Is there evidence for omitting the water control in fish early-life stage toxicity tests when solvents are used?

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Background

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

Using a solvent control alone would substantially reduce the number of animals used by 14% (80 fish) in the **fish early life stage toxicity** study (FELS; OECD Test Guideline 210 or US EPA OCSPP 850.1400; Figure 1).

Using collected and simulated FELS data, this project (Project 2.55 on the OECD Test Guidelines Programme work plan) is investigating whether **using only the solvent control affects the determination of EC_x** (concentration causing x% effect) **and NOEC** (No Observed Effect Concentration). This research provides a statistical basis for the revision of protocols and regulatory practice.

It cannot be evaluated whether there is an interaction between the solvent and the test chemical, unless the chemical is tested in the absence of a solvent.

Combination effects between solvents and test chemicals tend to be additive and the low toxicity of widely used solvents is well known.[1-5]
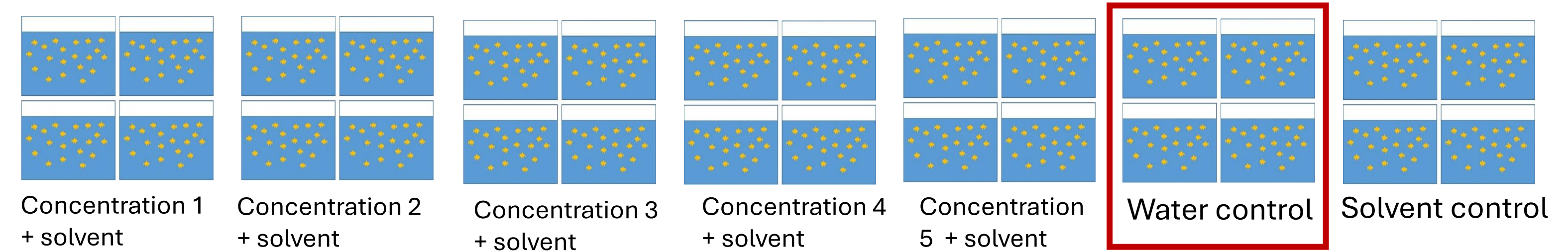
Concentration 1 + solvent | Concentration 2 + solvent | Concentration 3 + solvent | Concentration 4 + solvent | Concentration 5 + solvent | Water control | Solvent control

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 measurement endpoints from FELS studies** (Table 1) using freshwater Fathead Minnow or Rainbow Trout, or marine Sheepshead Minnow with the solvent dimethylformamide (DMF) has been analysed.

Investigations based on **both collected and simulated FELS data using Statistical Analysis System (SAS® 9.4) software** (Table 2) include:

- Analysis of the control data distributions (means, between- and within-replicate variances) for water, solvent, and pooled controls for FELS' endpoints 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.

- Exploration of model selection criteria and model averaging on EC_x estimation in relation to the choice of controls to increase the likelihood of obtaining a useful EC_{10} estimate.

Table 1. Endpoints analysed in FELS studies

Discrete endpoints	Continuous endpoints
Time to hatch, % hatch	Length
% survival (embryos, larvae)	Weight (wet/dry)
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

Are there systematic differences between solvent and water controls in collected data using Fathead Minnow / DMF and data from Oris *et al.* (2012)[6]?
- For all responses, some studies exhibited differences >5%.
- For all except length, some study differences >10% were detected. For dry and wet weight and survival, this was the case in 67, 58, 36% of studies, respectively.

However, **neither control is consistently higher or lower than the other.** Only dry weight and hatching are 2.5% lower, and 1% higher, respectively, in the water control than in the solvent control.

What is the influence of the choice of control on statistical power?

80% power to detect commonly accepted levels of effects using only the solvent control. EC_{10} estimation is generally possible if there is ≥15% maximum effect, often possible if 10%. Case studies for the three fish species with DMF show very little difference in power among control choices.

Is the NOEC influenced when using the water, solvent or pooled controls?

- 1000 wet weight datasets were simulated for a shallow concentration-response curve with homogeneous variance across treatment groups, normal distributions within treatment groups, and treatment range 0-100 ppm.
- The NOEC determined from the water (W) and pooled controls (P) is much more likely (34.1% and 9.9%, respectively) to be 0 than under the solvent control (S; 1.4%).
- **Use of water control increases likelihood of an unrealistically low NOEC.** Using the water control (W), a high percentage of NOECs found result from tiny effects (e.g., 0.02%) that were statistically significant.

% effect at 0, 6.25, 12, 25, 50, 100 ppm
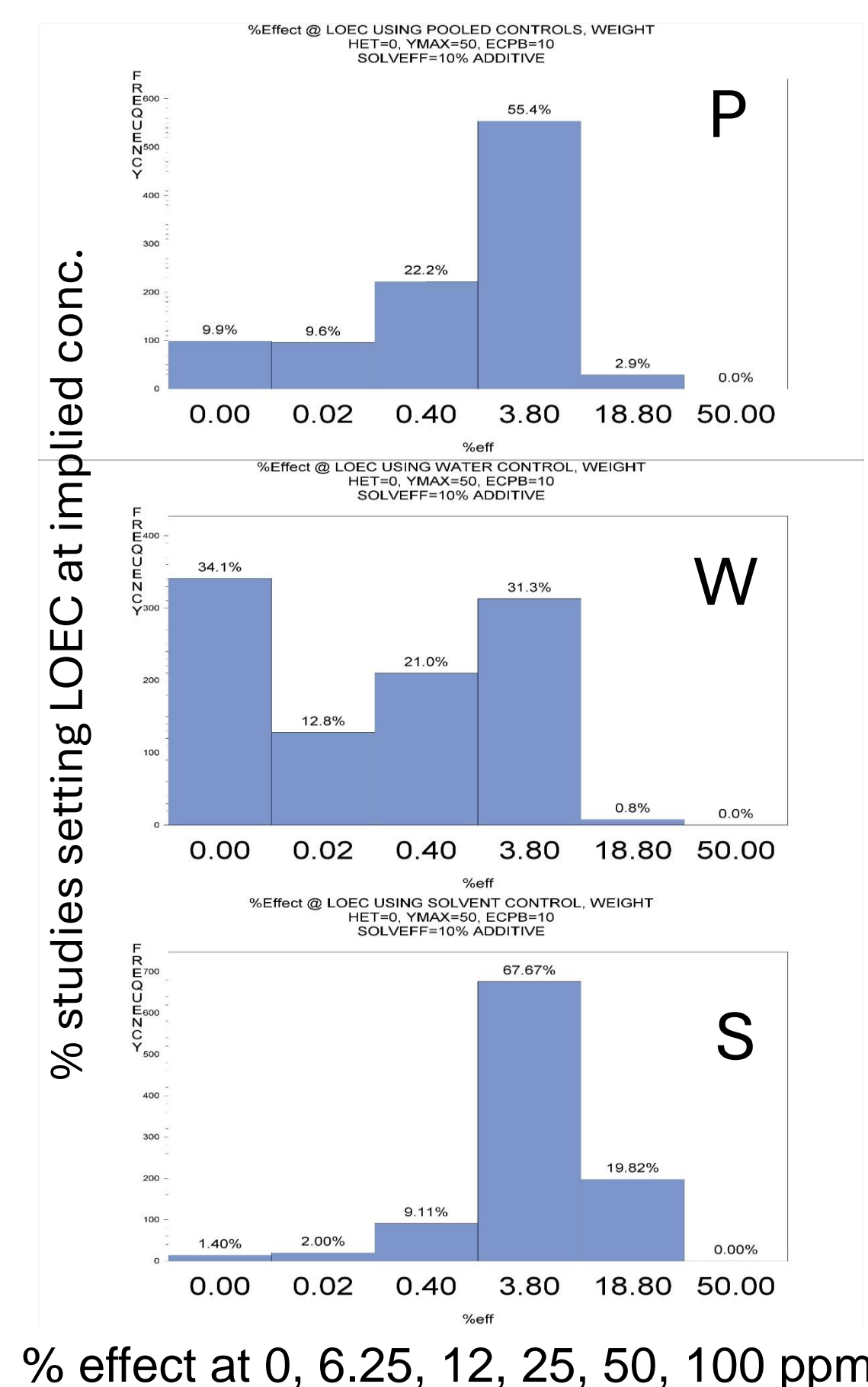
Is EC_x regression influenced when using the water, solvent or pooled controls?

Simulated Fathead Minnow length data with 20% decrease at high concentration in moderately steep conc.-response with 10% solvent effect additive to, and in same direction as treatment effect, variance homogenous, n=200.
True EC_{10}=64.5 (blue line)

Distribution of EC_{10} estimates:
- Using pooled controls (P), distribution is shifted & skewed left.
- Using water control (W), distribution is strongly shifted and skewed left.
- Using solvent control (S), distribution is symmetric and centered near true value.
- Case studies for the three fish species with DMF show that the confidence interval width for EC_{10} estimates varies little among control choices.

EC_{10} estimates based on solvent control (S) are centred closer to the true value than those based on water (W) or pooled (P) controls.
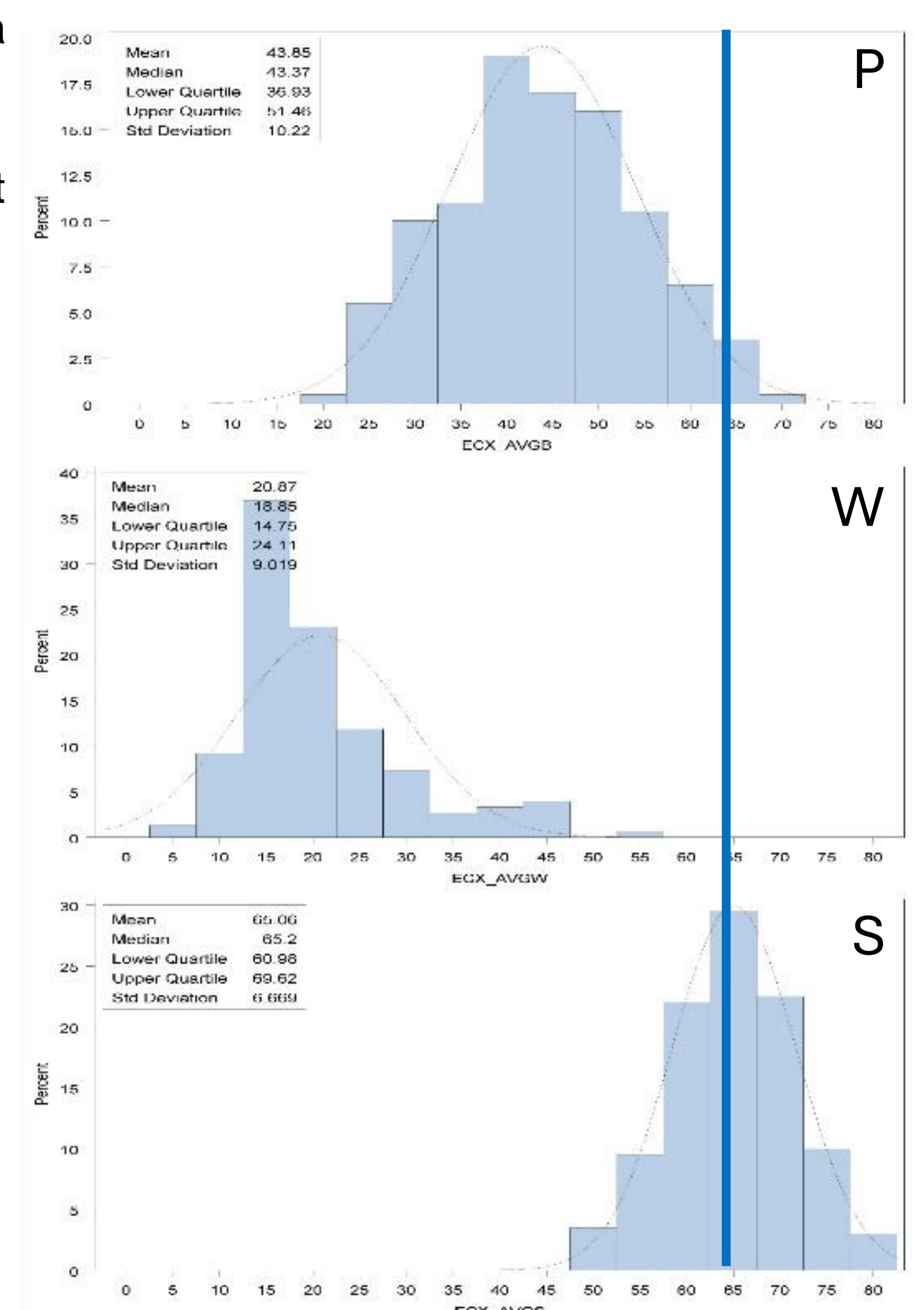
Taken together, this is evidence based on DMF supporting the omission of the water control and using only the solvent control in FELS studies.

Acknowledgements

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