

# How we can make ecotoxicology more valuable to environmental protection

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1 **How we can make ecotoxicology more valuable to environmental**  
2 **protection?**

3

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24 **ABSTRACT**

25

26 There is increasing awareness that the value of peer-reviewed scientific literature  
27 is not consistent, resulting in a growing desire to improve the practice and  
28 reporting of studies. This is especially important in the field of ecotoxicology,  
29 where regulatory decisions can be partly based on data from the peer-reviewed  
30 literature, with wide-reaching implications for environmental protection. Our  
31 objective is to improve the reporting of ecotoxicology studies so that they can be  
32 appropriately utilized in a fair and transparent fashion, based on their reliability  
33 and relevance. We propose a series of nine reporting requirements, followed by a  
34 set of recommendations for adoption by the ecotoxicology community. These  
35 reporting requirements will provide clarity on the experimental design and  
36 conditions, chemical identification, test organisms, exposure confirmation,  
37 measurable endpoints, how data are presented, data availability and statistical  
38 analysis. Providing these specific details will allow for a more full assessment of  
39 the reliability and relevance of the studies, including limitations.

40 Recommendations for the implementation of these reporting requirements are  
41 provided herein for practitioners, journals, reviewers, regulators, stakeholders,  
42 funders, and professional societies. If applied, our recommendations will improve  
43 the quality of ecotoxicology studies and their value to environmental protection.

44

45 *Keywords* – publications, quality, reliability, relevance, risk assessment, reporting  
46 recommendations, peer review

47 **Introduction**

48 There is widespread and growing concern that the quality, usability, and reporting  
49 of published peer-reviewed research is not as good as it could, and should, be.  
50 This can undermine the credibility and functioning of the scientific endeavor  
51 (Alberts et al., 2014; Forbes et al., 2016) and is a conversation that has spread  
52 beyond just the scientific community (e.g. The Economist, 2013). Poor science  
53 and reporting also come with steep economic costs. For example, it has been  
54 estimated that irreproducible results in the biomedical literature cost 28 billion  
55 USD in America alone, each year (Freedman et al., 2015). In addition to the  
56 direct economic costs from repeating poorly conducted studies that report  
57 spurious results, poor quality research delays and hinders protection of the  
58 environment, which is an underlying reason for conducting ecotoxicology  
59 research. By performing and reporting poor science, as a discipline we are failing  
60 to achieve our existential goal.

61

62 Only recently has attention been focused on the quality of published  
63 ecotoxicology studies (e.g. Klimisch et al. 1997; Durda and Preziosi 2000; Hobbs  
64 et al. 2005; Schneider et al. 2009; Brady, 2011; Agerstrand et al. 2014; Warne et  
65 al. 2015). A core issue is that ecotoxicology studies often do not report key  
66 information required to make a judgment on the quality of the studies (Harris and  
67 Sumpter, 2015). Harris et al. (2014) suggested twelve principles that they  
68 considered should be addressed in an ecotoxicology study. They then applied  
69 three of the most objective of these principles (i.e.. measurement of exposure

70 concentrations, study repeated, and more than a single exposure tested) to 200  
71 randomly chosen research papers, published in 2013, in three reputable journals  
72 covering the field. They concluded the quality of published ecotoxicology  
73 research was poor, with less than half, and often less than 25%, fulfilling the  
74 criteria. In particular, often less than 25% of papers provided information  
75 demonstrating that results were repeatable, with the majority of papers reporting  
76 results from only one experiment.

77

78 To further evaluate the quality of published ecotoxicology studies, we objectively  
79 assessed current reporting requirements of journals publishing ecotoxicology  
80 studies. This involved conducting an ISI Web of Science search using the topic  
81 keyword 'ecotoxicology', and years published '2014 – 2015'. The initial search  
82 generated 176 journals that published 'ecotoxicology' studies. We then employed  
83 a cut-off requiring greater than two 'ecotoxicology' articles published in 2014 –  
84 2015. This cut-off left 31 journals, and for those we then screened the 'guide to  
85 authors' for three basic criteria we deemed fundamental to paper quality in  
86 ecotoxicology. These criteria were: 1) expectations around statistical analysis  
87 (e.g., adequate replication); 2) analytical verification of exposure concentrations;  
88 and 3) availability of Supplemental Information (as a mechanism of providing  
89 data and other information required for critical analysis of relevance and  
90 reliability). We found that relatively few journals provided guidance on our three  
91 criteria, and only one journal met all three (Figure 1). This exercise suggests that  
92 guidance on publication standards provided by peer-reviewed journals requires

93 improvement. We do acknowledge that many journals rely on reviewers to  
94 assess publication quality, including use of our search criteria (i.e., analytical  
95 confirmation of exposure concentrations, statistical guidance). However, relying  
96 on expert judgment alone can be problematic, as it is often inconsistently applied  
97 between reviewers (e.g., depends on the reviewers' expertise and availability, as  
98 well as their own biases (Mahoney, 1997)) and is coupled with the needs of a  
99 journal to fill issues and increase impact factors. Baseline expectations around  
100 reporting and conductance of studies by all journals, along with mechanisms to  
101 facilitate a full review by future readers (e.g., through availability of Supplemental  
102 Information), are required in order to elevate the quality of the ecotoxicology  
103 literature as a whole (Moermond et al. 2016).

104

105 Overall, the reporting of both the methodology and results in ecotoxicity studies  
106 appears to be incomplete and inadequate (Ågerstrand et al., 2011a, 2011b,  
107 2014). This can decrease the likelihood that studies are cited by other authors, or  
108 used for regulatory purposes (ECHA, 2012). Examples of missing or insufficiently  
109 reported aspects include the types and performance of controls, analytical  
110 methods and exposure confirmation, test system design, information about  
111 statistical evaluations and statistical power, and presence of possible  
112 confounding factors. As a reader of a peer-reviewed publication it can be  
113 challenging to decide whether missing information is due to insufficient reporting  
114 or inadequate design and performance of the experiment. Regardless of the

115 cause, missing information decreases the value of ecotoxicology publications and  
116 may lead to a paper being omitted from subsequent interpretative work.

117

118 Only when studies are reported in a transparent and detailed way is it possible  
119 for a reader (e.g. a regulator who wants to use the results in the publication to  
120 ensure adequate protection of the environment) to judge the quality/reliability of  
121 the paper. Until recently, word limits of peer-reviewed journals caused authors to  
122 economize on the details of methods and materials, in order to leave room for  
123 descriptions of the results and discussion. This is no longer necessary, since it is  
124 possible to publish supporting information online where all aspects of a study can  
125 be reported in sufficient detail and raw data provided (e.g. Meyer and Francisco,  
126 2013). In this work we recommend a set of minimal reporting requirements. We  
127 then suggest a variety of strategies to maximize uptake of these requirements  
128 into all published ecotoxicology papers by those most able to create the  
129 necessary change within the peer review process (e.g., authors, journals and  
130 peer reviewers).

131

## 132 **RELEVANT REPORTING REQUIREMENTS FOR ECOTOXICITY STUDIES**

133

134 To ensure detailed and transparent reporting of peer-reviewed publications,  
135 reporting recommendations can be used as a tool for designing, performing,  
136 analyzing and reporting ecotoxicity studies (Ågerstrand et al., 2014; Moermond et  
137 al. 2016). This has also been suggested in other areas, e.g. in biomedical



138 research, to increase the value of publications and reduce waste from the  
139 reporting of poorly written research articles (Glasziou et al., 2014, Chan et al.,  
140 2014). Using a systematic reporting tool also has the potential to shorten time  
141 needed for peer-review and to decrease the number of questions from peer-  
142 reviewers, thereby increasing the chance of a paper getting published. It is a  
143 great loss in terms of scientific knowledge, but also from the viewpoint of animal  
144 welfare and economic resources, when peer-reviewed literature cannot be used  
145 in hazard and risk assessment of chemicals. Below we provide, in no particular  
146 order of importance, nine general reporting requirements to help enhance the  
147 quality, credibility, and usability of the ecotoxicology literature and a checklist for  
148 both authors and peer reviewers to employ when writing and assessing  
149 ecotoxicology studies (see and example in Table 1).

150

## 151 **1. Test Compound Source and Properties**

152

153 Test substances or products may have more than one component contributing to  
154 their toxicity and varying the amounts of these components can affect toxicity. As  
155 manufacturing processes change over time, the composition of a substance and  
156 its toxicity may also change. Ecotoxicology data derived from a historical form of  
157 a test substance may not be relevant to current forms of the test substance. As  
158 an example, the US EPA required an upper limit of 0.1% be established for DDT  
159 and its related impurities ( $\Sigma$ DDT, i.e. DDT, DDE and DDD) for all dicofol technical  
160 active ingredients by 1987 (US EPA, 1998). Unless otherwise specified, any data

161 produced prior to this date could have been conducted with technical active  
162 ingredients containing greater levels of contamination and therefore inaccurate  
163 estimates of the biological properties of the current product.

164

165 In another example, the pesticide cyfluthrin contains eight isomers, of which four  
166 are more biologically active. The related pesticide, beta-cyfluthrin, only contains  
167 the four more biologically active isomers. When the isomer profile is known, the  
168 exposure and ecotoxicity of both substances can be compared by correcting for  
169 isomer-equivalents, as was done by the US EPA for the aquatic risk assessment  
170 for cyfluthrin and beta-cyfluthrin (US EPA, 2013).

171

172 The following are the minimum recommended reporting requirements for test  
173 substance identification, where available:

- 174 • Technical name (e.g. International Union of Pure and Applied Chemistry  
175 (IUPAC) or registration no., Chemical Abstract Service number (CAS),  
176 batch number) and formulated product, brand or trade names;
- 177 • Source, purity, and composition of the test substance; specifically, percent  
178 active ingredient including levels or ratios of components and isomers and  
179 impurities

180

181 Basic physico-chemical property information (e.g., aqueous solubility, acid  
182 dissociation constant (pKa), dissociation constant (Kd), octanol-water partition  
183 coefficient (Kow), organic carbon-water partition coefficient (Koc), vapour

184 pressure (VP) or Henry's Law Constant (HLC), Bioconcentration Factor (BCF),  
185 Bioaccumulation Factor (BAF) and Biomagnification Factor (BMF)) can ensure  
186 studies are designed to minimize losses (e.g., volatilization, degradation or  
187 adsorption to test vessels; see Section 5 Exposure Confirmation) as well as  
188 identify the potential for chemical reactions to occur under different test  
189 conditions (e.g., ionization in relation to pH; see Section 4 Experimental  
190 Conditions). For example it might be relevant to test:

- 191 • a substance with an environmentally relevant pKa at various pHs to  
192 account for any differential uptake between its neutral and ionized form.
- 193 • a rapidly degrading, volatilizing, or strongly partitioning substance in a  
194 flow-through test system to ensure constant exposure concentrations.

195

## 196 **2. Experimental Design**

197

198 Details of the experimental design should be stated or, if the design is complex, a  
199 figure can often more efficiently and accurately explain the design (e.g. Figure 2  
200 from Dellinger et al., 2014). It is important that the experimental design should  
201 permit the hypothesis to be tested and the objectives to be met. This can include  
202 incorporating test conditions and species reflective of where the data will be  
203 applied by regulators (See Section 3: Test Organism Characteristics and Section  
204 4: Experimental Conditions). For example, the European Food Safety Authority  
205 (EFSA 2013) requires that the test conditions and species are reflective of the  
206 regions undergoing assessment, and this lack of congruency (particularly in soil

207 tests) has been a major factor in its rejecting certain studies in its evaluations.

208 Experimental design features that should be reported include:

- 209 • Hypotheses and objectives of the study should be clearly stated, even if  
210 the hypothesis is as simple as 'compound X causes a 50% reduction in  
211 egg-laying relative to control at a concentration less than its aqueous  
212 solubility'.
- 213 • The number of treatments and the nominal concentrations;
- 214 • The number and types of controls (e.g., positive, negative, or solvent). If a  
215 solvent carrier is used then its concentration in each treatment and control  
216 should be equal and stated.
- 217 • The degree of replication of each treatment and control and an  
218 explanation of whether they are true replicates or pseudo-replicates.
- 219 • The methods for creating and storing the stock and working solutions and  
220 the duration of storage.
- 221 • The exposure regime for the test substance (e.g. static, semi-static or  
222 flow-through) with details about the renewal regime and method.
- 223 • The method/design for determining the order in which test organisms are  
224 added to test vessels and the placement of test vessels (e.g. randomized,  
225 stratified random or Latin square design).
- 226 • The frequency of exposure (e.g., how often the test substance is  
227 administered or renewed) and type of samples analyzed to determine  
228 toxicant concentrations.

- 229       • Route of exposure to test organism should be clearly stated (e.g. via the  
230       diet, via the media, etc.).
- 231       • Details of all quality assurance and quality control procedures conducted  
232       as part of the study (e.g. whether the design was blind or double blind,  
233       whether scoring, data entry and calculations were conducted by one, or  
234       more than one individual, and see Section 4: Experimental Conditions as  
235       they relate to water quality, etc.).

236

### 237 **3. Test Organism Characteristics**

238

239 Test organisms may be obtained from a variety of sources, including in-house  
240 cultures or wild populations. Providing details on where they were obtained, how  
241 they were maintained, and as much information as possible on the control  
242 performance of the test species, is useful when determining if a chemical, or  
243 mixture of chemicals causes an effect and if that effect can readily and reliably be  
244 detected. The following points should be considered and reported, where  
245 applicable:

- 246       • Species selection – Justify the selected organism (e.g. ecological  
247       relevance and/or relevance to hypothesis)
- 248       • Identity of the species – Report the common and scientific name of the  
249       species, general type (e.g., plant), its source, and strain (if appropriate).  
250       Provide the DNA Bar-Code details if available. When dealing with new or  
251       cryptic species, genetic identification is recommended, e.g., the alga

252 *Oophila* sp. (Baxter et al., 2015) or the *Hyalella azteca* species complex  
253 (Leung et al., 2016).

254 • Source – In-house cultures, wild populations and, where applicable, to  
255 include method of collection (e.g., collection of fertilized eggs, or animals  
256 from the wild) and their subsequent handling.

257 • Life-history – The stage of the life-cycle, the age and sex of the test  
258 organisms, and their size or mass at the beginning of the experiment,  
259 should be provided.

260 • Husbandry – All procedures related to maintaining the organisms in good  
261 condition should be stated. Overviews of welfare and ethical approvals  
262 need to be reported, especially those that may influence observed  
263 responses (e.g., degree of enrichment, groupings). Outbreaks of disease  
264 or unexplained mortality/morbidity, including their incidence and severity,  
265 and how these were treated, must be reported.

266 • Test species performance – Available historical data on endpoints (e.g.,  
267 growth rates, reproduction) used in the experiment should be provided,  
268 enabling the data collected during the experiment to be put into context.  
269 For example, a detectable change in growth may not be deemed  
270 biologically significant when compared to historical control data of the  
271 performing laboratory (Länge et al., 2001). Control performance should be  
272 reported in order to permit comparison to validation criteria.

273

#### 274 **4. Experimental Conditions**

275 In addition to experimental design (See Section 2: Experimental Design) the  
276 general conditions of the experiment, facilities, and operating regime should be  
277 provided, as the interaction of the test organism and testing/exposure  
278 environment influence the outcome of the study. Where applicable, the following  
279 should be reported:

- 280 • General testing facilities – For example, growth chamber (make, model),  
281 tank (dimensions, capacity, rate of water change); mesocosm (location,  
282 volume, flow rate), greenhouse (location, size), field location (GPS  
283 coordinates, general climatic/environmental information, for duration of  
284 study).
- 285 • Test conditions – All available details on relevant experimental parameters  
286 such as light intensity, photoperiod, and temperature should be reported  
287 as means, with the variability.
- 288 • Source, type and composition of test media – e.g. water, commercially  
289 available media, soil, sediments, including known background  
290 contaminants.
- 291 • Test media parameters – All measurements that can influence test  
292 organism health or change endpoint responses should be reported (e.g.  
293 dissolved oxygen concentration, temperature, pH, salinity) as means with  
294 an estimate of variability (i.e., confidence intervals). In addition, report the  
295 properties of the test media that may influence interpretation of the test  
296 results (e.g. dissolved organic carbon where binding of test material is  
297 expected).

- 298 • Dosing mechanism – e.g. via the diet, peristaltic pump, spray application.
- 299 • Details of acclimation – For both the test system and test organisms. In
- 300 terms of the test system, this is to demonstrate that the conditions are
- 301 stable prior to introduction of test organisms. This is of particular
- 302 importance in mesocosm and sediment studies. In terms of the test
- 303 organisms, this is to ensure survival and growth of test organisms during
- 304 the experimental conditions (further details see Section 3 Test Organism
- 305 Characteristics).
- 306 • Feeding – Information should include the type of food, source, amount
- 307 provided and frequency of feeding. In the case of commercial foods,
- 308 detailed reporting of characteristics as required. The concentration of any
- 309 contaminants should be included, where relevant.
- 310 • Number and density of organisms – This may be influenced by purpose of
- 311 the test or experimental design (e.g., test power, see Section 8: Statistical
- 312 Analysis). However, test design should enable normal behavior of the test
- 313 organism. Density of the test organisms will determine adequate feeding
- 314 requirements and acceptable loading.
- 315 • Good Laboratory Practices (GLP) studies – When data are from a GLP
- 316 study, this should be expressly stated in the publication, as well as the
- 317 location of the raw data.
- 318 • Quality Assurance and Quality Control (QA/QC) activities – (e.g.
- 319 calibration of laboratory equipment) and the results of these should be



320 reported. If Standard Operating Procedures (SOPs) are used provide  
321 location of these.

322

## 323 **5. Exposure Confirmation**

324 Characterization of exposure in an ecotoxicity test is critical to facilitating  
325 publication, ensuring the stated hypothesis is being addressed, reducing  
326 uncertainty in the observed relationships, and allowing risk assessors to  
327 incorporate the data into their evaluations. Strong analytical support provides  
328 confidence that the stated chemical was the one that was used, that it was found  
329 in the test vessels at the concentrations targeted, in the appropriate  
330 compartments, with an understanding of the true duration of exposure. There are  
331 numerous examples in the scientific literature where the lack of analytical  
332 confirmation has resulted in erroneous conclusions, costly follow-up work, and  
333 retractions of published works (e.g., Ricaurte, 2003).

334

335 What follows are minimum reporting requirements around exposure confirmation  
336 for conducting standard laboratory ecotoxicology tests, but they can also be  
337 applied to *in vitro*, micro- and mesocosm, and field studies.

- 338 • Provide sufficient instrumental and details around your analytical  
339 approach, or a suitable reference, that supports the approach taken.
- 340 • Report any relevant QA/QC undertaken during the sampling and analysis  
341 (e.g., blanks, storage studies, internal standards, recovery efficiency, and  
342 specific storage preservation techniques) and the results of the QA/QC.

- 343 • Report your limits of detection, quantification, or reporting (LOD, LOQ, and  
344 LOR, respectively) and their variance.
- 345 • State clearly what samples were analyzed (e.g., stocks only, exposure  
346 vessels, pooled or unpooled) and the timing or frequency of  
347 measurements. This is important when interpreting the relevance of the  
348 observed response in light of actual exposure duration, regardless of test  
349 length.
- 350 • State the media type and the volumes sampled, as well as storage  
351 conditions and time till analysis.
- 352 • Report your values in metric units as the target analyte, and not as the  
353 formulated product. Where applicable, provide means of measured values  
354 and standard deviations/errors.
- 355 • State clearly whether subsequent statistical analyses and interpretations  
356 rely on nominal or measured values (See Section 8), and whether these  
357 exposures values have been corrected for recovery.
- 358 • Prepare a plan for archiving your original data (See Section 9).

359

360 There are some scenarios where robust analytical support is not feasible or for  
361 which analytical methods below the required reporting requirements are unlikely  
362 to be met. Still, in the case of substances where routine measurements and  
363 protocols for analysis do exist, as well as very cost effective and relatively simple  
364 analytical approaches (e.g., enzyme-linked immunosorbent assay; ELISA), no  
365 standard toxicity test should be performed without some exposure confirmation. If

366 you are unable to provide a level of analytical support that gives risk assessor's  
367 confidence in the data, this will seriously reflect on the value of conducting the  
368 study.

369

## 370 **6. Endpoints**

371

372 The reporting of information around test endpoints is especially important when  
373 assessing the relevance and reliability of the data. While endpoints, such as  
374 mortality, reproduction and growth, are familiar to the majority of ecotoxicologists,  
375 there are many that are less familiar (e.g., genomic and metabolic tools). This  
376 can lead to misunderstandings and misinterpretations of the significance and  
377 ecological relevance of the data that can and should be avoided. What follows  
378 are reporting recommendations around endpoints in ecotoxicology tests:

- 379 • State all endpoints monitored in the study, regardless of the observed  
380 response (e.g., avoid reporting only 'differences')
- 381 • Define the endpoint in order to remove ambiguity (e.g., what is a  
382 'malformation'?)
- 383 • Justify the selection of your endpoints (also see Section 2 Experimental  
384 Design) and their statistical power (see Section 8 Statistical Analysis).
- 385 • Express clearly when and how the endpoint was monitored and recorded  
386 (e.g., blind evaluations of behavior; See Section 2 Experimental Design)  
387 and how these data are presented in the paper (e.g., Tables,  
388 supplemental information)

- 389       • Report other observations that may have relevance, but were not an  
390           explicit part of the original study design (e.g., lesions in fish), as this can  
391           inform future work and be hypothesis generating.

392

## 393 **7. Presentation of Results and Data**

394

395 Presentation of results is an important aspect of ecotoxicology that is often not  
396 discussed but has implications for assessing the utility of peer-reviewed  
397 literature. The primary purpose of Figures and Tables is to convey as much  
398 information as possible, in a manner that is simple to understand. For excellent  
399 examples of good graphics, see Tufte (1997, 2001). It is equally important to  
400 report figures that allow an assessment of the statistical interpretation and  
401 inference. To facilitate this we recommend:

- 402       • Create figures that provide readers with greater ability to assess data  
403           distributions and variability (e.g., scatterplots, histograms, box plots, etc.)  
404           for each time-point. It is common for researchers to rely upon graphs  
405           displaying a mean  $\pm$  standard deviation or standard error. However, this  
406           has been shown to be problematic because different distributions of data  
407           can be represented in the exact same way when relying solely upon bar  
408           charts and line charts (Weissgerber et al. 2015). Furthermore, traditional  
409           bar charts can disguise outliers in data, which can be important,  
410           particularly for studies with small sample sizes (Weissgerber et al. 2015).

- 411 • Employ appropriate scales (e.g., do not truncate or break axes to over-  
412 emphasize effect sizes or differences relative to controls).
- 413 • Provide details in figure or captions specifying the statistical test employed  
414 (if applicable), degree of replication, level of statistical significance (if any).
- 415 • Confidence intervals, with alpha-level, should accompany summary  
416 statistics in figures and tables, instead of standard deviation and standard  
417 error. Confidence intervals are preferred over standard deviations or  
418 standard errors because measures of uncertainty are not always  
419 symmetric about the mean. This is especially relevant when the data are  
420 transformed for analysis and the results are expressed in back-  
421 transformed values. It also applies when some non-normal error structures  
422 are used, such as Poisson or binomial. Only in the case of normally  
423 distributed data, will standard deviations provide equivalent information to  
424 confidence intervals.
- 425 • Inclusion of statistically and biologically significant, as well as non-  
426 significant results, will allow for a balanced understanding of the full range  
427 of responses.
- 428 • It is useful to report summary data (e.g., end-points, estimates, ranges,  
429 etc.) in table-format, and greater attempts should be made to include as  
430 much data as practical, e.g., in Supplemental Information (See Section 9  
431 Raw Data).

- 432       • For field studies it is helpful to provide maps with relevant information  
433           (e.g., GPS coordinates, scale bars, orientation, regional context, etc.) that  
434           may enable a reader to understand the spatial context of a study.

435

## 436 **8. Statistical Analysis**

437

438 A well-conducted experiment can have its meaning distorted if poor statistical  
439 methods are used in the interpretation. Consequently, care should be taken in  
440 the selection and interpretation of statistical tests and models, and subsequent  
441 reporting of the approaches employed. Considerations include the following:

- 442       • Provide a statistical flow chart that includes any preliminary data checks to  
443           satisfy test or model requirements and accommodations (e.g.,  
444           transformations or robust methods) of data problems, the handling of  
445           multiple controls (e.g., solvent and negative controls), and indicate how  
446           statistical tests or models are selected and why (e.g., OECD 2006, 2010).
- 447       • Provide justification for any transformations (e.g., logarithm) used and  
448           whether/how it affects the analysis results.
- 449       • Power of the planned hypothesis testing procedure to find effects or the  
450           ability of a regression model to estimate EC<sub>x</sub> reliably should be reported  
451           (See Section 4: Experimental Conditions). This can be done partly through  
452           the use of historical control data (See Section 3 Test Organism  
453           Characteristics).

- 454 • Good estimation of the control mean is important since all tests and  
455 estimates are in relation to that mean, so if there were more replicates in  
456 the control than in treatment groups or other special considerations of the  
457 control, make clear what was done and why.
- 458 • Statistical outliers should be identified and their effect on conclusions  
459 should be stated.
- 460 • In reporting sub-lethal effects in a study with substantial mortality in high  
461 treatment groups or loss of subjects/replicates for other reasons, report  
462 any adjustments made to the tests or models (e.g., weighting) to avoid  
463 over-interpretation resulting from small sample size.
- 464 • Explain how the statistics account for the actual experimental set-up (e.g.,  
465 individual, paired or group housing, expected monotone dose-response or  
466 deviations therefrom, such as hormesis)
- 467 • For complex models (for regression or hypothesis testing) with multiple  
468 potential explanatory variables, any model selection method that  
469 sequentially adds or removes terms to arrive at a final model should be  
470 described and, if possible, verified by alternative approaches to avoid  
471 unintentional bias.
- 472 • Results should be reported in the original units and with no more  
473 significant digits than the raw data justify. For example, if the data are  
474 measured with two significant digits to the right of the decimal point, it is  
475 pointless to report means to five decimal points. That implies a level of  
476 precision not justified by the data. Moreover, the quality of the

477 measurements determines how many significant digits are meaningful. If  
478 the equipment being used to measure a response is accurate only to the  
479 nearest 0.1, but reports five digits past the decimal point, data should be  
480 reported only to the nearest 0.1. Summary statistics should be reported as  
481 means and confidence intervals (not standard deviations or standard  
482 errors) with an explanation of how confidence intervals were determined.  
483 This is especially important if transformed data were analyzed, so  
484 confidence intervals are not symmetric about the mean.

- 485 • While expressing change from control (percent of control) is useful for  
486 presentation, data should also be presented as actual recorded values.

487

488 There is disagreement about the appropriateness of calculating and  
489 implementing certain measures of toxicity, specifically no observed effect  
490 concentrations (NOECs) and lowest observed effect concentrations (LOECs)  
491 (Green et al., 2012; van Dam et al., 2012 and references therein). Still, they are  
492 necessary for some responses and datasets and are commonly used in many  
493 risk assessment frameworks, and so we have provided specific reporting  
494 requirements below.

- 495 • Power to detect a specified size effect should be stated.
- 496 • Confidence intervals for the mean response at the NOEC and LOEC  
497 should be reported along with the percent change from control.
- 498 • If the response is non-monotonic (e.g., hormesis), indicate how this was  
499 addressed in the statistical analysis.



500

501 Effective concentrations (EC<sub>x</sub> point estimates where x is typically no more than

502 50) also have specific reporting requirements. These are:

503 • Define percent change from control (the 'x' in EC<sub>x</sub>) as to whether it applies  
504 to raw or transformed data.

505 • Report when EC<sub>x</sub> estimates are extrapolations, including extrapolations  
506 below the lowest tested positive concentration/dose.

507 • Report what exposure values (e.g., measured versus nominal) were used  
508 in your estimates

509 • Model selection and goodness-of-fit criteria should be specified.

510 • The minimum effect size that a regression model can reliably estimate  
511 should be determined and reported. For example, if the standard error of  
512 the control mean is 20% of that mean, then the estimation of EC<sub>x</sub> for x  
513 <20 is likely unjustified.

514 • Confidence intervals should be reported for EC<sub>x</sub> and all model parameters  
515 and explain why any parameters not significantly different from zero were  
516 included in the model, as these can indicate possible model problems.

517

## 518 **9. Raw Data**

519

520 Ideally, all data that are important to the determination of a toxicity value should

521 be archived in a manner that they can later be made available for validation

522 and/or re-evaluation. However, the minimum data that should be archived (for  
523 example, in the Supplementary Information/material section of journals) are:

- 524 • The replicate and treatment identification.
- 525 • The measured and nominal chemical concentrations for each analysis.
- 526 • The non-transformed (e.g., non-logged, non-normalised) biological effects  
527 data at the level of the unit of measurement. For example, data on  
528 individuals if that is what is measured or data for groups of individuals  
529 when pooled and then measured.
- 530 • All measured values for experimental conditions that are known to affect  
531 the toxicity or bioavailability of the chemical.

532 The reason for archiving the above data is to increase the usability and longevity  
533 of impact. For example, risk assessors may require the raw data of papers that  
534 are crucial to their ecological risk assessments or regulatory decision-making.  
535 They may require these data in order to re-analyse them or to confirm the  
536 estimate of toxicity, especially as risk protection goals change through time, and  
537 across jurisdictions.

538

539

540 **WHAT CAN DIFFERENT STAKEHOLDERS DO TO IMPROVE THE**  
541 **REPORTING AND IMPACT OF OUR SCIENCE?**

542

543 All of us who produce, review, publish and use ecotoxicology data play a vital  
544 role in improving the quality and reporting of our science. There are also

545 immense benefits to everyone involved in this endeavor should we get it right.  
546 There are a number of actions we can take now to move the discipline forward so  
547 that our shared goal of environmental protection is accomplished.

548

549 **A. What Journals Can Do:**

550 Some of the benefits to journals from better ecotoxicology studies would be faster  
551 peer reviews, reduced likelihood of damaging retractions, and increased impact  
552 factors. By demonstrating a commitment to the best ecotoxicology, journals can  
553 distinguish themselves from predatory publishers (Bohannon; 2013; Kolata,  
554 2013). Below, we provide advice and guidance to journal publishers, editors-in-  
555 chief, associate editors, reviewers and authors.

- 556 1. Journals should work with all stakeholders to create clear minimal  
557 standards for publishing ecotoxicology studies in concert with the peer  
558 review process. This could be implemented through a formal checklist for  
559 authors prior to submission. Ideally, all journals would implement the same  
560 standards. Journals could screen submissions using their checklist and if  
561 their criteria are not met, the paper will not be reviewed.
- 562 2. Reporting author contributions, source of funding, and other possible  
563 conflicts of interests, with no addition to author list after submission.
- 564 3. Journal training of reviewers and the use of the checklist of publishing  
565 criteria (same checklist as journal submission) to facilitate review.
- 566 4. Open discussion/critique of papers and mechanisms for discussion, such  
567 as letters to the editor in online forums.

- 568 5. Create more effective mechanisms for corrections by authors, and clear  
569 reporting of retractions, with the reasons, for published papers.
- 570 6. Facilitate obtaining data reported in papers (e.g., supplemental  
571 information, figures with extractable data). Supplemental Information  
572 should download with the PDF of the paper (e.g., as it does with  
573 Proceedings of the National Academy of Sciences, USA).
- 574 7. Encourage submission of good quality papers that contain negative  
575 findings, or that replicate (or fail to) previous studies.

576

577 **B. What Scientists/Practitioners Can Do:**

578 The benefits of improving the conductance and reporting by scientists (whether  
579 academic, government, industry, consultants and contract labs, or non-  
580 government organizations (NGOs), and students) include less time, resources  
581 and money wasted repeating previous work that was poorly performed by others,  
582 fewer animals being used, studies being reviewed more rapidly and by better  
583 journals, greater inclusion of publications in decision-making processes. Articles  
584 with greater impact can also lead to increased funding to do more good science.

585 To achieve these goals we recommend:

- 586 1. Work towards enhancing the training of all practitioners prior to the  
587 conduction of any ecotoxicology study.
- 588 2. Prior to starting the study, draw in appropriate expertise to ensure greatest  
589 possible quality (e.g., statisticians, chemists).

- 590 3. Create a checklist of a good quality study prior to commencing  
591 experimental work (see Section A: What Journals Can Do; Example in  
592 Table 1) and have a plan to meet those requirements.
- 593 4. Develop internal laboratory QA/QC procedures and training.
- 594 5. Attempt to verify unusual results (e.g., replicate study).
- 595 6. Acknowledge the limitations of your data and do not over-interpret the  
596 results.
- 597 7. Cite good quality and appropriate science. As a general rule of thumb, do  
598 not cite retracted papers.
- 599 8. Have a mechanism for storing data (historical assay performance, etc.)  
600 and a means to share that with others. For example, graduate students  
601 can include raw data within their thesis appendices.
- 602 9. Push for a set of consistent screening toxicity test methods across  
603 jurisdictions and standard test organisms so that replication is facilitated  
604 and these lower tier data have the widest possible uptake by regulators.  
605 With this recommendation, we are not attempting to stifle scientific inquiry,  
606 but to reduce the ambiguity that can arise when different investigators ask  
607 the same initial screening question, e.g., what is the response of the  
608 duckweed *Lemna minor* when exposed to a chemical?
- 609 10. Become familiar with testing standards standards (e.g., Japanese Ministry  
610 of Agriculture, Forestry and Fisheries (JMAFF), Office of Chemical Safety  
611 and Pollution Prevention (OCSPP), Organisation for Economic Co-  
612 operation and Development (OECD) and the American Society for Testing

613 and Materials (ASTM))for your test organisms, so that you are aware of  
614 performance requirements and minimal expectations around experimental  
615 design.

616 11. Investigate whether or not regulatory bodies have criteria for evaluating  
617 published literature information (e.g., ECHA 2012, EFSA 2013, US EPA  
618 2011) and report this information in your study.

619 12. Practitioners should gain a better understanding of GLP and its role in  
620 improving the quality of reports for risk assessment (see Borgert et al.,  
621 2016).

622 13. Encourage conversations and consultation with diverse data users (e.g.  
623 NGOs, media, industry).

624

### 625 **C. What Data Users Can Do:**

626 The benefits to data users from the better reporting of ecotoxicology includes the  
627 context required to interpret and integrate the results with all other information  
628 evaluated during a risk assessment, less uncertainty in the decision-making  
629 process, studies and data that can be used across regulatory jurisdictions, saving  
630 time and money, and positions based on data that will be more broadly accepted.

631 It is acknowledged, however, that given the precautionary approach taken in  
632 most risk assessment frameworks, even if a study does not report all  
633 experimental details, it may still be considered if it provides information on a  
634 potential relevant risk not assessed by other data (e.g., toxicity to a non-standard  
635 sensitive species/population or vulnerable ecosystem). Those who read and use

636 the ecotoxicology literature, such as risk assessors, regulators, NGOs, policy  
637 makers, risk managers, scientists/practitioners play an integral role in improving  
638 the quality and reporting of ecotoxicology. This can occur through:

- 639 1. Setting and making available clear expectations for well-conducted  
640 studies, guidance to assess those studies, and the outcomes of those  
641 assessments.
- 642 2. Engaging with the totality of the data and justify the inclusion/exclusion of  
643 studies and explain how information from multiple studies is assessed,  
644 weighted and integrated together.
- 645 3. Consistently use and cite the best science available.
- 646 4. Conduct outreach amongst practitioners and data users to communicate  
647 what your data needs are and why.
- 648 5. Work towards setting consistent toxicity test methods across jurisdictions.

649

#### 650 **D. What Other Stakeholders Can Do:**

651 In this instance, we are thinking primarily of the public and media, and what they  
652 can do to help ensure better ecotoxicology. Some simple things would be to:

- 653 1. Cite good quality and appropriate science in an unbiased fashion.
- 654 2. Encourage consultation with the range of scientists/practitioners and data  
655 users (academics, government, industry).
- 656 3. Work towards building a stronger scientific understanding of what the  
657 strengths and limitations of peer reviewed literature in general are,  
658 become comfortable with uncertainty, and acknowledge that well-

659 conducted and reported studies will trump poorly reported and conducted  
660 studies.

661

662 **E. What Funders Can Do:**

663 We acknowledge that the vast majority of the ecotoxicology that is done is a  
664 result of funding, whether from government, industry, or other sources, such as  
665 NGOs. As bodies that decide which work will be performed, it is vital they ensure  
666 that they strive to support the highest quality science, and that it is reported  
667 properly. The benefit to funders will be the creation of data that will allow for the  
668 widest reach by all users, enhancing the value of limited financial resources. To  
669 facilitate this, funders should:

- 670 1. Create minimum requirements for conducting studies and reporting of data  
671 prior to funding approval.
- 672 2. Provide funding so that open source publishing and repositories for raw  
673 data can be maintained.
- 674 3. Promote ethics and integrity among grantees.

675

676 **F. What Professional Societies Can Do:**

677 Many of us involved in the field of ecotoxicology are also members of  
678 professional societies (e.g., Society of Environmental Toxicology and Chemistry)  
679 that can bring together ecotoxicologists from all stakeholder groups, as well as  
680 provide forums such as dedicated journals for the dissemination of new data.



681 Through these societies we can promote better conductance and reporting of  
682 ecotoxicology studies through:

- 683 1. Better and ongoing training of scientists/practitioners (e.g., free short  
684 courses on best practices for the conducting and reporting of studies at  
685 annual meetings).
- 686 2. Promoting ethics and integrity for students and supervisors in their  
687 research activities.
- 688 3. Advocating for a set of consistent toxicity test methods across jurisdictions  
689 that will be the agreed initial screen characterization of the toxicity of a  
690 compound to a particular organism.
- 691 4. Promoting civil and open discussion/critique of papers and mechanisms  
692 for discussion, such as special sessions at annual meetings.
- 693 5. Ensuring society journals are working with publishers, authors, and  
694 reviewers to improve the reporting of new and negative data.

695

## 696 **DISCUSSION**

697

698 We believe that our recommended reporting requirements (which also inform  
699 practice), coupled with our recommendations to promote communication among  
700 users, will improve the overall quality of ecotoxicology. We acknowledge that our  
701 recommendations do not begin to address adequately the issue of relevance for  
702 the studies themselves (i.e., asking the right question). However, when a user of  
703 ecotoxicology data has identified a series of studies as highly relevant, our

704 recommendations should help him or her to distinguish those that are of the  
705 greatest quality and how well they have been performed to address the question  
706 of interest (i.e., what is the reliability (aka quality) of the data). We also  
707 acknowledge that the requirements we propose are neither definitive nor fixed.  
708 As the types of studies we conduct change (e.g., new protocols, new classes of  
709 chemicals of concern, *in silico* methods) the reporting requirements might change  
710 as well. Finally, we acknowledge the need in some cases for exclusivity of data  
711 and feel this is a question of striking the right balance. An appropriate mechanism  
712 (e.g., registration protections without the need to keep data from competitors or  
713 designating a neutral third party to handle any sensitive information) for sharing  
714 can be created so that proprietary data generated for risk assessment and  
715 regulators can be examined by all stakeholders. Regulatory agencies are tasked  
716 with making scientifically-informed decisions on behalf of the public, and  
717 therefore need to use and be seen using data of the highest quality, but also  
718 communicating why those data are selected, to ensure public trust and reduce  
719 perceptions of possible bias (Forbes et al., 2016).

720

721 In summary, we have identified crucial areas where the quality of research and  
722 publication can be strengthened. These have been addressed through a set of  
723 broad recommendations for everyone involved in the discipline. If these are  
724 applied, ecotoxicology and its application in environmental protection will  
725 improve.

726

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737

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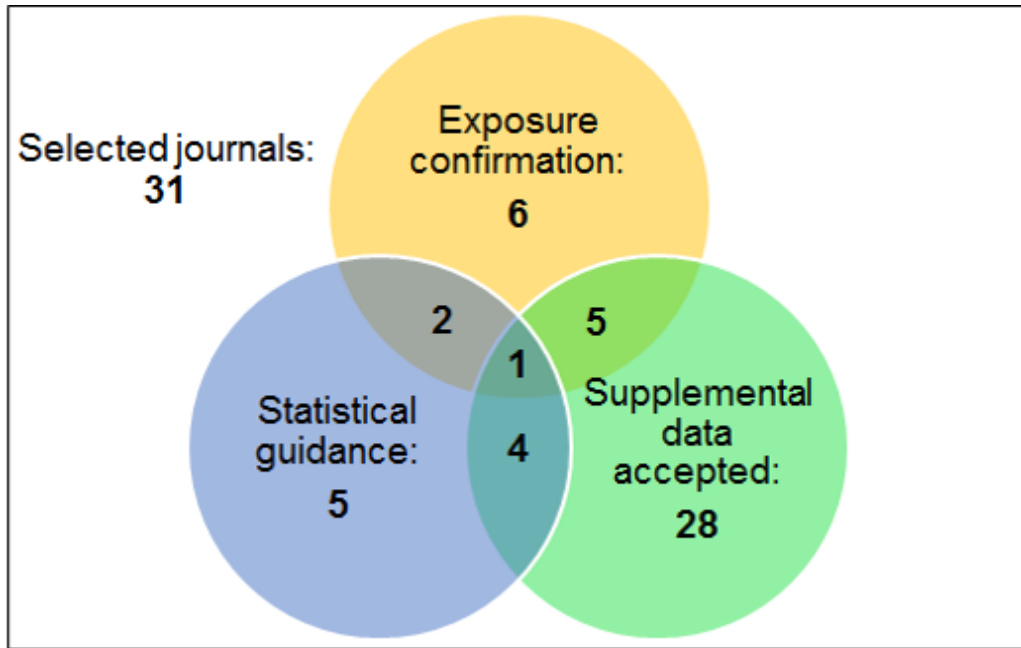
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## FIGURES



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**Figure 1:** Number of journals that provide reporting requirements of 1) expectations around statistical analysis (blue circle), 2) confirmation of exposure concentrations (yellow circle), and 3) availability of supplemental data (green circle). Journals were selected using an ISI Web of Science search using the topic keyword 'ecotoxicology', and years published '2014 – 2015' ( $n = 172$ ). Journals were further required to publish more than two 'ecotoxicology' articles in 2014 – 2015 ( $n = 31$ ).

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**Table 1:**

The model checklist provided below will assist authors and peer reviewers of ecotoxicology studies to improve their reporting and assessment.

<b>Reporting Requirement</b>	<b>Met?</b>
<b>1. Test Compound Source and Properties</b>	
Source and purity provided?	
Technical name?	
<b>2. Experimental Design</b>	
Hypotheses, if any, stated?	
Number of treatments and their exposure levels?	
Number and type of controls?	
Duration of exposures?	
Number of replicates?	
<b>3. Test Organism Characteristics</b>	
Name, source, and strain of species reported?	
Control performance criteria met?	
Husbandry protocols listed?	
<b>4. Experimental Conditions</b>	
General test conditions reported?	
Source and condition of media?	
Acclimation and feeding?	
<b>5. Exposure Confirmation</b>	
Clear statement of which samples were analyzed?	
Method LOD and LOQ provided?	
Nominal or measured used in subsequent analyses?	
<b>6. Endpoints</b>	
All endpoints monitored, regardless of response, provided?	
Clear definitions and measurement units provided?	
<b>7. Presentation of Results and Data</b>	
All data, regardless of statistical significance is discussed?	
Untransformed data provided?	
<b>8. Statistical Analysis</b>	
Statistical flowchart?	
Transformations justified?	
All outliers are reported	
Justification for model selection and variables?	
NOE-LOEC: power of test and percent change reported?	
ECx: Model estimates and confidence intervals provided?	
<b>9. Raw Data</b>	
Nominal and measured concentrations provided?	
Untransformed response by replicate available in some form?	

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