Progressing statistical analysis for regulatory ecotoxicology: developments, processes, and opinions

Raoul Wolf[1,*], David R. Fox[2,3], and S. Jannicke Moe[4,5]

[1]Norwegian Geotechnical Institute (NGI), Sandakerveien 40, 0484 Oslo, Norway

[2]University of Melbourne, Parkville, Victoria 3010, Australia

[3]Environmetrics Australia, Beaumaris, Victoria 3193, Australia

[4]Norwegian Institute for Water Research (NIVA), Økernveien 94, 0579 Oslo, Norway

[5]IEAM Deputy Editor

*Corresponding author: Raoul Wolf. E-mail: raoul.wolf@ngi.no

Editor's Note: This article is part of the special series "Statistical Analysis of Ecotoxicology Data for Regulatory Purposes." The series provides an overview of approaches in statistical ecotoxicology and reflects recent developments, processes and debates. The papers represent viewpoints from academia, industry and government.

Data availability statement

No data were generated or used in the creation of this brief communication article.

Funding

R.W. acknowledges funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 101036756, project ZeroPM: Zero pollution of persistent, mobile substances. S.J.M. received funding from the Research Council of Norway (contract number 342628/L10).

Conflicts of interest

The authors declare no competing conflicts of interest.

Acknowledgements

The authors thank John Toll for his constructive and insightful comments on a draft version of the article.

Progressing statistical analysis for regulatory ecotoxicology: developments, processes, and opinions

Abstract

Statistical methods and computational tools applicable to ecotoxicology have developed and improved over recent decades, but regulatory risk assessments haven't kept pace, instead relying on outdated statistical methods and tools. Sessions in recent SETAC meetings dedicated to the development of statistical methods and tools for ecotoxicology have generated a high level of interest and discussion. Ecotoxicologists have expressed interest in the revision of the 2006 OECD document No. 54, "Current approaches in the statistical analysis of ecotoxicity data: a guidance to application" because they believe that the guidance is no longer reflective of contemporary statistical methods and tools; its revision is ongoing. Against this background, a call for papers for Special Series in IEAM was launched in autumn 2024 with manuscript submissions accepted until summer 2025. The purpose of this Special Series was to survey the statistical ecotoxicology landscape and reflect on recent developments, processes, and opinions. The editors of the Special Series welcomed and encouraged contributions and viewpoints from all employment sectors. The resulting series comprises eleven papers (seven original articles, three brief communications, and a workshop synthesis), with authors from academia, industry, government, and other research organizations. The series covers topics such as: recent progress in concentration-response modelling and hypothesis testing, including use of generalized linear models; strengths and weaknesses of established toxicity metrics (EC_X, NOEC, BMD); application of toxicity metrics for species sensitivity distribution (SSD) modelling; added values of mechanistic effect modelling; and case studies highlighting opportunities and challenges related to the various statistical methods. This introductory paper aims to give an overview of the papers in the Special Series, summarizes their main topics and methods, and addresses further challenges to progress statistical analysis for regulatory ecotoxicology.

Keywords: concentration-response modelling, generalized linear and additive models, mixed-effect models, hypothesis testing, regulatory statistics

Key points:

1) The ongoing revision of guidance for statistical analysis of ecotoxicity data has inspired scientific developments and debates involving industry, government, academia and other research organizations.
2) This paper introduces a Special Series of eleven papers, which cover novel approaches, applications, and assessments of methods related to concentration-response modelling, hypothesis testing, and derived toxicity metrics for regulatory use.
3) Updated guidance on statistical analysis should be followed by revised recommendations for experimental design for ecotoxicity studies.

Introduction

Statistical methods and computational tools applicable to ecotoxicology have developed and improved over recent decades, but risk assessments for regulatory purposes have not kept pace and rely on outdated statistical methods and tools that are no longer considered state of the art (Laskowski, 1995; Moe et al., 2025; OECD, 2002; Wasserstein & Lazar, 2016). OECD document No. 54 ("Current approaches in the statistical analysis of ecotoxicity data: a guidance to application") was published in 2006 and contained practical recommendations for statistical analysis of experimental test results in ecotoxicology (OECD, 2006). In the 20 years since its release, statistical best practices have evolved, also within ecotoxicology.

Since 2024, the revision of OECD document No. 54 is ongoing, and a revised version is expected for publication in 2027. The initial phase of this revision process, led by the German Environment Agency (*Umweltbundesamt*; UBA), was publicly presented as a poster during the SETAC Europe 34th Annual Meeting (Daniels et al., 2024). In September 2024, the UBA also organized a workshop with a hybrid part to present the initiative and an in-person part to identify and discuss priority topics, as well as an additional online recap event for this workshop (Daniels et al., 2025a). Inspired by this initiative, the guest editors of this Special Series have expressed both short-term wishes and long-term visions for the revision of OECD document No. 54 and beyond (Moe et al., 2025).

Set against this background, the purpose of this Special Series is to provide an overview of the statistical ecotoxicology landscape and reflect on recent developments. Contributions to this Special Series were invited from all employment sectors, i.e., academia, industry, government, and other research organizations. The Special Series was announced through the SETAC website, LinkedIn posts by the guest editors, the online recap event for the UBA workshop, and SETAC Annual Meetings in 2024 (North America) and 2025 (Europe).

Special Series articles summary

The Special Series contains seven original articles, three brief communications, and a workshop synthesis, which showcase current progress in statistical analysis for regulatory ecotoxicology, and exploratory research that will inform future progress, with a strong link to the currently ongoing revision of OECD document No. 54. Article 1 (workshop synthesis) provides broad perspectives for the current developments within the field. Articles 2–6 cover various aspects of concentration-response models for deriving toxicity metrics (EC_X and BMDL). Article 7 addresses the use of such metrics in species-sensitivity distribution (SSD) studies. Articles 8 and 9 focus on developments for deriving no observed effect concentrations (NOECs). Article 10 discusses a method for analyzing ecotoxicity data from field studies. Finally, Article 11 highlights mechanistic effect modelling as a complementary approach to empirical data analysis. Below we provide a summary of each of the eleven articles.

1. Daniels et al., 2025a: “**High time to update statistical guidance in ecotoxicology—a workshop synthesis on the revision of OECD document No.**

54". This paper sets the scene for the Special Series, by providing background information on the revision process and main steps and outcomes through summer 2025. It provides a thorough summary of the workshop activities, debates and main outcomes. The workshop identified the following key topics for improved guidance, as ranked by the number of notecards: (1) improved user-friendliness, (2) guidance on model selection and test settings, (3) scope of OECD document No. 54, (4) improved assessment concepts and statistical approaches, e.g., generalized linear models (GLMs), equivalence testing, alternative toxicity metrics, Bayesian approaches; (5) quality assessment and diagnostics of tests and methods, (6) TK-TD (toxicokinetic-toxicodynamic) modelling and GUTS (General Unified Threshold model of Survival); and (7) software use. The paper highlights the dilemma between scientific drive for progress and regulatory need for standardization, which is a common theme for the following papers.

2. Hill et al., 2025: "**Multilevel modeling of concentration-response data can improve risk assessment: a case study of copper effects on fish**". This study applies a multi-level or mixed generalized non-linear model to account for different sources of variation in a concentration-response model. The authors highlight the multi-level approach for making better use of available data from multiple experiments. This paper also shows the efficient use of a generalized model for fitting a concentration-response model by logistic regression, although with constraints on the shape (symmetrical tails).
3. Erickson et al., 2026: "**Implications of asymmetric concentration-response relationships on effect concentration estimation: A case study regarding the chronic toxicity of NaCl to *Ceriodaphnia dubia***". The case study explores the importance of asymmetry in concentration-response relationships using logistic regression. The authors offer both symmetrical and asymmetrical versions of their model. The symmetric version of their model is similar to the model used by Hill et al. (2025); the asymmetric version allows for skewed tails. Toxicity metrics in the lower tail of the concentration-response relationship, e.g., EC_{10}, were more sensitive to the symmetry assumption than EC_{50} estimates.

4. Daniels et al., 2025b: "**Four-parameter nonlinear regression and maximum achievable effect in ecotoxicology: just visually appealing or relevant for risk assessment?**". This paper explores another aspect of concentration-response models, namely a maximum achievable effect level, as represented by the fourth parameter in a four-parameter regression (4PR). While 4PR models can be analyzed in custom-built tools, the resulting EC_X value, i.e., relative EC_X, can deviate from the absolute EC_X. The authors call for higher transparency regarding the analysis and reporting of relative versus absolute EC_X.
5. Baalkilde & Jensen, 2025: "**Hybrid method benchmark dose estimation with a heterogeneous variance structure**". The benchmark dose (BMD) concept is now established in ecotoxicology but was not covered in OECD document No. 54 in 2006. This paper discusses the importance of proper variance structure in a concentration-response model and demonstrates how the variance structure assumption can affect the resulting lower benchmark dose (BMDL). This approach can be seen as a compromise between a non-linear concentration-response model and a GLM.
6. Cedergreen et al., 2026: "**How normalization of reporter gene *in vitro* assays affects the results**". This paper highlights the importance of rigorous experimental control validation and data normalization in *in vitro* bioassays, particularly when applied to environmental samples, e.g., wastewater. The authors also call for more investigations of how exposure duration affects the response of *in vitro* test systems.
7. Hu et al., 2026: "**Ecotoxicological Risk of Sulfate in Baltic Sea Brackish Water Assessed Using Model-Averaged Species Sensitivity Distribution**". This paper applies a toxicity metric, i.e., EC_{10}, in SSD models to derive predicted no-effect concentrations (PNECs) for environmental risk assessment. Key methodological aspects in this study include the model-averaging of SSDs, and evaluation of alternative assessment factor scenarios to account for different types of uncertainty.
8. Schimera et al., 2025: "**The role of background variability for interpreting biological relevance and statistical significance in Collembola soil field studies**". Based on a field experiment, this study explores the use of closure principal computational approach test (CPCAT) for increasing power in hypothesis

testing. Shortcomings of this approach are identified for field studies with high background variability, e.g., over-dispersed data compared to a Poisson distribution. The authors emphasize the importance of study- and sampling-specific control data for the assessment of biological relevance.

9. Gao et al., 2026: “**The role of statistical power in context: implications for regulatory practices**”. This paper contains a critical review of current statistical practices for hypothesis testing in ecotoxicology, followed by data examples and power comparisons for various statistical approaches. Focusing on NOECs, the authors stress the need for robust methodologies that can accommodate both the complexities of ecological data and the practical requirements of regulatory frameworks.
10. Vermeiren et al., 2026: “**Getting most out of ecotoxicological field data with generalized linear and additive mixed models: a 6-step analysis framework**”. The authors demonstrate the advantages of GLMs and generalized additive models (GAMs) for analyzing responses with different data types, e.g., counts, proportions and continuous data, as well covariates and other potential factors from field studies within a single analysis while considering the repeated sampling. An analysis framework is proposed to guide the use of GLMs and GAMs in ecotoxicology, supported by the long tradition of these methods in ecology.
11. Jager, 2025: “**It’s about time: moving away from statistical analysis of ecotoxicity data**”. This brief communication points out a general shortcoming for statistical methods for ecotoxicology: that toxicity metrics derived from concentration-response models represent a fixed exposure duration, rather than the full exposure pattern as a function of time and argues that mechanistic effect models can better support a more comprehensive concentration-time-response analysis.

Conclusion and recommendations

Statistical practices in ecotoxicology are in flux, and the revision of OECD document No. 54 serves as a catalyst for a critical review of current practices. The contributions to this

Special Series cover many priority topics that were presented during the UBA online recap event, including, for example:

- Parametrization of concentration-response models (Daniels et al., 2025b; Hill et al., 2025; Erickson et al., 2026).
- Use of GLMs – in particular multilevel, i.e., GLMMs –, both for concentration-response models (Hill et al., 2025) and for hypothesis testing (Vermeiren et al., 2026).
- Various data treatment-related issues, e.g., variability (Baalkilde & Jensen, 2025) and scaling (Cedergreen et al., 2026).
- Additional challenges related to analysis and interpretation of field studies (Vermeiren et al., 2026; Schimera et al., 2025).
- Discussions on the appropriateness and relevance of NOECs (Gao et al., 2026; Schimera et al., 2025).
- The use of derived toxicity metrics in downstream assessments (Hu et al., 2026).
- Shortcomings of traditional statistical approaches in ecotoxicology, compared to mechanistic effects models (Jager, 2025).

Several other topics related to statistical analysis have been discussed at the UBA workshops (Daniels et al., 2025a) as well as in other fora. In our view, the following topics merit more in-depth coverage: more extensive use of generalized and multi-level principles for concentration-response modelling; added value of probability-based Bayesian approaches; and equivalence testing as an alternative to traditional hypothesis testing.

Moreover, in the wider context of regulatory risk assessments, the type of toxicity metrics addressed in this series (e.g., EC_X, BMD, and NOEC) are only steppingstones on the path from data to decisions. The conceptual linkage between uncertainties in ecotoxicological studies and regulatory risk assessments would also benefit from statistical scrutiny. Furthermore, updated statistical methods and best practices should influence and optimize guidance on experimental designs.

Two decades after the first guidance documents were released by OECD (2006) and ISO (2006), momentum is finally being built up to improve the use of statistical approaches for regulatory ecotoxicology. Important landmarks, such as the revision of OECD document No. 54, will contribute to improved practices and will advance data literacy within ecotoxicology, both inside and outside of the regulatory context. Further long-term improvements will require continued efforts by the statistics community in ecotoxicology and beyond: better collaboration with regulatory authorities; the crucial role of statistics in supporting the 3 Rs (replace, reduce, and refine the use of animals for toxicity testing); robust and transparent computational solutions; and investment in educational possibilities. Statistical practices have come a long way since OECD document No. 54 was first released 20 years ago, and we are looking forward to the exciting new chapters in the field of statistics for regulatory ecotoxicology.

References

Baalkilde, J. R., & Jensen, S. M. (2025). Hybrid method benchmark dose estimation with a heterogeneous variance structure. *Integrated Environmental Assessment and Management*, vjaf168. https://doi.org/10.1093/inteam/vjaf168

Cedergreen, N., Mandava, G., & Lundqvist, J. (2026). How normalization of reporter gene *in vitro* assays affects the results. *Integrated Environmental Assessment and Management*, vjag012. https://doi.org/10.1093/inteam/vjag012

Daniels, B., Gräff, T., Kotschik, P., & Walter-Rohde, S. (2025a). High time to update statistical guidance in ecotoxicology—A workshop synthesis on the revision of OECD document no. 54. *Integrated Environmental Assessment and Management*, vjaf113. https://doi.org/10.1093/inteam/vjaf113

Daniels, B., Gräff, T., Walter-Rohde, S., & Kotschik, P. (2024). Activities to revise the OECD Document No. 54 (and ISO/TS 20281) on statistical analysis of ecotoxicity data. In *Statistics for Ecotoxicology and Environmental Fate – From Tried and Tested over New and Exciting Methods to Machine Learning* [Poster]. SETAC Europe 34th Annual Meeting, Seville, Spain.

Daniels, B., Hommen, U., & Ratte, M. (2025b). Four-parameter nonlinear regression and maximum achievable effect in ecotoxicology: Just visually appealing or relevant for risk assessment? *Integrated Environmental Assessment and Management*, vjaf085. https://doi.org/10.1093/inteam/vjaf085

Erickson, R. J., Mount, D. R., Forsman, B. B., Highland, T. L., Hockett, J. R., Jenson, C. T., & Norberg-King, T. J. (2026). Implications of asymmetric concentration-response relationships on effect concentration estimation: A case study regarding the chronic toxicity of NaCl to *Ceriodaphnia dubia*. *Integrated Environmental Assessment and Management*, vjag014. https://doi.org/10.1093/inteam/vjag014

Gao, Z., Hothorn, L. A., Settles, L., & Green, J. W. (2026). The Role of Statistical Power in Context: Implications for Regulatory Practices. *Integrated Environmental Assessment and Management*, vjag011. https://doi.org/10.1093/inteam/vjag011

Hill, R., Pyper, B., & Engelking, S. (2025). Multilevel modeling of concentration-response data can improve risk assessment: A case study of copper effects on fish. *Integrated Environmental Assessment and Management*, vjaf138. https://doi.org/10.1093/inteam/vjaf138

Hu, X., Leppänen, M. T., Lehtonen, K. K., Karjalainen, A. K., Järvistö, J., Mäkinen, M., Samarawickrama, R., Strode, E., & Karjalainen, J. S. (2026). Ecotoxicological Risk of Sulfate in Baltic Sea Brackish Water Assessed Using Model-Averaged

Species Sensitivity Distribution. *Integrated Environmental Assessment and Management*, vjag010. https://doi.org/10.1093/inteam/vjag010

Jager, T. (2025). It's about time: Moving away from statistical analysis of ecotoxicity data. *Integrated Environmental Assessment and Management*, vjaf009. https://doi.org/10.1093/inteam/vjaf009

Laskowski, R. (1995). Some Good Reasons to Ban the Use of NOEC, LOEC and Related Concepts in Ecotoxicology. *Oikos*, *73*(1), 140–144. https://doi.org/10.2307/3545738

Moe, S. J., Fox, D. R., & Wolf, R. (2025). Updating statistical practice in ecotoxicology: Reflections and recommendations. *Integrated Environmental Assessment and Management*, *21*(3), 475–476. https://doi.org/10.1093/inteam/vjaf029

OECD. (2002). Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data. *OECD Series on Testing and Assessment*. https://doi.org/10.1787/9789264078284-en

OECD. (2006). Current Approaches in the Statistical Analysis of Ecotoxicity Data: A guidance to application (annexes to this publication exist as a separate document). *OECD Series on Testing and Assessment*. https://doi.org/10.1787/9789264085275-en

Schimera, A., Marx, M. T., Bottoms, M., Ernst, G., Gao, Z., Hanisch, J., Jakoby, O., Kimmel, S., Neuwoehner, J., Sharples, A., Staab, F., Thorbek, P., Wittwer, T., & Yan, L. (2025). The role of background variability for interpreting biological relevance and statistical significance in Collembola soil field studies. *Integrated*

Environmental Assessment and Management, vjaf171. https://doi.org/10.1093/inteam/vjaf171

Vermeiren, P., Wittwer, T., Coffinet, S., von Blanckenhagen, F., & Jakoby, O. (2026). Getting most out of ecotoxicological field data with generalized linear and additive mixed models: A 6-step analysis framework. *Integrated Environmental Assessment and Management*, vjag018. https://doi.org/10.1093/inteam/vjag018

Wasserstein, R. L., & Lazar, N. A. (2016). The ASA Statement on p-Values: Context, Process, and Purpose. *The American Statistician*. (world). https://amstat.tandfonline.com/doi/abs/10.1080/00031305.2016.1154108