Reducing the number of controls in fish early life stage toxicity tests when solvents are used

Christopher Faßbender,*[1] John W. Green,[2] John Handley,[3] Thomas H. Hutchinson,[4] Lennart Weltje,[5] Daniel Faber,[6] Gilly Stoddart[1]

*corresponding author: christopherf@thepsci.eu
[1]PETA Science Consortium International e.V., (PSCI) Stuttgart, Germany
[2]John W Green Ecostatistical Consulting LLC, Newark, DE, USA
[3]JH Consulting, Derby, UK
[4]Reckitt Benckiser Group PLC, Hull, UK
[5]BASF SE, Agricultural Solutions – Ecotoxicology, Limburgerhof, Germany
[6]Bayer AG, Division Crop Science, Environmental Safety, Monheim am Rhein, Germany

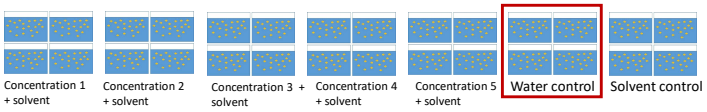
Introduction

When a test chemical requires a solvent to facilitate its dissolution for aquatic toxicity testing, a water control and a solvent control are required.

The use of only one control would substantially reduce the number of animals used by 17% (80 fish) in the **fish early life stage toxicity** (FELS; OECD Test Guideline 210 or US EPA OCSPP 850.1400) study (Figure 1). Using collected and simulated FELS data, the United States and PSCI are leading an investigation to determine whether **using only the solvent control affects the determination of EC_x** (concentration causing x% effect) **and NOEC** (No Observed Effect Concentration; Project 2.55 on the OECD Test Guidelines Programme work plan). If not, this provides a statistical basis for the revision of protocols and regulatory practice.

Use of the water control cannot help identify effects of the test chemical, because the test chemical is not tested in the absence of solvent. Furthermore, combination effects between solvents and test chemicals tend to be additive. Also, the low toxicity of widely used solvents is well known.[1-5]

Figure 1. The FELS study uses 560 fish if both controls are included.



Statistical approach

A **database of control and concentration-response data for all endpoints from FELS studies** (Table 1) using fathead minnow, rainbow trout or sheepshead minnow with the solvent dimethylformamide (DMF) is being analysed.

Ongoing investigations based on collected and simulated FELS data using SAS 9.4 software include:

- Analysis of the control data distributions (means, between- and within-replicate variances) for water, solvent, and pooled controls for any endpoint to **identify systematic differences between the two controls.**
- Analysis of concentration-response data to **investigate the effect of the choice of control (water, solvent or pooled) on the estimated treatment effect (NOEC, EC_x regressions)** and develop respective concentration-response curves to give side-by-side comparison of results.
- Example computer simulation results show the implications of control choice (Table 2). We explored model selection criteria and model averaging on ECX estimation in relation to the choice of controls.

Table 1. Endpoints analysed in FELS studies

Discrete endpoints	Continuous endpoints
Time to hatch, % hatch	Length
% survival (embryos, larvae)	Weight
Behavioural / morphological abnormalities	Survival proportions treated as continuous
Time to swim-up (rainbow trout)	

Table 2. Computer simulations.

Responses	Models
Continuous (length, wet/dry weight)	Bruce-Versteeg, 3-parameter log-logistic, Brain-Cousens hormetic, and four exponential models.
Quantal responses (survival, abnormalities)	Bruce-Versteeg model was replaced by probit model. The other non-hormetic models listed can be used but with a conditionally binomial error structure in a generalized non-linear mixed model (GNLMM) with adjustment for overdispersion as needed.
Time-to-event (first or last day of hatch or swim-up)	Limited variation in values usually makes regression impractical, but GNLMM with Poisson error structure is sometimes useful. More often, only NOEC methods (Jonckheere-Terpstra test) are needed.

Results & Discussion

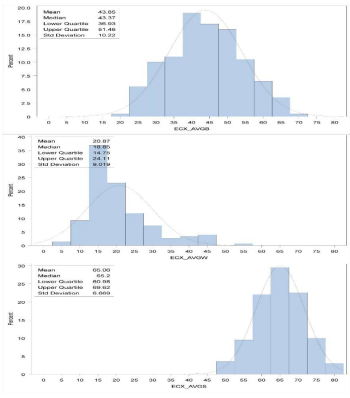


Figure 2. EC_{10} estimates for moderate chemical effect for fathead minnow length; 20% decrease in high dose simulated in moderately steep dose-response with 10% solvent effect additive to treatment effect.

True EC_{10} = 64.5.

- Using pooled controls, distribution is shifted and skewed left (top panel).
- Using water control, distribution is strongly shifted and skewed left (centre panel).
- **Using solvent control, distribution is symmetric and centered near true value** (bottom panel).

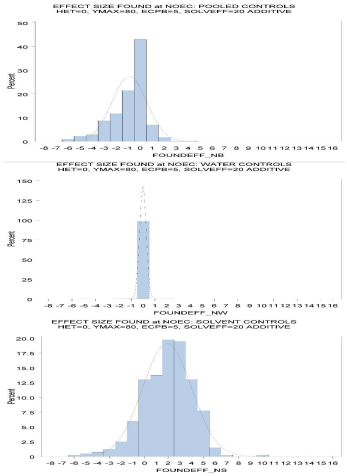


Figure 3. Effect of control choice on NOEC for fathead minnow length, solvent effect in same direction to treatment effect; 20% decrease in high dose, 20% additive effect.

- Using pooled controls, the observed effect at the NOEC is 0 or negative in most simulated studies (top panel).
- Using water control, the NOEC=0 in 99% of simulated studies even though the simulated effect was 0-7% in the lowest test concentration (centre panel).
- **Using solvent control, a significant effect is always found at least in the high dose and the effect found at the NOEC < 10%** (bottom panel).

The false positive rate is high when using the pooled or water control. Using the solvent control, it is controlled at 5%

1) Simulations based on additivity demonstrate that the power (for NOEC) and sensitivity (for $EC_{10/20}$) properties obtained from using only the solvent control are comparable to those when there is no solvent and only the water control is used.
2) In some cases, power can be higher using only the water control or pooled water and solvent controls, but only at the expense of false positive rates as high as 40%.
3) **$EC_{10/20}$ estimates tend to be less variable when only the solvent control is used**, and this is further enhanced through model averaging.
4) The sensitivity of $EC_{10/20}$ estimates is indicated by their distribution and relation to the known true value underlying the simulation, **and sensitivity is shown to improve using only the solvent control**.

Figures 2 and 3 show examples for fathead minnow length. Similar results were found for other responses and species and for EC_{10}LB estimates (lower bound of the confidence interval of the EC_{10} value).

If there is a solvent effect additive to the treatment effect, serious bias occurs if water or pooled controls are used in modelling.

These findings provide evidence supporting the omission of the water control and using only the solvent control in FELS studies.

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The views, conclusions, and recommendations expressed in this poster are those of the authors and do not necessarily represent the policies or positions of the PETA Science Consortium International e.V., the International Council on Animal Protection in OECD Programmes, Charnwood Molecular LTD, Reckitt Benckiser Group PLC, BASF SE, or Bayer AG.

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