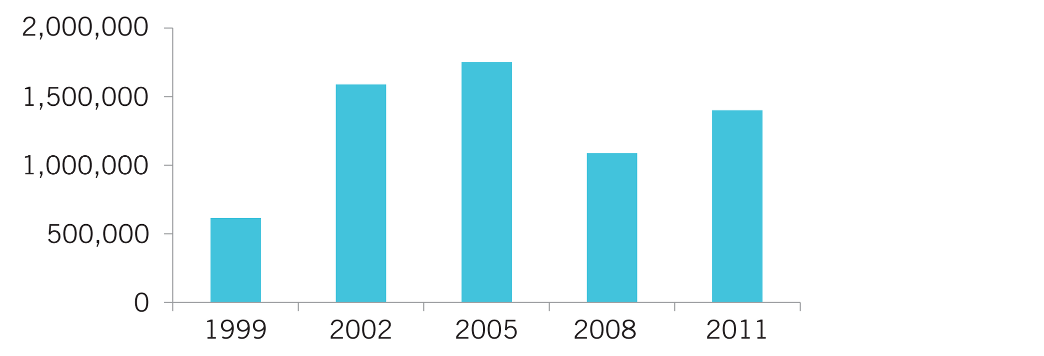
INTRODUCTION

- 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 THE EUROPEAN UNION FOR SCIENTIFIC PURPOSES2



EXPERIMENTAL DESIGN

- In aquatic toxicity 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 added. As the solvent can infuence 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 signifcant animal welfare implications.

NUMBER OF FISH USED IN OECD TEST GUIDELINES FOR REACH

OECD Test Guideline (TG)	# fish per control	# test concentrations	# fish per test concentration replicate	# 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. aquatic exposure)[7]	36	2	4	9 time points	114	36 (25)

AIMS

- Statistical evaluation of historical and simulated data to determine whether one of the controls can be eliminated from aquatic toxicity studies when a solvent is used
- Promote use of advanced techniques to avoid the use of solvents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USE ONLY ONE CONTROL WHEN A SOLVENT IS ADDED

POSSIBLE SOLVENT EFFECTS

- Additive
- Subtractive
- Synergistic
- Antagonistic


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Current study design (ie, use of two controls) does not allow these interactions between the solvent and test chemical to be determined.

If only one control is used when a solvent is necessary, the solvent control is preferred, and the water control should be eliminated.

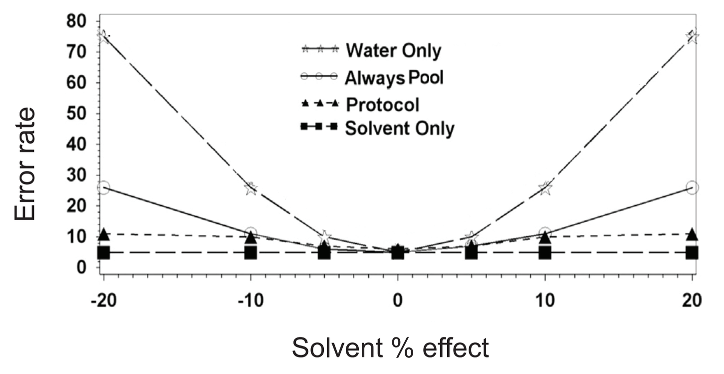
CONTROLS USED FOR STATISTICAL ANALYSIS VARY ACCORDING TO REGION,[8] GUIDELINE AND TEST RESULTS.

- Water control
- Solvent control[9]
- Always pool water and solvent controls
- Always pool water and solvent controls unless they differ significantly, in which case use only the solvent control, or vice versa.[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: The probability (%) of indicating a significant treatment effect when there is actually no treatment depends on the choice of control.[11]



ALTERNATIVES TO USING SOLVENTS

Direct Dosing[12]

- Prolonged stirring
- High-shear mixing
- Solvent evaporation
- Flow-through systems
- Temperature adjustment
- Large water volumes
- Ultrasonification
- pH adjustment

Passive Dosing

- Excess chemical added to inert carrier (eg, silica gel) moves passively into water.[13,14]
- Equilibrium-partitioning occurs between polymer and water
- Losses are compensated for by additional release.

Other Generator Systems for Poorly Water-Soluble Substances

- Liquid-liquid saturation units[15]
- Saturator columns (generate saturated solutions without undissolved or emulsified material)

SUMMARY

- The use of solvents for toxicity testing of poorly water-soluble substances in fish currently requires the use of additional fish in solvent controls.
- The need for a water control is scientifically questionable when a solvent is used.

Advantages of Using a Single Control	Disadvantages of Using a Single Control
• About 14 to 25% fewer animals when solvent used • All animals included in analysis • Fewer resources (eg, time and money) • Single approach adopted • Lower false-positive rate	• There is a decrease in power in some instances. • Revisions to TGs and regulatory requirements will be needed.

- A review of historical and simulated data will be needed to determine which controls are required.
- Regulatory harmonisation and mutual acceptance of data will be required to implement changes to controls used in regulatory testing.
- Methods that avoid solvents altogether are available and significantly reduce fish use.
- Animal welfare must be considered when determining control requirements.
- Existing control data are needed from companies to advance this project.

NEXT STEPS

FUTURE WORK

- Collect control data from fish TGs.
- Conduct statistical simulations to determine whether the water control can be eliminated when solvents are used.
- Promote international harmonisation in use of controls.
- Revise and harmonise TGs and regulatory requirements for controls.

CONTRIBUTE DATA TO THIS PROJECT

- Control data for statistical simulations are needed.
- If you can contribute control data in the presence and absence of a solvent for OECD fish TGs, please go to the QR code or contact **GillyS@piscltd.org.uk** for more details.

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