Environmental Toxicology and Chemistry—Volume 42, Number 9—pp. 2019–2028, 2023
Received: 24 January 2023 | Revised: 23 February 2023 | Accepted: 13 March 2023

Hazard/Risk Assessment

Introducing the No-Significant-Effect Concentration

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Abstract: The no-effect concentration (NEC) is the preferred threshold metric for single-species toxicity tests applied to derive safe concentration thresholds for contaminants in the environment for use in species sensitivity distributions. However, the NEC is only suitable when concentration–response (C-R) data exhibit a threshold response. We describe an alternative toxicity estimate, the no-significant-effect concentration (NSEC), which is better suited to C-R data for which the response is a monotonically decreasing function of concentration and no threshold effects are evident. We use a flexible, three-parameter sigmoidal function to describe the C-R relationship and detail both Bayesian and frequentist approaches to estimation and inference for the NSEC. While the NSEC is conceptually linked to the traditional no-observed-effect concentration (NOEC), it is a substantial improvement over the NOEC because it decouples the estimate from being directly dependent on the placement of treatment concentrations as well as admitting statements of precision of the resulting toxicity estimate. *Environ Toxicol Chem* 2023;42:2019–2028. © 2023 Commonwealth of Australia and The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Species sensitivity distribution; no-observed-effect concentrations (NOECs); Concentration–response modeling; Ecosystem protection; Ecotoxicology; Toxicity estimate

INTRODUCTION

The species sensitivity distribution (SSD) has been a cornerstone of ecotoxicological practice for the past 30 years (Kooijman, 1987; Stephen et al., 1985; van Straalen & Denneman, 1989). It was introduced by ecotoxicologists to overcome (or at least reduce) the subjectivity associated with the arbitrary scaling of laboratory toxicity estimates to derive "safe" or "protective" concentrations of contaminants in the natural aquatic environment. At its heart, the SSD is nothing more than a theoretical cumulative distribution function fitted to a sample of toxicity values obtained from a small number of "randomly" selected species. A low-order quantile (typically 1%, 5%, 10%, or 20%) from the fitted distribution is used to establish an upper concentration limit for a specific chemical in the receiving environment. This limit is referred to either as the concentration which is hazardous to *x*% of all species or the concentration that is protective of (100 – *x*)% of all species.

Although the SSD methodology elevated the statistical rigor associated with the protection of the aquatic environment, it has not been without controversy. While not wishing to revisit the debates surrounding the use of SSDs, it is fair to say that the most troublesome aspects are (1) the pathologically small sample sizes involved; (2) the lack of any biological, ecological, or environmental theory to inform the selection of SSD distributional form; (3) the inevitable nonrandomness of species selection; and (4) the choice of toxicity metric(s) used to fit the SSD, which is the subject of this communication. Further discussion about the strengths and weaknesses of the SSD methodology may be found in Fox (2016) and references therein.

Despite these issues, the SSD is the only available objective and quantitative means by which species protection values can be derived (Fox et al., 2021), and it remains a critical tool for environmental regulation in Australia (Warne et al., 2018) and elsewhere (British Columbia Ministry of Environment and Climate Change Strategy, 2019).

The primary use of the SSD is to estimate the concentration below which a high fraction of all species is expected to show no "effect" (e.g., death). Thus, the data on which the SSD is

This article includes online-only Supporting Information.

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Published online 21 March 2023 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5610

based are the highest concentrations at which different species are not adversely affected by the toxicant under investigation. An obvious candidate for this metric was the no-observed-effect concentration (NOEC). The NOEC is the largest test concentration for which the observed difference between the mean response at that concentration and the mean response for the controls is not statistically significant. The NOEC is widely used as a measure of the no-effect toxicity value.

Again, we do not wish to dwell on the long and sustained criticisms of the NOEC as a measure of chronic toxicity. These have been well documented in the ecotoxicology literature (Fox, 2008; Van Der Hoeven et al., 1997), with more recent calls for them not to be used at all (Fox & Landis, 2016; Van Dam et al., 2012; Warne & Van Dam, 2008). The main concerns with NOECs are that (1) they are constrained to be one of the test concentrations chosen by the researcher, (2) poor experiments favor larger NOECs (i.e, more liberal rather than more stringent values), (3) they make no use of the relationship between concentration and response, (4) statements of precision are not possible, and (5) they are dependent on the selection of a significance level for statistical testing. A measure which is immune to the drawbacks (1–5) is the no-effect concentration (NEC; Fox, 2010). The NEC is the concentration at which there is a response and can be estimated as one of the parameters in a threshold model. An example is the model used by Pires et al. (2002), which relates the response (Y) to concentration (x) such that Y is constant from $x = 0$ up to a threshold, γ (the NEC), and thereafter exhibits an exponential decay (Fox, 2010; Pires et al., 2002).

While the NEC has found wide applicability and can be readily estimated using both frequentist (e.g., the R package drc; Ritz et al., 2015) and Bayesian (Fisher et al., 2020, 2023; Fox, 2010) methods, it is predicated on the assumption that the biological response exhibits a threshold effect at low concentrations (Figure 1)—an assumption that is not always supported by the data or biological theory.

In the present study, we describe the no-significant-effect concentration (NSEC) as a simple alternative to the NEC in situations where there is no threshold effect in the response (Figure 1). The NSEC is similar in spirit to the NOEC while avoiding most of the drawbacks mentioned above. The NSEC is estimated from a monotonically decreasing model as the highest concentration for which the difference between the predicted response at that concentration is statistically insignificant from the predicted response at zero concentration.

The remainder of our study is structured as follows: We begin with a brief review of both effect and no-effect toxicity metrics to provide context. We follow with a conceptual introduction to the NSEC as well as a detailed section outlining NSEC estimation and inference. We describe both the frequentist and Bayesian approaches and illustrate this using worked examples. Finally, we discuss some of the issues associated with the use and estimation of the NSEC.

A BRIEF REVIEW OF EFFECT AND NO-EFFECT TOXICITY METRICS

The effect concentration

The ECx is that concentration at which the predicted response (survival, growth, etc.) represents an x% "effect" relative to the control. When concentration–response (C-R) data exhibit a smooth decline, the EC10 is generally considered a good/acceptable estimate of a "low" toxicity effect (Warne et al., 2015, 2018). It also has the advantage of being relatively easy to compute (Van Der Hoeven et al., 1997). However, its

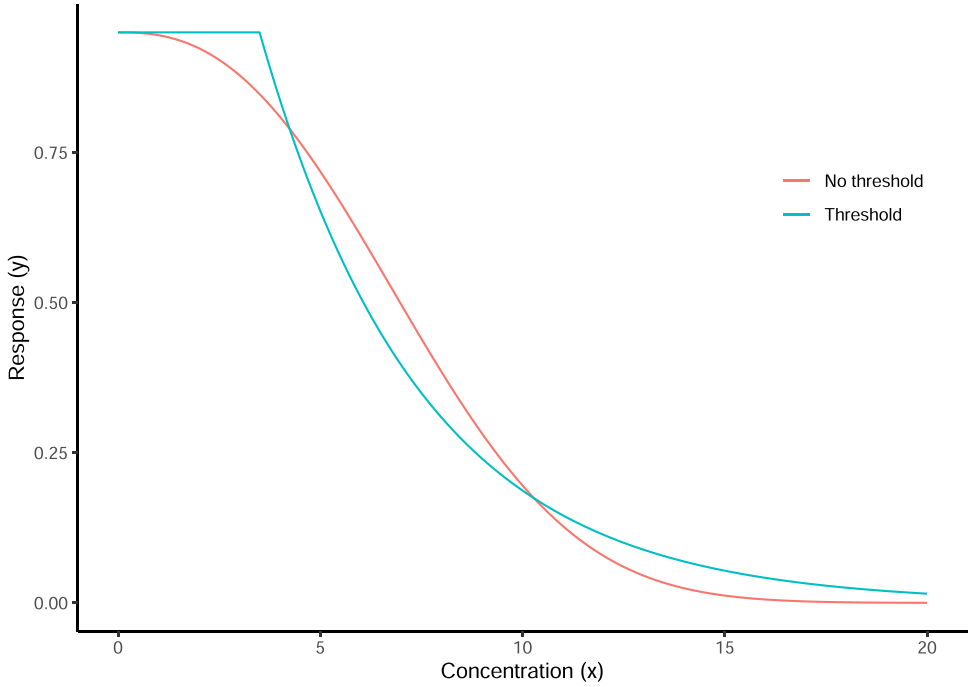

FIGURE 1: Conceptual comparison of a concentration–response curve with (cyan) and without (red) a threshold effect.

use in SSD modeling is more problematic. By definition, an EC*x* represents an effect and is thus conceptually inconsistent with the objective of SSD modeling, which aims to protect some high fraction of all species in an ecosystem by not causing any effect (Warne et al., 2018). Nevertheless, EC*x* data are routinely used in SSD modeling, although this remains a divisive issue (Green et al., 2013).

The NEC

As previously mentioned, the estimation of an NEC assumes the existence of a threshold effect in the C-R data. Of all the measures of no or low toxicity, the NEC is the preferred metric (Warne et al., 2018). Various C-R threshold models have been proposed (e.g., those found in the R package "drc"; Ritz et al., 2015), although they all have the generic form of Equation 1.

$$y = \theta_0[1 - I(x; \theta_1)] + f(x; \Theta)I(x; \theta_1) \quad (1)$$

In Equation 1, $\Theta = \{\theta_0, \theta_1, \ldots, \theta_{p-1}\}$ is a vector of parameters, θ_1 is the NEC, θ_0 is the response for concentrations below the NEC, $f(x; \Theta)$ is a function that describes the response at concentrations above the NEC, and $I(.)$ is the indicator function:

$$I(x; \theta_1) = \begin{cases} 1 & \text{if } x > \theta_1 \\ 0 & \text{otherwise} \end{cases} \quad (2)$$

A Bayesian approach to estimation and inference for the NEC (γ in Equation 3) of the simple exponential-threshold model described in Pires et al. (2002) was outlined by Fox (2010). This model has the functional form given by Equation 3

$$y_i = \mu(x_i; \alpha, \beta, \gamma) + \varepsilon_i$$

where

$$\mu(x_i; \alpha, \beta, \gamma) = \alpha \exp[-\beta(x_i - \gamma)I(x_i; \gamma)]; \{\alpha, \beta, \gamma\} > 0$$

and

$$\varepsilon_i \sim N\left(0, \sigma_\varepsilon^2\right) \quad (3)$$

The parameters of Equation 3 are also readily estimated using maximum likelihood methods in the "drc" package via the function NEC.3 (Ritz et al., 2015).

The NOEC

The NOEC is that concentration, c_l, among the set of concentrations $\{c_0 < c_1 < \cdots < c_k\}$ such that the mean responses at concentrations $\{c_1 < c_2 < \cdots < c_l\}$ are statistically indistinguishable from the mean response at concentration c_0. The identification of c_l is usually made using Dunnett's test following a one-way analysis of variance of the null hypothesis: $H_0 : \mu_0 = \mu_1 = \cdots = \mu_k$.

THE NSEC

Jurisdictions around the world have grappled with providing guidance on which toxicity metric(s) should be used for SSD modeling. In Australia and New Zealand the most recent advice is that a NEC is the preferred measure, followed by a low-order "effect" metric such as the EC*x*, the *x*% inhibition concentration, or *x*% lethal concentration, with $x \leq 10$ and the NOEC being the least preferred (Warne et al., 2015, 2018). There are difficulties with each of these, which we discuss in turn.

Firstly, we agree that the NEC should be the default measure *when the C-R data indicate a threshold in the response*. In the absence of any discernible threshold or where biological considerations rule this out, the estimated NEC is not an estimate of a concentration for which there is "no effect." In other words, the estimated NEC is spurious. Secondly, using a low-order effect concentration is, as already mentioned, logically inconsistent with the objectives of SSD modeling; and, on that basis, we recommend against using EC*x* data for SSD modeling. Finally, although one of us (D. R. Fox) along with many others has been part of the chorus of calls to stop using NOECs, we acknowledge that the inertia to heed those calls may in part be because the NOEC has a certain intuitive appeal. This is perhaps tied to the "NO" part of the NOEC acronym—it is a concentration below which no (statistically) discernible effect (relative to a notional "control") was *observed*. *Statistical* significance has long been used by environmental scientists as a surrogate for *biological* significance owing to the difficulty in defining the latter.

In view of the foregoing, we describe an alternative toxicity metric, the NSEC, to cater for those instances where the empirical dose–response relationship has no threshold and/or a threshold effect is biologically untenable. Figure 2 illustrates the relationships between the various toxicity metrics as well as the Bayesian NSEC estimate, which we discuss later. The idea was initially proposed by Bellio et al. (2000) and further developed by Chèvre et al. (2002), who termed the concept the *statistical-no-effect concentration*. While proposed more than 20 years ago, the concept has gained little traction in the ecological community, despite its potential value in providing a no-effect toxicity estimate in the absence of threshold effects. In the following sections, we describe the NSEC and provide details of both Bayesian and frequentist methods of inference.

ESTIMATION AND INFERENCE FOR THE NSEC

To motivate the discussion, consider the fish growth data given in Table 1 and shown in Figure 3. The data are growth rate, over a period of months at different concentrations of a pollutant. These data are hypothetical but serve as a useful starting point for the introduction of the NSEC concept based on data suitable for a normally distributed error function, for which computations can be simplified.

Using ordinary least squares (OLS), the parameters of a sigmoidal response model given by Equation 4 can be estimated. The resultant fit is shown by the red line in Figure 3.

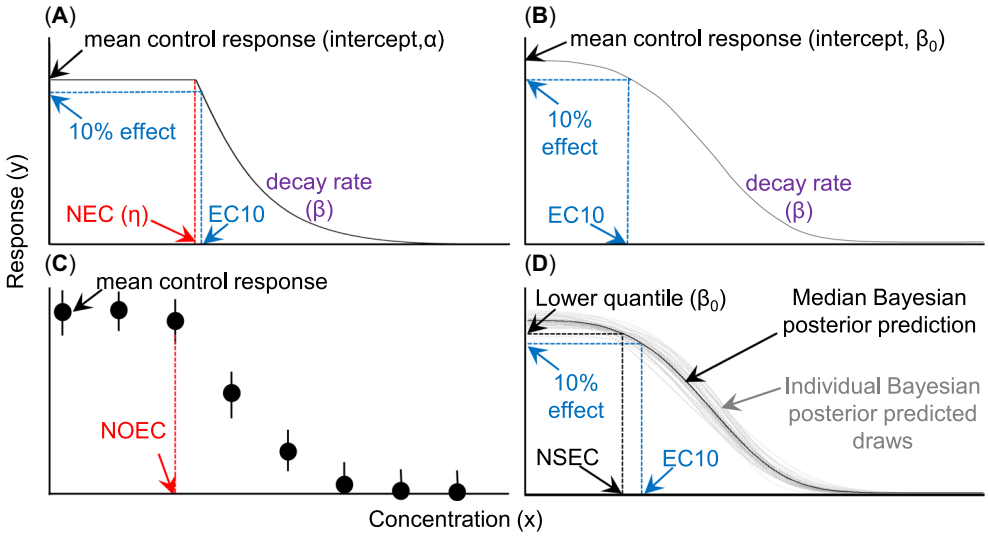

FIGURE 2: Conceptual diagrams of commonly used toxicity estimates, including (**A**) no-effect-concentration (NEC) model–based concentration–response (C-R) curve, showing the difference between a NEC and 10% effect concentration (EC10) toxicity estimate; (**B**) sigmoidal model–based C-R curve, showing the EC10; (**C**) treatment-based analysis estimating the no-observed-effect concentrations; and (**D**) sigmoidal model–based C-R curve, showing the EC10 and the derivation of an interpolated no-significant-effect concentration, with the dashed black line indicating the lower first percentile of the Bayesian posterior predictions for the control. NOEC = no-observed-effect concentration; NSEC = no-significant-effect concentration.

In addition, with the assumption of a normally distributed error function, confidence intervals for the parameter estimates as well as the mean response can be computed (blue lines in Figure 3).

$$Y_i = \beta_0 \exp\left[-\beta_1 x_i^{\beta_2}\right] + \varepsilon_i$$
$$\{\beta_0, \beta_1, \beta_2\} > 0;\ \varepsilon_i \sim N\left(0, \sigma_\varepsilon^2\right) \quad (4)$$

It is evident from Equation 4 that the β_0 parameter represents the mean response at zero concentration (the "control" group). For the fitted model in Figure 3, $\hat{\beta}_0 = 6.2$ with a standard error of 0.3059. Upper and lower limits of the 90% confidence interval for β_0 are [5.67, 6.73]. Thus, a response as low as 5.67 is indistinguishable from the mean response of the control group at the 5% level. A (modeled) mean response of 5.67 corresponds to a concentration of 5.38, and this concentration becomes the NSEC. By way of comparison, the NOEC estimated using a one-sided Dunnett's test at the 5% level is 11.

This example illustrates how the NSEC can be routinely computed using standard statistical software for a ratio-level response. While this is appropriate when the endpoint in a toxicity test is a measured quantity such as weight or length, modifications are required for quantal responses such as number of hatchlings, germination success, survival, or some categorical measure of impact.

In the following sections, we develop both Bayesian and frequentist methods to estimate the NSEC for a dichotomous response variable.

TABLE 1: Fish growth across a range of concentrations of an unknown pollutant

	Growth (cm)		
Concentration	Rep 1	Rep 2	Rep 3
0	6.59	6.14	7.19
1	4.91	5.03	6.25
2	5.89	7.44	6.11
5	4.51	6.75	5.69
11	4.52	5.52	3.71
25	2.54	1.76	0.21
50	0.10	0	0.91

There are three independent replicates for each concentration treatment.
Rep = replicate.

Frequentist estimation

We assume a data structure of the following form: {Y_i = number of "successful" outcomes out of n_i replications of an experiment at concentration x_i}.

Our assumed model is given by Equation 5 and is one which we have frequently fitted to C-R curves in our own ecotoxicological testing.

$$Y_i \sim bin(\pi_i, n_i)$$

where

$$\pi(x_i; \beta_0, \beta_1, \beta_2) = \beta_0 \exp\left[-\beta_1 x_i^{\beta_2}\right];\ \{\beta_0, \beta_1, \beta_2\} > 0 \quad (5)$$

Equation 5 is an example of a generalized nonlinear model in which the probabilities, π_i, are not expressible in the form $g(\pi_i) = \eta_i$, where η_i is a linear function of the parameters and $g(\cdot)$ is some monotone, differentiable function. Nevertheless, it

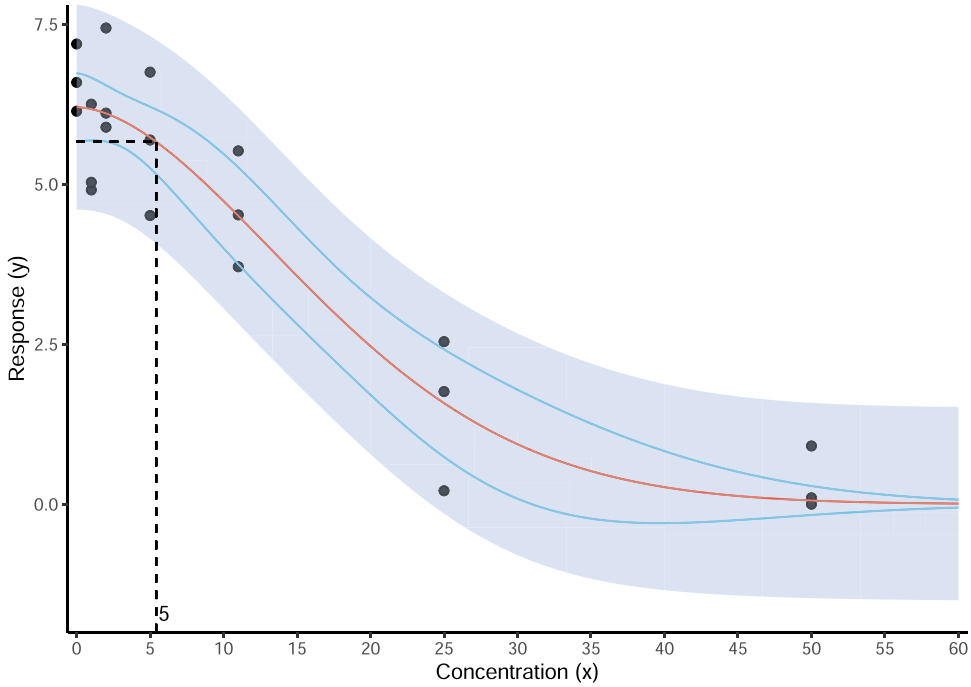

FIGURE 3: Fish growth data (solid circles). Red curve is estimated mean response function given by Equation 4. Blue lines denote limits of 90% confidence interval for mean response; 90% prediction band is represented by the shaded region. Horizontal dashed line is at the lower limit of the confidence interval for concentration = 0, and vertical dashed line is at the corresponding concentration.

is relatively straightforward to develop the necessary equations to obtain the maximum likelihood estimates (MLEs) for the parameter vector $\mathbf{B} = (\beta_0, \beta_1, \beta_2)^T$ as described in Supporting Information, Appendix A.

We illustrate the computations necessary for estimating the NSEC using maximum likelihood (frequentist) methods with an example that relates to assessing the impact of treated effluent on germination success. The data in Table 2 represent the number of successful germinations, Y_i, out of N_i seedlings of a species of macroalga when exposed to various concentrations, x_i, of a treated effluent. Three replications of the experiment were performed at each concentration. While these data are based on real assays, they are from historic confidential reports which cannot be shared publicly. They do, however, serve as a useful example to demonstrate the frequentist derivation of the NSEC for a binomial example.

The assumed model for Y_i is given by Equation 5. The OLS estimates of the parameters of Equation 5 are $\hat{\mathbf{B}}^{(OLS)} = \{0.8035; 9.865 \times 10^{-5}; 3.0786\}$. Using the R code in Supporting Information, Appendix A, the MLE is computed as $\hat{\mathbf{B}}^{(OLS)} = \{0.79997; 3.5472 \times 10^{-4}; 2.6278\}$. A plot of the data and fitted curves is shown in Figure 4A.

Although the fitted curves of Figure 4A are very similar in appearance, the toxicity estimates from the OLS fit are between 5% and 10% higher than those from the MLE fit (Table 3). For comparison, the NOECs have also been listed in Table 3, and the inadequacy of this toxicity estimate is evident. For example, at the 99% level of confidence, the NOEC is 30% to 40% higher than either of the NSEC values. Furthermore, the NOEC shows no variation, with confidence levels between 80% and 95%. In fact, the NOEC for 55% confidence is the same as the NOEC for 95% confidence (=10), whereas the $NSEC^{(MLE)}$ for 55% confidence is 4.14.

TABLE 2: Number of successful germinations (response) out of *n* seeds of a species of macroalga when exposed to various concentrations of a treated effluent

Concentration	Rep 1	Rep 2	Rep 3
0.1			
Seeds	13	14	12
Germinations	9	11	10
5			
Seeds	12	8	8
Germinations	8	7	8
10			
Seeds	10	7	11
Germinations	8	4	9
15			
Seeds	8	12	13
Germinations	6	7	6
20			
Seeds	11	11	7
Germinations	2	3	2
25			
Seeds	13	10	11
Germinations	1	1	2
30			
Seeds	9	12	13
Germinations	1	1	1

Three replications of the experiment were performed at each concentration.
Rep = replicate.

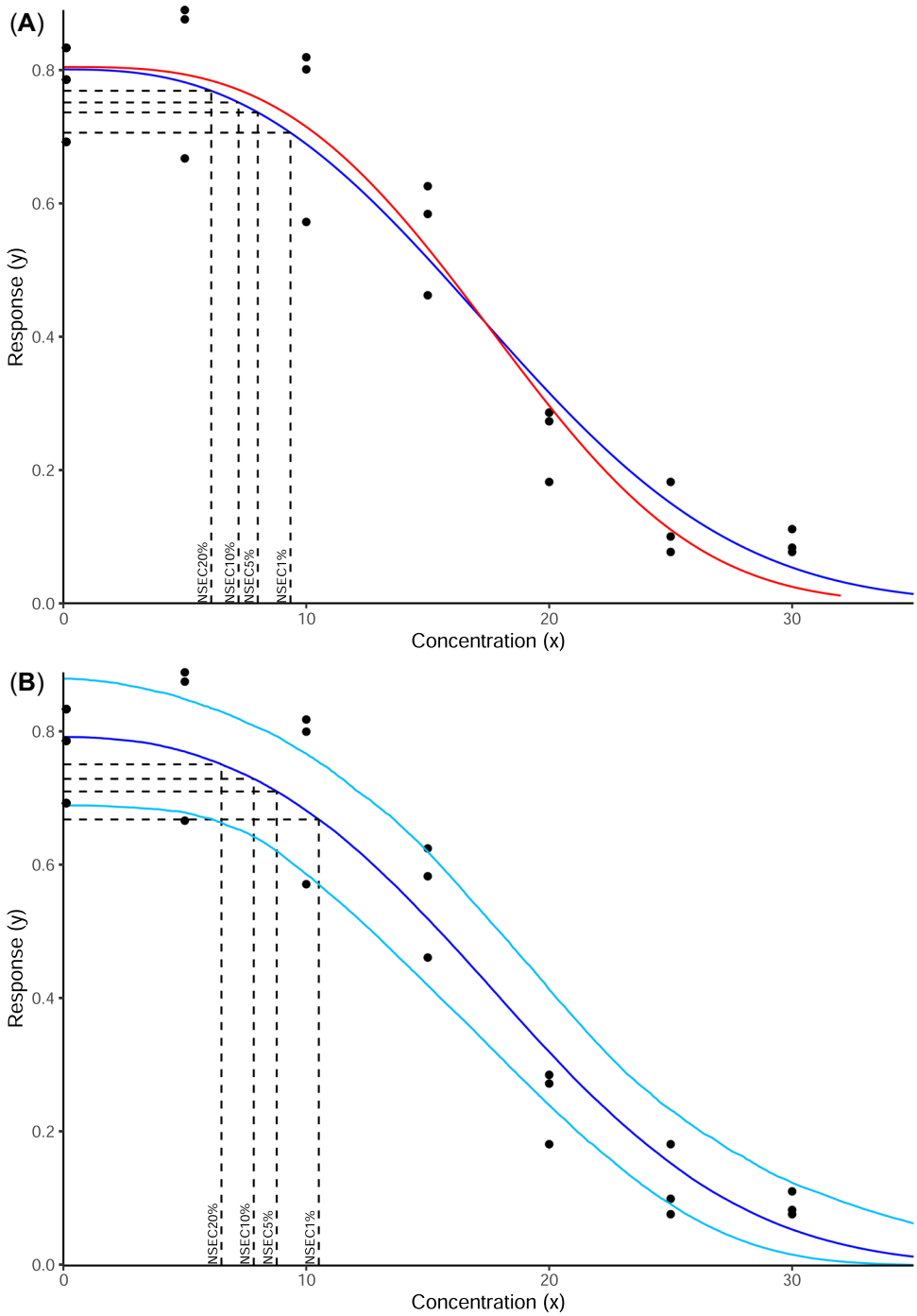

FIGURE 4: Concentration–response model of Equation 5, with parameters estimated by ordinary least squares (red curve, **A**), maximum likelihood estimate (MLE; blue curve, **A**), and Bayesian Markov Chain Monte-Carlo (MCMC; blue curve, **B**). Vertical lines are (left to right) no-significant-effect concentrations at 20%, 10%, 5%, and 1% estimated from the MLE fit (**A**) and MCMC fits (**B**).

In the following sections we describe a more flexible Bayesian approach to the estimation of an NSEC by accommodating any user-specified model and error structure.

Bayesian estimation

It is true that frequentist methods have dominated the ecotoxicological landscape, although the pace of development and uptake in the use of Bayesian methods has accelerated over the past 10 years (see Billoir et al., 2008; Fisher et al., 2020, 2023; Pollino & Hart, 2005). This is largely attributable to the ready availability of Bayesian software tools including WinBUGS (Lunn et al., 2000), JAGS (Plummer, 2003), and Stan (Carpenter et al., 2017). Still, impediments to the widespread uptake of these Bayesian software tools exist (Fisher et al., 2019). There is a range of "enabler" packages such as R2WinBUGS, R2jags (Su et al., 2015), and RStan (2022) that allow these Bayesian tools to be called within R. While these still require model specification,

TABLE 3: Comparison of the no-significant-effect concentration estimate for the data shown in Figure 4 (Table 2)

	Lower confidence bound (%); significance level			
Toxicity estimate	99; 0.01	95; 0.05	90; 0.10	80; 0.20
NSEC				
MLE (present study)	10.7	9.14	8.22	6.93
OLS	11.2	9.82	8.97	7.75
MCMC (present study)	10.5 (6.15–13.6)	8.78 (0–12.1)	7.84 (0–11.5)	6.51 (0–10.7)
NOEC				
Dunnett's test	15	10	10	10

Included are estimates based on 99%, 95%, 90%, and 80% bounds of the control (equivalent to a 0.01, 0.05, 0.10, and 0.20 significance level, respectively). The NSEC was calculated using OLS, MLE, as well as Bayesian MCMC. The NOEC values based on Dunnett's test are shown for comparison.
OLS = ordinary least squares; MCMC = Markov chain Monte Carlo; MLE = maximum likelihood estimate; NOEC = no-observed-effect concentration; NSEC = no-significant-effect-concentration.

which does involve some nontrivial coding, the rapid expansion of these and similar packages within the R community is greatly increasing accessibility of Bayesian methods. We are involved in several projects aimed at even further simplifying C-R model fitting using Bayesian tools (Fisher et al., 2020, 2023).

In the present study, we used the R2Jags package to fit the Bayesian version of the sigmoidal model in Equation 5 to the data from Table 3 as a way of demonstrating the Bayesian implementation of the NSEC concept. The full R code for running this example can be found in Supporting Information, Appendix B.

We start by defining the BUGS model ASCII file required to run JAGS:

```
model
  {
    # likelihood
    for (i in 1:N)
    {
      theta[i]<-b0*exp(-b1*(x[i])^b2)
      # response is binomial
      y[i]~dbin(theta[i],trials[i])
    }
    # specify model priors
    b0 ~ dunif(0.0001,0.999)
    b1 ~ dgamma(0.0001,0.0001)
    b2 ~ dnorm(1, 0.0001)
  }
```

The components of the BUGS model are a likelihood function; a function describing the relationship between the probability of a "success" and concentration; and priors distributions for the model parameters. The mean response, theta, is modeled as a function of *x* (concentration) via the exponential function given in Equation 5. We specify the priors for the intercept (b0) as uniform (0.0001, 0.999), for the exponential slope coefficient (b1) as gamma (0.0001, 0.0001), and for the exponent (b2) as normal (1, 0.0001). Running this model in R is relatively straightforward using the jags function in the R2jags package (Su et al., 2015) and can be achieved using the following R code:

```
library(R2jags)
dat <- read.csv("table 2.csv")
model_file <- "jags_model.txt"

# create jags model data list
mod.dat <- list(
x = dat$concentration, # concentration
y = dat$response, # response (successes)
N = nrow(dat), # Sample size
trials = dat$n # binomial trials
)
params <- c("b0", "b1", "b2")
fit <- jags(data = mod.dat, parameters = params, model = model_file)
```

The data are passed to JAGS as a list of the concentration data (*x*) and the response data (*y*), with trials specified as the number of observations per replicate. The sample size is the total number of rows in the data (Supporting Information, Appendix B). We fitted the model using the default initial values generated by JAGS: three chains, 2000 iterations, and a burn-in of 1000.

From the model fit the posterior draws of the three parameters b0, b1, and b2 are obtained, with median estimated values of $\hat{\mathbf{B}}^{(MCMC)} = \{0.791946;\ 2.4976 \times 10^{-4};\ 2.7381\}$. We can calculate posterior predicted values for a vector of concentration data (x.seq) for the mean response from the three parameters, with the following code:

```
parameter_posteriors <- do.call("cbind", fit$BUGSoutput$sims.list[params])
colnames(parameter_posteriors) <- params
head(parameter_posteriors)

x.seq <- seq(0, 35, by=0.01)
y.pred <- apply(parameter_posteriors, MARGIN = 1, FUN = function(r){
r[1] * exp(-r[2] * (x.seq)^r[3])
})
m.vals <- apply(y.pred, MARGIN = 1, FUN = quantile, probs = 0.5)
up.vals <- apply(y.pred, MARGIN = 1, FUN = quantile, probs = 0.975)
lw.vals <- apply(y.pred, MARGIN = 1, FUN = quantile, probs = 0.025)
```

These are summarized as a median across all posterior draws (red line, Figure 4B), with the upper and lower confidence limits calculated as the 0.025 and 0.975 quantiles, respectively (shaded blue area, Figure 4B).

To estimate NSEC using the JAGS output, we first obtain the appropriate quantiles of the predicted *y*-intercept, b0, which is an estimate of the response at the control treatment:

```
control_quantiles <- quantile(parameter_posteriors[, "b0"],
                              probs = c(0.01, 0.05, 0.10, 0.20))
```

We then use backward interpolation to solve for x given each control quantile value (rearranging Equation 4):

```
NSEC_vals <- apply(parameter_posteriors, MARGIN = 1, FUN = function(r){
    sapply(control_quantiles, FUN = function(p){x <- (-log(p/r[1])/r[2])^(1/r[3])})
})
```

We obtain a median of these interpolated values from all 3000 available posterior draws as the estimate of NSEC (Table 3). The upper and lower 95% credible intervals of the NSEC can also be obtained as the 0.025 and 0.975 quantiles of the posterior predicted values of the NSEC obtained from each posterior draw, noting that the lower bound will be 0 for any significance level greater than the 0.025 quantile (Table 3). The Bayesian Markov Chain Monte Carlo (MCMC)–based estimates for these data are very similar to the MLE estimates, albeit slightly lower (Table 3).

DISCUSSION

We highlight an alternative toxicity estimate, the NSEC, suitable for estimating a NEC surrogate when there is no evidence of a threshold effect in the C-R data. The method is related to the NOEC, in that the estimated no effect is defined as the concentration at which there is no statistically significant difference in the response relative to the control concentration. The NSEC resolves three of the five main concerns identified in the introduction associated with the use of NOECs. Specifically, NSECs are not constrained to be one of the test concentrations chosen by the researcher, they make use of the relationship between concentration and response, and statements of precision are possible. In this regard, the NSEC can clearly be considered a substantial improvement over the NOEC as a toxicity estimate of no effect. The method retains the intuitive appeal of the NOEC related to it being a concentration below which no (statistically) discernible effect (relative to a notional "control") was *observed*. In this sense, the NSEC can still be considered a valid measure of no effect, unlike the EC*x*—which by definition must represent some arbitrarily defined "effect" and is logically inconsistent with the objectives of SSD modeling.

The NSEC does not, however, overcome all of the five issues identified with the NOEC. First, poor experiments will favor larger NSECs (i.e., more liberal rather than more stringent values) because the increased stochastic error will result in wider confidence bands and thus higher, less conservative estimates of "no effect." Second, the estimated NSEC will be very much influenced by the degree of variability in the control. Adequately describing this variability requires, among other things, ensuring that an appropriate statistical distribution is used for modelling the response variable (Bolker et al., 2009; Harrison et al., 2018; Szöcs & Schäfer, 2015). The chosen distribution should result in a good fit, be consistent with the likely data-generation process, and appropriately reflect the variance apparent in the control treatment. In addition, for quantal response data, it is critical to check for under- or overdispersion in the response variable and to contemplate alternatives to the standard binomial or Poisson distributions (e.g., beta-binomial [Griffiths, 1973] or negative binomial [Bliss & Fisher, 1953]), if necessary. Furthermore, like the NOEC, the NSEC will be sensitive to sample size. Because an experiment with low replication will yield greater uncertainty in the estimation of the control, an NSEC-based toxicity estimate will be less conservative. Such issues related to the impact of experimental design are well understood in the case of the NOEC (Green et al., 2018) and can presumably be resolved similarly for the NSEC through clear guidance on best practice experimental and statistical approaches for estimating valid NSEC values. While a formal treatment of optimal design considerations for estimating the NSEC is beyond the scope of our study, techniques do exist (Fox, 2016).

Second, like the NOEC, the NSEC is dependent on the selection of a significance level for statistical testing. The use of p values in science has been widely criticized (Wasserstein & Lazar, 2016; Wasserstein et al., 2019), and there have been calls to move beyond p value–based hypothesis testing in ecotoxicology (Erickson & Rattner, 2020). However, for the NOEC (and therefore the NSEC), the use of p values is directly linked to their intuitive appeal relative to the use of EC*x*. This is because defining an appropriate level of statistical significance is perhaps easier than defining a biologically relevant "effect" (Green et al., 2013). In our example, as the defined level of significance is decreased from 99% to 80%, we see the expected corresponding decrease in the estimated NSEC concentration, and the toxicity estimate becomes more conservative. This would be equivalent to effectively decreasing the significance value in a Dunnett's test–based NOEC analysis (as we have done in Table 3) and is counter to the current Australian recommendations, which indicate that the significance level in NOEC testing must be less than at least 0.05 (Warne et al., 2018). In our example, we used a "significance value" as low as 1%, which is considerably lower than 0.05. The appropriate level of significance to use for NSEC will be dependent on the specific risk context with due consideration in the context of relative Type 1 and Type 2 error rates. While higher values could be considered (and would yield lower, more conservative NSEC estimates), if the significance value (alpha) for testing relative to the controls is higher than the value used to calculate the lower bound of the NSEC estimate (typically 2.5% for a 95% credible interval), then the estimated lower bound for the NSEC will be zero. Such an outcome potentially validly reflects the underlying fact that the confidence bounds of a true no-effect value may in fact contain 0 because, for smooth curves, a decline in the response may occur at the lowest concentration.

The Bayesian MCMC-based estimates of NSEC were very similar to the MLE estimates in our example, although this is not always expected to be the case. There are several advantages of using Bayesian methods for calculating NSEC values. First, it is relatively easy within the currently available Bayesian packages (JAGS [Plummer, 2003], Stan [Carpenter et al., 2017]) to generalize the approach to a broad range of likelihood functions (e.g., Poisson, gamma, beta, negative binomial,

beta-Binomial) to accommodate the wide range of response types commonly used in ecotoxicology (Szöcs & Schäfer, 2015). This is critical given our above discussion highlighting the importance of using the appropriate statistical distribution to model the response to properly quantify uncertainty. While our example focused on a single three-parameter sigmoidal function, there is a wide range of possible monotonically decreasing functional forms that can be used to model C-R data in ecotoxicology (Ritz et al., 2015). Expanding the range of models that can be used is very simple within the Bayesian framework using the currently available packages in R. Indeed, this is already being achieved in two recently developed C-R packages (Fisher et al., 2020, 2023; https://github.com/open-AIMS/bayesnec). The Bayesian approach also has two other advantages in C-R modeling, previously noted by Krull (2020): (1) direct inclusion of uncertainty in the estimates, which can be drawn directly from the posterior distribution, and (2) the fact that prior information can be adjusted by using expert elicitation, information from the literature, or previous experiments. Even when weakly informative priors are used, their inclusion in the model-fitting process can contribute to the accuracy of the method and improve the stability of model fitting (Krull, 2020).

CONCLUSIONS

The NSEC provides an alternative to the NEC toxicity estimate for data that do not exhibit threshold effects, which can be calculated using both frequentist and Bayesian approaches. The NSEC is conceptually similar to the traditional NOEC but superior in two key ways: (1) it decouples the estimate from being directly dependent on the placement of treatment concentrations by using model-based extrapolation, and (2) it provides the additional advantage of yielding a confidence bound around the resulting toxicity estimate.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5610.

Acknowledgments—We thank A. Negi, F. Flores, G. Batley, and J. Van Dam for helpful comments on an early draft of the manuscript. The present study was funded through the Australian Institute of Marine Science (AIMS) internal project (003125, 003518). Members of the AIMS Tropical Ecotoxicology and Risk Assessment team provided valuable feedback in the development of the NSEC concept, trialing early implementations of the approach. Open access publishing facilitated by The University of Western Australia, as part of the Wiley - The University of Western Australia agreement via the Council of Australian University Librarians.

Author Contributions Statement—**Rebecca Fisher**: Conceptualization; Data curation; Formal analysis; Software; Writing—original draft; Writing—review & editing. **David R. Fox**: Data curation; Formal analysis; Methodology; Software; Visualization; Writing—original draft; Writing—review & editing.

This article has earned both an Open Data and an Open Materials badge for making publicly available the digitally shareable data necessary to reproduce the reported results. The data are available at https://github.com/open-AIMS/NSEC_ETandC. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

Data Availability Statement—The data used for analyses in the present study are all freely available and contained within the article's tables. In addition, we have made all the data and R code to reproduce the materials presented available on github at https://github.com/open-AIMS/NSEC_ETandC.

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