# Methods for estimating no effect toxicity concentrations in ecotoxicology.

**Running header:** Estimating low- and no-effect thresholds

**Authors:** R. Fisher1,2, D.R. Fox3,4, A. Negri1, J. van Dam1, F. Flores1

**Affiliations:**

1 Australian Institute of Marine Science, Crawley, Australia  
2 University of Western Australia, Crawley, Australia  
3 Environmetrics Australia Pty Ltd, Beaumaris, Vic 3193, Australia  
4 University of Melbourne, Parkville 3010, Australia

**Corresponding author:** Rebecca Fisher

**Email:** [r.fisher@AIMS.gov.au](mailto:r.fisher@AIMS.gov.au)

## Abstract

A range of new statistical approaches are being developed and/or adopted in ecotoxicology, that when combined, have the potential to greatly improve the estimation of no-effect toxicity values from Concentration-Response (CR) experimental data. In particular, we compare the existing No-Effect-Concentration (NEC) threshold-based toxicity metric with an alternative No-Significant-Effect-Concentration (NSEC) metric suitable for when CR data do not show evidence of a threshold effect. Embedded within a model averaging approach, these metrics can be combined to seamlessly yield estimates of no-effect concentrations, along with estimation of their uncertainty. The outcome is a framework for CR analysis that is robust to uncertainty in the appropriate model formulation, and for which resulting no-effect toxicity estimates can be confidently integrated into risk assessment frameworks, such as the Species Sensitivity Distribution (SSD).

## Introduction

The species sensitivity distribution (SSD) describes the cumulative potential for harm to a range of species as the concentration of a contaminant or other stressor increases (Posthuma et al. 2001). SSDs underpin the derivation of protective concentrations (PCx) for x % of all species, or hazardous concentrations (HCx) for 100-x% of all species, that are applied in most current formal water quality guideline value (GV) derivations (Fox et al. 2021). The response values for the SSD are the toxicity estimates extracted from individual species concentration-response (CR) experimental data. Estimates of protective concentrations from the SSD is critically dependent on the input data, particularly when sample sizes are small, and a high level of protection is desired (PC 95 and PC 99). Within some jurisdictions there is substantial formal guidance on the SSD derivation itself with strict scrutiny and criteria around the methods used (Warne et al. 2015; Warne et al. 2018). However, there is comparatively little advice surrounding the methods adopted to derive the underlying toxicity estimate data. Under the conservative SSD paradigm, toxicity estimate data must necessarily represent no-effect concentrations.

There is a wide range of statistical metrics have been adopted to estimate low- or no effect toxicity values for use use in SSDs. These include the No-Observed-Effect-Concentration (*NOEC*); Effect-Concentration of a defined percentage *x* (typically 10%, *EC10, LC10*) and the No-Effect-Concentration (*NEC*, (Pires et al. 2002; Fox 2010)). There is considerable debate in the literature regarding the validity and value of different approaches for estimating endpoint values (Fox 2008; Warne and Van Dam 2008; Fox 2012; Green et al. 2013; Fox and Landis 2016a, 2016b). All three methods are used in practice with each having strengths and weaknesses across the myriad of situations that arise in CR modelling. The current Australian guidelines indicate that: *‘… the preferred order of statistical estimates of chronic toxicity to calculate default and site-specific GVs is: chronic NEC, EC/IC/LCx where x≤10, BEC10, EC/IC/LC15–20, and NOEC. While all of these acceptable statistical estimates of toxicity are not numerically the same, they are all treated as equivalent for the purposes of deriving GVs …’* (Warne et al. 2018). However, the recommendation that “effect” concentrations are allowable in an SSD is logically inconsistent with SSD modelling (Fisher and Fox in review). Since affected versus not affected (HCx or PCx) is a binary outcome, to be consistent, the input data for the SSD must represent no effect toxicity estimates. This is the reason that an NEC is the preferred toxicity metric for SSD modelling.

The NEC is estimated using a threshold model (Pires et al. 2002; Fox 2008; Fox 2010), and represents the maximum concentration of no impact on a given species, thereby providing a toxicity measure that is ideal for incorporation into SSDs aimed at estimating protective concentrations of contaminants. While the toxicity estimate is generally considered the preferred measure for inclusion into SSDs, CR data do not necessarily exhibit threshold-like responses and applying a threshold model in this case will lead to poor outcomes (Krull 2020). This phenomenon has occurred in our own work, were at times the NEC model may fail to fit using standard packages such as drc in R (Ritz et al. 2015), or when it does fit successfully, may yield values that are higher than even the EC10 (Negri et al. 2021). In this case, a threshold model of response is clearly not appropriate, and the NEC will not be a good estimate of the no-effect concentration.

More recently Fisher and Fox (in review) suggested an alternative to the NEC, the No-Significant-Effect concentration (NSEC), suitable for estimating ‘no effect’ concentrations for data that do not exhibit a threshold response, but rather decline smoothly with increasing contaminant concentration. 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’ (SNEC). The method involves fitting a non-linear model to the CR data and evaluating the concentration at which the predicted value of the response becomes significantly different to the control concentration, based on the estimated variability and a pre-defined level of statistical significance. 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. The method also allows an estimate of precision of the resulting toxicity estimate. While Fisher and Fox (in review) provide a thorough description of the mathematical derivation of the frequentist version of the NSEC, as well as implementation of the Bayesian equivalent, the concept has yet to be evaluated more extensively using simulated and case study data.

In addition to the range of toxicity estimates that are used in SSDs, there are an even wider range of possible CR models that may be used to derive them. For example, the popular frequentist CR modelling package drc (Ritz et al. 2015) contains 23 possible non-linear functions that can be used, as does our recently developed Bayesian CR modelling package bayesnec (Fisher et al. 2023). These include multiple threshold models, as well as a wide array of models representing a smooth decline with increasing toxicant concentration. While there may be biological scenarios suggesting a threshold model is appropriate in some cases, this is not always well known, is not always obvious based on the data at hand, and given the specific experimental design. This makes it difficult to a-priori determine the best model to use for a given set of data. Given the many possible curves that may be used to describe the data, model uncertainty needs to be considered in any given analysis. Model averaging represents an approach that can provide a robust way of accommodating model uncertainty. Model averaging is achieved by fitting a candidate set of plausible models to the data and obtaining weighted averaged predictions of the metrics of interest (Burnham and Anderson 2002). Weights are based on the relative fit of each model to the data; thus the underlying data drive the selection of the best approach for estimating the no-effect toxicity value. Model averaging is currently being considered as a potentially more robust framework for SSD modelling (Fox et al. 2021) and is widely used in ecology (Dormann et al. 2018).

Here we explore estimation of no-effect-concentration toxicity values using the recently suggested NSEC toxicity measure in combination with NEC estimates, within a model averaging framework. We begin with a simplified simulation study including only two alternative models, an NEC threshold model and a simple sigmoidal decay function. We then present a case study using real data showing how the NSEC and NEC estimates, when combined using model averaging can yield seamless estimates of no-effect concentrations, along with estimation of their uncertainty.

## Simulation study

We constructed a simulation study to compare toxicity estimates obtained using NEC, ECx and the NSEC, across a range of experimental designs with differing replication. Simulations were based on four alternative scenarios representing four different theoretical concentration response relationships of a binary response endpoint. This included two scenarios based on the three-parameter NEC exponential decay threshold model of Fox (2010), with the parameters α (top, y intercept), β (exponential decay rate) and τ (the NEC threshold, see equation 1).

 where

 and

 (1)

We also included a three-parameter sigmoidal decay model representing a smooth decline with concentration (see equation 2). This includes that same parameters α (top, y intercept) and β (exponential decay rate), and additional parameter influencing shape of the decay function.

 (2)

These models were used to generate predicted data for a given concentration (x), using the following two R functions for the NEC threshold and sigmoidal and models respectively:

NEC3param <- function(x, NEC, top, beta) {  
 pre.index <- which(x <= NEC)  
 post.index <- which(x > NEC)  
 x.seq.pre <- x[pre.index]  
 x.seq.post <- x[post.index]  
   
 y.pred <- rep(NA, length(x))  
   
 y.pred.pre <- top  
 y.pred.post <- top \* exp(-beta \* (x.seq.post - NEC))  
   
 y.pred[pre.index] <- y.pred.pre  
 y.pred[post.index] <- y.pred.post  
   
 return(y.pred)  
}

ECxsigmoidal <- function(x, top, beta, d) {  
 y.pred <- top \* exp(-beta \* (x)^d)  
   
 return(y.pred)  
}

Graphical user interface

Description automatically generated

Figure 1. The four CR curves generated for the simulation study, including two NEC and two sigmoidal curves (based on equations 1 and 2 respectively). For all four scenarios the y-intercept parameter (mean value of the response for the control) was set at 0.9, and for the two NEC models we used the same exponential decay rate of 0.75. In all plots NEC and EC10 are shown in dashed red and blue lines respectively. Note that there is no theoretical NEC for smooth sigmoidal curves.

Parameters were selected for α, β, γ and δ (Table 1) to yield the four curves shown in Figure 1. Simulated data sets were randomly generated using these four curves based on a binomial distribution, to represent a hypothetical binary endpoint (e.g. survival) with a mean value of 90% success for the control (α, y-intercept). This was achieved by generating theoretical predicted probabilities for two theoretical experimental treatments sequences with either 8 (low density design) or 12 (high density design) treatment concentration values distributed evenly from 0.01 to a maximum hypothetical value of 10 concentration units. We applied the base R function *rbinom()* to randomly simulate binomial data at each treatment level for the predicted probability, with varying levels of n (5 or 10 *replicates*) and size (10 or 20 binomial *trials*, representing the number of individual test organisms within each replicate). This combination of experimental conditions resulted in 8 different sets of simulated data with the total number of ‘trials’ in each experiment ranging from 400 to 2400. This simulation process was repeated 100 times for each design, for all four model scenarios.

Table 1. Scenario parameters used in simulations.

| scenarios | α (top, intercept) | β (beta) | γ (NEC) | δ (d) | N treatments |
| --- | --- | --- | --- | --- | --- |
| Sigmoidal 1 | 0.9 | 5.0e-03 | - | 3.5 | 8 |
| Sigmoidal 1 | 0.9 | 5.0e-03 | - | 3.5 | 12 |
| NEC 1 | 0.9 | 7.5e-01 | 2.5 | - | 8 |
| NEC 1 | 0.9 | 7.5e-01 | 2.5 | - | 12 |
| Sigmoidal 2 | 0.9 | 1.0e-08 | - | 9.0 | 8 |
| Sigmoidal 2 | 0.9 | 1.0e-08 | - | 9.0 | 12 |
| NEC 2 | 0.9 | 7.5e-01 | 6.0 | - | 8 |
| NEC 2 | 0.9 | 7.5e-01 | 6.0 | - | 12 |

We used the R package jagsNEC <https://github.com/open-AIMS/NEC-estimation> (Fisher et al. 2020) to fit both the 3 parameter NEC and sigmoidal models to the randomly simulated data. Both models were fit in a single call to fit.jagsMANEC, with model.set set to c("NEC3param", "ECxsigmoidal"), which represent the two original model types used to generate the two NEC and sigmoidal scenarios (see equation 1 and equation 2). Posterior estimates for the NEC were obtained directly from the NEC model fit for each simulated dataset as the estimated parameter. Posterior estimates for NSEC were obtained with the function extract\_NSEC (Fisher et al. 2020) which implements the Bayesian method for estimating No-Significant-Effect-Concentrations, as described in Fisher & Fox (Fisher and Fox in review) (Fisher and Fox in review). We also obtained model averaged posterior estimates of EC10, EC5 and EC1 using the function extract\_ECx (Fisher et al., 2020).

### Comparing model weights

The jagsNEC package uses DIC based model weights to generate model averaged estimates of the toxicity estimates of interest, such as EC10 and NSEC values. To compare NSEC estimates across the different scenarios, we first wanted to establish if the model weighting procedure works effectively in these examples.

Chart, scatter chart

Description automatically generated

Figure 2. DIC based model weight for the NEC3param model for each scenario as a function of the total sample size (number of replicates x number of individuals) and the number of experimental treatment levels (n.treatments).

We found that the underlying generating model usually had the highest weight for most simulated datasets (Figure 2). There were some exceptions, with a few simulations resulting in high weight for the model not used to generate the data, particularly for low sampling and treatment density (Figure 2).

There was a tendency for the data generated from the second sigmoidal scenario to yield a higher weight for the NEC model, particularly when there are few treatments (Figure 2). This tendency persists, even with quite high sample density within treatments. There was also a tendency for data generated using the first NEC scenario to have a high weight for the sigmoidal model when sampling density is lower, with this occurring more frequently when there are few treatments (Figure 2).

### Comparing NSEC estimates

Box and whisker chart

Description automatically generated

Figure 3. Estimated model averaged NSEC value based on a 99% certainty value for each scenario as a function of the total sample size (total.reps, number of replicates x number of individuals) and the number of experimental treatment levels (n.treatments). Also shown are the true toxicity estimates for each scenario, including EC1, EC10, EC5 and NEC. Note that there is no theoretical NEC for sigmoidal curves, thus no horizontal red lines are shown for these scenarios.

Estimated NSEC values are more variable with lower sample density across all four scenarios (Figure 3). Estimated NSEC values are very close to NEC estimates for data simulated using a NEC-type model. At lower sample sizes uncertainty in NSEC estimation appears to bias towards an underestimate of NEC for data based on an underlying NEC model (i.e. estimated NSEC is lower than NEC) (Figure 3). This is due to the fact that the NSEC is based on a lower bound of a confidence limit. For the NEC models the three ECx values (1, 5 and 10) all fall relatively close to the true NEC, and all represent relatively precise estimates of low-effect concentration, with only minimal range between the 1% and 10% effect values, due primarily to the relatively steep decline used in the simulations (Figure 3).

For the sigmoidal models there is no true theoretical NEC, so there is no horizontal red line on Figure 3 for these curves. The three ECx values differ markedly across their range from the EC1 to the EC10, with the second sigmoidal scenario showing a slightly tighter range of values (Figure 3) due to the slightly sharper decline (Figure 1) and reflecting the high dependency of ECx on the shape of the curve. As for the NEC modelled data, estimates of NSEC for sigmoidal data show greater variability with low sample density (Figure 3). However, for the sigmoidal models, NSEC values also tend to decline as sampling density increases (Figure 3). For these scenarios, the estimated NSEC values fall across the range of EC1 to EC10 (Figure 3).

So far, we have compared our NSEC estimates to the true known toxicity estimate values of our simulated data. But how do these compare to those equivalent toxicity estimates as they would be estimated using the same Bayesian model fits used to estimate the NSEC values? When NSEC is estimated for data based on a NEC model, there is a good relationship with medians of estimated NEC values from the same data, with many simulations estimating these as near equivalent and centered around the true NEC values (two upper plots, Figure 4). There is a tendency for NSEC estimates to be lower than equivalent NEC estimates from the same model, suggesting NSEC is conservative relative to NEC (Figure 4), at least given the experimental designs simulated in this study. This tendency is apparent as a bias downwards, with points below the 1:1 black line indicating when NSEC estimates are lower than NEC (Figure 4). This bias towards lower toxicity estimates is greatest for the second NEC model and for the simulations based on highly replicated experiment data (Figure 4).

For data generated using a sigmoidal model NSEC estimates are generally much lower than estimates for NEC based on the same dataset (two lower plots, Figure 4). Estimates of NEC for data generated using these two sigmoidal models are quite high relative to even the EC10 estimate, with most estimates falling on the higher side of the true EC10 value for these curves (most points are to the right of the yellow vertical line, Figure 4). In contrast the NSEC estimates from these simulated data span the range of true EC1 to EC10 values (horizontal lines, Figure 4). The estimated NSEC value is always lower than the estimated NEC value for these sigmoidal models, given the simulated experimental design, and therefore tends to be conservative (Figure 4).

Graphical user interface

Description automatically generated

Figure 4. Median estimated model averaged NSEC values based on a 99% certainty value for each scenario as a function of estimated NEC from the same data. Shown as horizontal and vertical lines are the true toxicity estimates for each scenario, including EC1, EC10, EC5 and NEC. Note that there is no theoretical NEC for the sigmoidal scenarios, although an ‘NEC’ estimate was obtained using the NEC model. The diagonal black line shows the 1:1 relationship indicating where NSEC estimates are equal to estimated NEC. Points are coloured according to the total sample size (total.reps, number of replicates x number of individuals).

These findings are confirmed via violin plots of all the toxicity estimates for data simulated for a well replicated (n.treatments=12, total.reps=2400) and poorly replicated experiment (n.treatments=8, total.reps=400) (Figure 5). All toxicity estimates are well aligned for data generated using an underlying NEC model (Figure 5). Poor replication in experiments based on the NEC model can result in highly variable estimates of EC10 and NSEC toxicity estimates, particularly for the second scenario when the NEC was situated further along the concentration axis (Figure 5). The estimation of the NEC parameter in these models seemed relatively more robust to deceases in sampling density based on these simulations (Figure 5).

Variation was much higher in all simulated toxicity estimates when sampling density is low for data based on a sigmoidal model (Figure 5). The expected differences between EC1, EC5 and EC10 were observed for simulations based on both high and low sampling density for the sigmoidal model and these estimates correspond well with their true values (Figure 5). As would be expected, estimates of NEC for the sigmoidal model were highly unreliable and generally higher than the EC10 estimates for these same data, due to the fact that the underlying model has no theoretical NEC (Figure 5). Estimates of NSEC were similar to EC10 estimates for simulations based on low sampling density and fell between the EC1 and EC5 estimates for simulations based on high sampling data (Figure 5).

The width of the estimated 95% confidence bands were consistent across all toxicity estimate types for the data based on the NEC model, although these were considerably wider for data with low sample density (Figure 5). The width of the estimated 95% confidence bands for the NSEC estimates were considerably higher than for the other toxicity estimates for the sigmoidal model, and this appeared true for both levels of sample density (Figure 5).

Chart

Description automatically generated with medium confidence

Figure 5. Median estimated model averaged toxicity estimates and width of the 95% confidence band for each scenario for a well replicated (n.treatments=12, total.reps=2400) and poorly replicated experiment (n.treatments=8, total.reps=400), where total.reps is number of replicates x number of individuals. Shown as horizontal lines are the true toxicity estimates for each scenario, including EC1, EC10, EC5 and NEC. Note that there is no theoretical NEC for the sigmoidal scenarios, although an ‘NEC’ estimate was obtained using the NEC model.

## Case study

Here we explore issues associated with deriving no- and low-effect toxicity estimates using real data. We use data from our recent publication (Negri et al. 2021) that was analysed via the same jagsNEC package (Fisher et al. 2020) as used in the simulation study. These data examined the response of eight tropical marine species to the water accommodated fraction of gas condensate from the Ichthys and Prelude gas fields off the tropical northwest coast of Australia. The main aim of the original study was to build an SSD following the standard guideline methods (Warne et al. 2015; Warne et al. 2018) to validate mixture toxicity modelling for petroleum hydrocarbons. Here we focus on the underlying CR curves and use these to explore issues with fitting NEC models to real data, as well as how the NSEC toxicity estimate performs in practice. The original data considered eight different species. However, three of these did not show a complete response at the highest concentration examined (*A. muricata*, *P. foliascens*, and *Rhodomonas salina*), and fourth exhibited evidence of hormesis (*Cladocopium goreaui*). For simplicity, these four species were excluded here, leaving the remaining four species (*A. millepora*, *S. variolaris*, *N. dorsatus* and *A. amphitrite*) to be used in our case study assessment.

Substantial details regarding the collection of these data including the experimental conditions and a description of each assay are described in the original study (Negri et al. 2021) and the extensive supplementary files <https://ars.els-cdn.com/content/image/1-s2.0-S0025326X21009334-mmc1.pdf>. Response data for assays based on a decline in the percentage of larvae successfully completing metamorphosis (*A. millepora* and *A. amphitrite*) or fertilization success (*S. variolaris*) were initially modelled using a binomial distribution. However, in all cases, these initial models were over dispersed. Instead, these were converted to a proportion and the data modelled as a beta distribution. Response data for assays based on specific growth rate were normalised if values exceeded one (*N. dorsatus*) by dividing by the maximum observed value (to reflect proportional decline) and then also modelled using a beta distribution. Concentration data were modelled on a log scale, as this was the natural scaling evident in the placement of treatments across the concentration range considered.

NECs and EC10s were estimated from CR curves generated in the R package jagsNEC (Fisher et al. 2020). This package uses a model averaging approach based on deviance information criterion (DIC) weighted averaged predictions across a potential candidate model set composed of a range of functional NEC models adapted from (Fox 2010), including models two NEC models (NEC3param, NEC4param) and a range of commonly used sigmoidal models (ECx models; ECx4param, ECxExp, ECxSigmoidal, ECxWeibull1 and ECxWeibull2, see Fisher et al. (2020) and the supplementary information for more details, including model formula). In all cases, chain mixing was assessed visually, and where models showed poor mixing, they were excluded from model averaged estimates of toxicity estimates. Only models with NEC as a specific parameter were used to obtain a DIC weighted model averaged estimate of NEC (i.e. both models with the prefix NEC). Estimates of 10% effect (EC10) and NSEC were calculated using DIC weighted model averaged estimates obtained from all successfully fitted models (both models with a prefix NEC and models with the prefix ECx, see (Fisher et al. 2020)).

The highest weighting model varied across the four species examined (Figure 6). For *A. amphitrite* there was strong weight for a single model – which was the original NEC 3-parameter model (Fox 2010) (Figure 6). For this species the model average based on “all” models was largely identical to the NEC model set, and the estimated values for the NEC, EC10 and NSEC were all very similar (with NEC marginally lower than the other two estimates), although the confidence bands for the NEC were substantially narrower than for the other two toxicity estimates (Figure 6). For the other three species there was substantial support for more than one model, and this generally included support for one of the “NEC” model types, as well as a smooth “ECx” model type (see Figure 6). For *N. dorsatus*, the EC10 estimate is nearly identical to the estimated NEC (blue vertical line overlaps the red vertical line), with the estimated NSEC corresponding to the lowest bound of the NEC (Figure 6). For the remaining two species the NEC estimate was definitively higher than the estimated EC10, and the NSEC estimate was lower than either the NEC or EC10 (Figure 6).

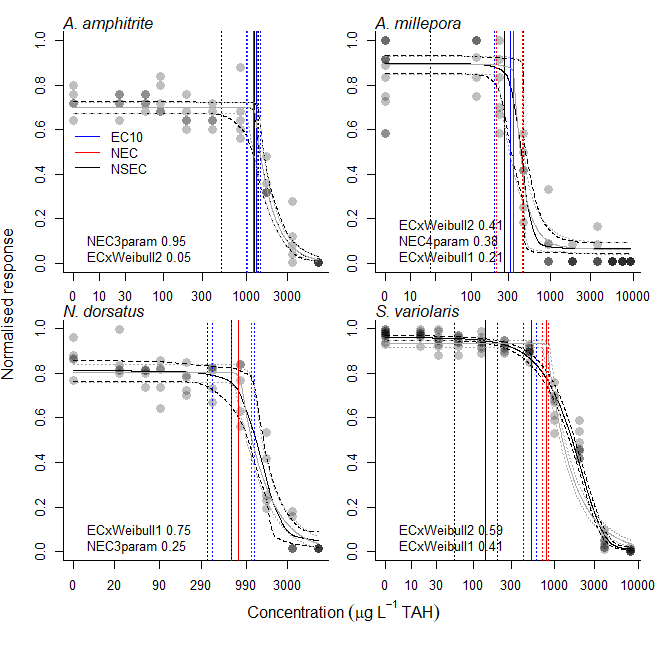


Figure 6. Concentration response relationships for the effects of 40% weathered Ichthys condensate WAF on four tropical marine species. Measured time-weighted average concentrations are expressed as Total Aromatic Hydrocarbons (TAH) on a log scale. Solid black curves are model-averaged Bayesian non-linear beta model fits with 95% credible intervals indicated by dashed lines. Darkgrey curves show the quivalent model-averaged fits using only NEC models. Binomial response data are the proportion of successes (A. millepora, and S. variolaris); growth rate response data taking values >1 are normalised relative to the maximum value (N. dorsatus); and growth rate response data taking values <1 are modelled on the original scale (A. amphitrite). The vertical red, blue and black lines indicate the estimated NEC, EC10 and NSEC values respectively, each with 95% confidence intervals (dashed lines based on posterior predictions).

# Discussion

The estimation of No-Effect-Concentrations are critical for deriving safe concentrations of thresholds in the environment. Threshold-based models for estimating toxicity estimates of no effect are ideal because they directly estimate the no effect concentration as a parameter in the model. However, some concentration-response data exhibit a gradual decline with concentration and cannot be appropriately modelled as thresholds (Fisher and Fox in review). Indeed, for both our simulation and the case study examples, when the 3-parameter NEC model of (Fox 2010) is fit to smooth sigmoidal data estimates of NEC are often higher than even the EC10 estimate. This occurs because the 3-parameter NEC model fit is unable to capture the gradual decline inherent in smoothly sigmoidal data and suggests that using the NEC model of (Fox 2010) is clearly not appropriate. In the original analysis of these case study data, we adopted the conservative approach of selecting the lower value of either the NEC or EC10 values (estimated using models properly capturing the smooth decline) for inclusion in the final SSD, which represents one solution for resolving this issue (Negri et al. 2021). Certainly, using the NEC as is in this case would result in the derivation of thresholds that are unlikely to be as protective of the community as intended. Estimates of the NSEC toxicity estimate presented here were always less than the NEC and EC10 in our case study, and usually less than both EC10 and EC5 in our simulations.

The fact that NEC models provide a poor fit to smoothly sigmoidal data resulting in NEC estimates higher in some cases than the EC10 is to be expected, because the NEC three-parameter model fitted here was not used to generate the underlying simulation data in this case. However, the general lack of alternative NEC step models currently available means that this is exactly how the NEC would be estimated from these data in practice. We had attempted to resolve this issue in the development of jagsNEC (Fisher et al. 2020), by including an additional model that expanded the original three-parameter model of (Fox 2010) to allow a sigmoidal decline in the response. However, using this model with real data often resulted in highly unresolved NEC estimates with extremely wide confidence bands (see Supplementary Information, Figure S1). There may be value in further developing the NEC modelling framework to allow for sigmoidal declines using other step model parameterisations. However, it seems likely that similar issues will occur for any sigmoidal model with a relatively flat upper asymptote as the transition point (step) of the NEC becomes unresolved.

For simulated data based on the underlying NEC model NEC, NSEC, EC1, EC5 and EC10 all produce very similar toxicity estimates. The relative similarity of all three ECx estimates occurs because of the sharp decay inherent in the exponential decay NEC model formulation of the underlying model used and is likely specific to the scenarios considered here. Overall, the simulation studies suggest that NSEC provides an estimate consistent with the true NEC for data based on an underlying NEC model, and if anything, when replication is high NSEC is conservative relative to NEC. For situations where the NEC model fits the data well, it is clear that NEC estimated as a parameter in a threshold model should be the preferred method, as this represents a true no-effect concentration. In the jagsNEC package that we have used for fitting our case study data (Fisher et al. 2020) a model averaging approach is used to provide ‘NEC’ estimates across a range of NEC and smooth sigmoidal models. For the sigmoidal models the NSEC is used as an estimate of the no-effect concentration, as a true NEC does not exist in this case. This model averaging strategy avoids the issue of having to decide a-piori which type of model fits the data better (NEC or sigmoidal) and instead uses an information theoretic approach (Burnham and Anderson 2002) to weight the resulting ‘NEC’ estimate, so it is based only on those models with the most support given the data. In this scenario, if the NEC model(s) fits the data better these will have high weight and the resulting estimate’ will be a true NEC estimate. Conversely, if the sigmoidal model(s) fit better, the resulting toxicity estimate will be based largely on a NSEC estimate.

As discussed in (Fisher and Fox in review) the derivation of NSEC is critically dependent on the estimation of uncertainty in the α (y-intercept) parameter. This means that the statistical methods used must be appropriate and able to accurately capture the level of variability observed in the control treatment. Furthermore, like the NOEC, NSEC will be sensitive to sampling effort. This is verified by our simulation study, which clearly shows that for data based on a sigmoidal model NSEC declines as sampling effort increases. This is expected, because experiments with low replication yield greater uncertainty in the estimation of α, which reduces the lower-bound estimate and results in higher estimates of the NSEC value. Clear guidance on best practice experimental and statistical approaches for estimating valid NSEC values are clearly warranted if these approaches are to be adopted (Fisher and Fox in review), and similar issues related to the impact of experimental design are well understood in the case of the NOEC (Green et al. 2018). In addition, it may be reasonable to adopt a similar approach to that of (Negri et al. 2021) where the estimated NSEC values are compared against a relevant ECx level, and the lower of the two adopted to ensure an appropriate level of conservatism is maintained, depending on the context of the analysis being undertaken.

# Conclusions

Overall it appears that the NSEC method proposed by (Fisher and Fox in review) has the potential to provide a useful estimate of “No-Effect” Concentration that can be used when response data do not show a clear threshold effect. Embedded within a model averaging approach, the NSEC and NEC can be combined to seamlessly yield estimates of no-effect concentrations, along with estimation of their uncertainty. The outcome is a framework for CR analysis that is robust to uncertainty in the appropriate model formulation, and for which resulting no-effect toxicity estimates can be confidently integrated into risk assessment frameworks, such as the Species Sensitivity Distribution (SSD).

# Acknowledgements

We thank Graeme Batley and Monique Binet for useful comments on the manuscript.

# References

Bellio R, Jensen J, Seiden P. 2000. Applications of likelihood asymptotics for nonlinear regression in herbicide bioassays. *Biometrics* 56:1204-1212.

Burnham K, Anderson D. 2002. *Model Selection and Multimodel Inference: A Practical Information‐Theoretic Approach, 2nd ed.* Springer, New York, NY, USA.

Chèvre N, Slooten KBv, Tarradellas J, Brazzale AR, Behra R, Guettinger H. 2002. Effects of dinoseb on the life cycle of Daphnia magna: Modeling survival time and a proposal for an alternative to the no‐observed‐effect concentration. *Environmental Toxicology and Chemistry: An International Journal* 21:828-833.

Dormann CF, Calabrese JM, Guillera-Arroita G, Matechou E, Bahn V, Bartoń K, Beale CM, Ciuti S, Elith J, Gerstner K, Guelat J, Keil P, Lahoz-Monfort JJ, Pollock LJ, Reineking B, Roberts DR, Schröder B, Thuiller W, Warton DI, Wintle BA, Wood SN, Wüest RO, Hartig F. 2018. Model averaging in ecology: a review of Bayesian, information-theoretic, and tactical approaches for predictive inference. *Ecological Monographs* 88:485-504. DOI: <https://doi.org/10.1002/ecm.1309>.

Fisher R, Barneche D, Ricardo G, Fox D. 2023. bayesnec: A Bayesian No-Effect- Concentration (NEC) Algorithm. R package version 2.1.0.2. <https://CRAN.R-project.org/package=bayesnec>.

Fisher R, Fox D. in review. Introducing the no-sigificant-effect-concentration (NSEC). *Environmental Toxicology and Chemistry*.

Fisher R, Ricardo G, Fox DR. 2020. Bayesian concentration-response modelling using jagsNEC. [*https://doiorg/105281/ZENODO3966864*](https://doiorg/105281/ZENODO3966864).

Fox D, van Dam R, Fisher R, Batley G, Tillmanns A, Thorley J, Schwarz C, Spry D, McTavish K. 2021. Recent Developments in Species Sensitivity Distribution Modeling. *Environmental Toxicology and Chemistry* 40:293-308.

Fox DR. 2008. NECS, NOECS and the EC {x}. *Australasian Journal of Ecotoxicology* 14:7-9.

Fox DR. 2010. A Bayesian approach for determining the no effect concentration and hazardous concentration in ecotoxicology. *Ecotoxicology and environmental safety* 73:123-131. DOI: <http://dx.doi.org/10.1016/j.ecoenv.2009.09.012>.

Fox DR. 2012. Response to landis and chapman (2011). [*https://doiorg/101002/ieam1264*](https://doiorg/101002/ieam1264).

Fox DR, Landis WG. 2016a. Comment on ET&C perspectives, November 2015—A holistic view. *Environmental Toxicology and Chemistry* 35:1337-1339.

Fox DR, Landis WG. 2016b. Don't be fooled—A no‐observed‐effect concentration is no substitute for a poor concentration–response experiment. *Environmental Toxicology and Chemistry* 35:2141-2148.

Green JW, Springer TA, Holbech H. 2018. *Statistical analysis of ecotoxicity studies*. John Wiley & Sons.

Green JW, Springer TA, Staveley JP. 2013. The drive to ban the NOEC/LOEC in favor of ECx is misguided and misinformed. *Integrated environmental assessment and management* 9:12-16.

Krull M. 2020. Comparing statistical analyses to estimate thresholds in ecotoxicology. *PloS one* 15:e0231149.

Negri AP, Brinkman DL, Flores F, van Dam J, Luter HM, Thomas MC, Fisher R, Stapp LS, Kurtenbach P, Severati A. 2021. Derivation of toxicity thresholds for gas condensate oils protective of tropical species using experimental and modelling approaches. *Marine Pollution Bulletin* 172:112899.

Pires AM, Branco JA, Picado A, Mendonça E. 2002. Models for the estimation of a ‘no effect concentration’. *Environmetrics: The official journal of the International Environmetrics Society* 13:15-27.

Posthuma L, Suter II GW, Traas TP. 2001. *Species sensitivity distributions in ecotoxicology. 1st Edition*. CRC press, Boca Raton. 616 pp.

Ritz C, Baty F, Streibig JC, Gerhard D. 2015. Dose-response analysis using R. *PloS one* 10:e0146021.

Warne M, Batley G, van Dam R, Chapman J, Fox D, Hickey C, Stauber J. 2018. Revised Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants – update of 2015 version. *Prepared for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality Australian and New Zealand Governments and Australian state and territory governments, Canberra, 48 pp*.

Warne M, Van Dam R. 2008. NOEC and LOEC data should no longer be generated or used. *Australasian Journal of Ecotoxicology* 14:1-5.

Warne MSJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW, Stauber JL. 2015. Revised Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants. Prepared for the Council of Australian Government’s Standing Council on Environment and Water (SCEW). Department of Science. *Information Technology and Innovation, Brisbane, Queensland*.