A Bayesian Approach for Determining the No Effect Concentration and Hazardous Concentration in Ecotoxicology

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ABSTRACT:

This paper describes a Bayesian modeling approach to the estimation of the no effect concentration (NEC) and the hazardous concentration (HC_x) as an alternative to conventional methods based on NOECs – the no *observed* effect concentration. The advantage of the proposed method is that it combines a plausible model for dose-response data with prior information or belief about the model's parameters to generate posterior distributions for the parameters – one of those being the NEC. The posterior distribution can be used to derive point and interval estimates for the NEC as well as providing uncertainty bounds when used in the development of a species sensitivity distribution (SSD). This latter feature is particularly attractive and overcomes a recognized deficiency of the NOEC-based approach. Examples using previously published data sets are provided which illustrate how the NEC / HC_x estimation problem is re-cast and solved in this Bayesian framework.

Keywords: species sensitivity distribution, NOECs, hypothesis testing, ecosystem protection.

INTRODUCTION

The Species Sensitivity Distribution (SSD) is a cornerstone of modern ecotoxicology and provides a basis for establishing guidelines, trigger values, and limits on concentrations of hazardous chemicals in animals and the receiving environment. In the context of water quality, use of the SSD is underpinned by the well-validated belief that aquatic species generally have different, although predictable responses to increasing concentrations of physical-chemical toxicants at the community/assemblage level. The familiar dose-response curves generated by laboratory experiments are used to estimate a variety of measures such as the LC_{50} (the concentration which is lethal to 50% of some defined population), the NEC (the maximum concentration which causes no adverse effect in a target organism), and EC_x (the concentration which affects x% of organisms in a dose-response experiment). Classical tools of statistical inference such as t-tests, ANOVA, and multiple comparison techniques are also widely used to estimate related statistical measures such as the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). One of the difficulties with conventional practice is that many of these statistics are being used interchangeably which, as argued by Fox (2009) not only creates problems of interpretation but obfuscates what is really being protected. A number of authors have denounced the ad-hoc procedures for setting safe environmental concentrations that are based on NOECs and LOECs and have argued for a more rigorous, model-based approach (Fox 2009, Jager et al. 2006, Kooijman 2006).

A vast literature has accumulated over the last twenty years in which the theoretical, computational, statistical, and socio-economic aspects associated with the identification of ‘safe’ concentrations have been discussed. Readers requiring more

49 detailed background information on the use of SSDs and their application in ecotoxicology
50 and within a regulatory framework will find the collection of papers in Posthuma et al.
51 (2002) a useful starting point. A good review of the statistical issues associated with
52 ecotoxicological risk assessment is provided by Van der Hoeven (2004) while more
53 recently Fox (2006, 2008) reviewed the use of statistical methods in ecological risk
54 assessment more generally.

55 The general problem addressed by this paper is: how does one set a realistic
56 threshold concentration on some contaminant or toxicant such that some arbitrary high
57 fraction of all species will be protected provided environmental concentrations do not
58 exceed the threshold? This is indeed not a new problem and has been studied extensively
59 by many researchers (Wagner and Løkke 1991, Kooijman et al. 1996, Aldenberg and
60 Jaworska 2000, Shao 2000). Despite a plethora of models and incremental refinements,
61 the numerous concerns with the NOEC-based procedures (Fox 1999, 2006, Newman et al.
62 2000, Isnard et al. 2001, Pires et al. 2002, Verdonck et al. 2003) that underpin current
63 practice in Australia, New Zealand, the United States, the Netherlands, and Denmark have
64 not been extinguished and as recently noted by Newman (2008), the current
65 ecotoxicological landscape is dominated by classical (i.e. frequentist) statistical methods.

66 Although a number of Bayesian papers have recently appeared in the
67 ecotoxicological literature (Aldenberg and Jaworska 2000, Verdonck et al. 2001, Grist et
68 al. 2006, Billoir 2008, Hickey et al. 2008) the framework by and large remains outside the
69 realm of conventional ecotoxicological practice. Readers wanting to learn more about
70 Bayesian statistics should consult any of a number of good introductory texts such as Lee,
71 2004 or McCarthy, 2007). The statistical profession spent many years and devoted many

journal pages to the debate over the legitimacy of the Bayesian paradigm. Early objectors strenuously refuted the notion of a 'prior' distribution, the incorporation of subjective assessment, and treatment of parameters as random quantities. Thankfully the old divisions between the 'Frequentist' and 'Bayesian' schools of thought have largely given way to a more pragmatic approach that accommodates multiple modes of statistical inference with the choice increasingly based on the notion of 'fit-for-purpose' rather than ideological or pedagogical constructs. Furthermore, the advent of high-powered desk-top computers and associated software such as WinBUGS (Lunn et al. 2000) has removed any lingering impediments to the Bayesian analysis of complex, real-world problems.

In light of these developments it is timely to revisit the role and place of Bayesian statistics in the context of determining hazardous concentrations for ecosystem protection. Some of the more serious limitations associated with conventional NOEC-based analyses center on: (i) the unknown (and perhaps unknowable) underlying distributional form for NOECs; (ii) the statistical method by which a NOEC is determined; (iii) the inability to represent uncertainty in the estimated NOEC; and (iv) the non-random selection of a small number of species (van der Hoeven, 1997; Crane and Newman, 2000). As will be demonstrated later in this paper, these issues are addressed through the use of posterior distributions to represent and describe uncertainty in the estimated no effect concentration (NEC). Uncertainty in the collection of NECs can be incorporated into a final estimate of a hazardous concentration to which a statement of 'confidence' (or the Bayesian analogue *credibility*) can be attached.

Our starting point is a flexible and realistic model for the raw data generated by a dose-response experiment – this is consistent with the recommendations of Kooijman et al.

(1996), Van der Hoeven (1997) and Van der Hoeven et al. (2004). In the remainder of the paper we describe the procedure and illustrate its implementation with the use of previously published data sets.

A BAYESIAN MODEL FOR THE NEC

A number of models have been proposed to describe the dose-response relationship in ecotoxicological studies. We have adopted 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 and thereafter exhibits an exponential decay. It is important to note that the incorporation of γ in our model does not presuppose the existence of a threshold – it simply allows for one to be estimated if that is what the data suggests. Pires et al. (2002) assumed Y was discrete (numbers of individuals) and hence used a Poisson probability model to describe stochastic variation in Y. We relax this assumption and allow Y to be either discrete or continuous (for example, percent mortality) having arbitrary probability function $g_Y(\cdot)$. The complete model is defined by equations 1 and 2.

$$Y_i \overset{d}{\sim} g_Y(\cdot) \tag{1}$$

$$E\left[Y_i \middle| x_i\right] = \mu_i = \alpha \exp\left[-\beta\left(x_i - \gamma\right) I(x_i - \gamma)\right] \tag{2}$$

with $I\left(z\right) = \begin{cases} 1 & z > 0 \\ 0 & z \le 0 \end{cases}$; $E\left[Y_i \middle| x_i\right]$ denotes the mathematical expectation of Y_i conditional on a given concentration x_i ; and the notation $\overset{d}{\sim}$ in equation 1 meaning "is distributed as".

Taken together, equations 1 and 2 assume the response at the i^{th} concentration, x_i follows some distribution $g_Y(\cdot)$ having mean μ_i. The form of equation 2 generates a response curve as shown in Figure 1.

< Insert Figure 1 here >

The parameters $\alpha, \beta,$ and γ in equation 2 have intuitive interpretations: α is a 'basal' response – that is, the response at zero /low-dose concentrations; β controls the rate of decay in the response; and γ is the NEC. Given data $\{x_i, y_i\}$ our objective is to estimate the parameters α, β, γ. A conventional regression-based approach would do this by: (i) assuming Y_i to be normally distributed with mean μ_i and some constant variance σ_ε^2; and (ii) use a least-squares (LS) or maximum likelihood (ML) criterion to find the 'best-fitting' parameter estimates.

The Bayesian formulation similarly requires specification of $g_Y(\cdot)$ but in addition assumes the parameter vector $\underline{\Theta} = \{\alpha, \beta, \gamma\}$ is a random quantity to which is assigned a *prior* distribution, $p(\underline{\Theta})$. The Bayesian method provides an updated version (the *posterior*) of $p(\underline{\Theta})$ through the use of Bayes' formula. In essence this computes $P\left[\underline{\Theta}\middle|data\right] \propto \int l\left[data\middle|\underline{\Theta}\right] P\left(\underline{\Theta}\right) d\underline{\Theta}$ where $l\left[data\middle|\underline{\Theta}\right]$ is the likelihood of the data given a parameter vector $\underline{\Theta}$. The specification of prior distributions affords the analyst with an opportunity to inject formally elicited expert opinion about each of the model parameters or as a mechanism for introducing personal belief. While the choice is arbitrary, a prior is chosen to be either informative or vague. *Informative priors* are used when our

understanding or expectation about the range of likely values for a parameter is well defined. For example, the choice of a normal distribution centered on what is believed to be the most probable value and having a small variance would constitute an informative prior. Conversely, a *non-informative* or *vague* prior is typically one that assigns equal weight to all values (such as a uniform distribution) or one that has a large variance. Other possibilities exist, such as Jeffrey's and improper priors but will not be considered here.

The computation of the integral associated with the derivation of the posterior distribution is invariably high dimensional and complex. It is generally easier (and often-times the only option available) to use numerical methods such as Markov Chain Monte Carlo (MCMC) methods that are suited to implementation on a desk-top computer. The WinBUGS program is a free software tool that uses a particular implementation of MCMC estimation known as Gibbs sampling (BUGS is an acronym for Bayesian inference Using Gibbs Sampling). WinBUGS was developed at the MRC Biostatistics Unit, Cambridge University and can be downloaded from http://www.mrc-bsu.cam.ac.uk/bugs/ . We have used the 'open source' version of WinBUGS available at the University of Helsinki's website http://mathstat.helsinki.fi/openbugs/ . WinBUGS has its own, reasonably intuitive programming language although novices may prefer (at least initially) to use the so-called 'doodle bugs' editor which allows the user to represent his or her model in a graphical format. The WinBUGS code is then automatically generated from the graphical representation. The Bayesian model development and parameter estimation procedure is illustrated in the following section.

Example – Estimation of a NEC for *Daphnia magna*

Biesinger et al. (1982) reported on a study into the chronic toxicity of mercury (Hg) to *Daphnia magna*. The compounds of mercury tested were mercuric chloride ($HgCl_2$), methyl mercuric chloride (MMC), and phenyl mercuric acetate (PMA). A range of Hg concentrations was prepared and for each toxicant the number, y_i out of an initial sample of n_i individuals surviving after 21 days was recorded against the $i^{th.}$ concentration. A summary of the data is shown in Table 1.

< Insert Table 1 here >

The response variable here is discrete and while the Poisson distribution used by Pires et al. (2002) could be adopted, this is perhaps not the most sensible choice given that some of the n_i were small and y_i is constrained to lie in the range $[0, n_i]$. A more natural candidate for $g_Y(\cdot)$ in this case is the binomial distribution (equation 3).

$$P[Y_i = y_i] = \binom{n_i}{y_i} \theta_i^{y_i} (1-\theta_i)^{n_i - y_i}; \quad 0 < \theta_i < 1, \quad 0 \le y_i \le n_i \tag{3}$$

Now, $E[Y_i] = \mu_i = n_i\theta_i$ and so equation 2 relates the quantity $(n_i\theta_i)$ to x_i. We can also consider *proportion* $p_i = Y_i / n_i$ surviving at concentration x_i. The expectation of p_i is θ_i, the *true* proportion, and thus equation 2 becomes

$$E[p_i] = \theta_i = \alpha \exp\left[-\beta\left(x_i - \gamma\right) I(x_i - \gamma)\right] \tag{4}$$

As noted above, there is flexibility in the choice of prior distributions. To reflect prior ignorance about the likely parameter values, we have chosen gamma priors with a large variance for each of the parameters α, β, γ in equation 4. The graphical representation for this model is shown in Figure 2 and the corresponding WinBUGS code in the Appendix.

< Insert Figure 2 here >

The model was run 100,000 times (after an initial 'burn-in' period of 10,000 iterations) for each toxicant with model outputs recorded at every tenth iteration (a process known as 'thinning' which reduces autocorrelation in the recorded data). Summary statistics for the resulting 10,000 MCMC iterations were gathered and are presented in Table 2. Additional diagnostics tools available in the WinBUGS software were used to check for convergence.

< Insert Table 2 here >

An example of the posterior distributions obtained from WinBUGS for phenyl mercuric acetate is shown in Figure 3 and the fitted model in Figure 4.

< Insert Figure 3 here >

< Insert Figure 4 here >

In a Bayesian analysis it is customary to use the median of the posterior distribution as a point estimate of a parameter. We thus obtain the following point estimates for the NEC: 3.27 μg Hg/L for mercuric chloride; 0.863 μg Hg/L for methyl mercuric chloride; and 1.792 μg Hg/L for phenyl mercuric acetate. The Bayesian analog of the Frequentists'

confidence interval is the Bayesian credibility interval. A $\xi 100\%$ credibility interval $(0 < \xi < 1)$ is an interval containing $\xi 100\%$ of the posterior distribution. Although not uniquely defined, we use the 2.5 and 97.5 percentiles of the posterior distribution to define a 95% credibility interval (Table 2 and Figure 4).

The attractive feature of the posterior distribution is that it summarizes the combined uncertainty in the estimated NEC arising from both stochastic variation and epistemic uncertainty (i.e. incomplete knowledge). This uncertainty can be incorporated into a more comprehensive Bayesian framework for multiple species from which an ecosystem HC_x (and its uncertainty) can be determined. The approach is explained in the following section with reference to recently published data on the toxicity of pond waters at a uranium mine in Northern Australia.

Example – Estimation of the HC_x for pond water at a Uranium mine

The Ranger mine located 230 km east of Darwin, Australia is one of the world's largest open-pit uranium mines. The mine site is located within the environmentally sensitive Kakadu National Park. On March 9, 2007 tropical cyclone 'George' impacted the Pilbara mining region in the far north-west of Western Australia. The Ranger mine was also affected by this system with nearly 850 mm of rain falling in the seven days to March 4, including 750 mm in one 72-hour period causing flooding of the mine and the release of water into the Magela Creek. In response to this event, the Australian government undertook toxicity studies as part of an examination of options for reducing the volume of pond water stored at the mine site. One of these options involved the direct release of untreated pond water from Retention Pond 2 (RP2) to Magela Creek (Hogan et al. 2008).

The toxicity studies reported by Hogan et al. (2008) were based on an analysis of the SSD obtained from five test species' IC_{10} (i.e. the concentration that results in a 10% inhibition of response relative to the control response) or equivalent data using the BurrliOZ software (www.cmis.csiro.au/envir/burrlioz/). With respect to RP2 water, Hogan et al. (2008) concluded that a dilution of 0.33% (approximately 1:300) would protect 99% of all species in Magela Creek. We have undertaken a Bayesian analysis of the data given in Appendix 5 of Hogan et al. (2008) using the methods outlined in this paper. Our results suggest that the Hogan et al. (2008) concentration is overly liberal (i.e. too high) and as such the *actual* fraction of species protected may be significantly *less* than the claimed 99%. Details of our analysis are provided below.

Hogan et al. (2008) used the following five test species: *Chlorella* (an alga); *Lemna aequinoctialis* (duckweed); *Hydra viridissima* (green hydra); *Moinodaphnia macleayi* (water flea); and *Mogurnda mogurnda* (purple-spotted gudgeon). The respective endpoints were: 72 hour cell division rate; 96 hour plant growth; 96 hour population growth; brood reproduction; and 96-hour survival. Three replicates of each species were exposed to either 6 or 7 dilutions of RP2 water except for the *Moinodaphnia macleayi* test which was based on 10 replicates. The complete set of data is available in Appendices 5.1-5.5 of Hogan et al. (2008) which can be downloaded from http://www.environment.gov.au/ssd/publications/ssr/197.html . An important feature of this data set is the results for *M. mogurnda* in which none of the animals showed any response at any of the 6 dilutions in the range 0-100%. The zero variance for these data is problematic for statistical distribution-fitting methods. To overcome this difficulty, Hogan et al. (2008) assumed a dilution of 50% as "a conservative toxicity estimate for *M.*

mogurnda". The lack of any response for *M. mogurnda* data also means that our model will result in an estimate of γ (the NEC) close to zero which is unrealistic. To maintain consistency with Hogan et al. (2008) we have also adopted a 50% value for the *M. mogurnda* NEC. Furthermore, and as pointed out by one of the reviewers of an earlier draft of this paper, the response variable for *Moinodaphnia* is discrete (number of neonates) thus calling into question the appropriateness of the normal probability model. In response, we note that: (i) the normal distribution was implicitly used by Hogan et al. (2008) and (ii) re-running the model assuming a Poisson response made little difference (in this case) to the estimated NEC.

Estimation of the No Effect Concentration

The response variables (Y_i) for each of the four species used by Hogan et al (2008) are assumed to be normally distributed as $Y_i \sim N\left(\mu_i, \sigma_i^2\right)$ with μ_i given by equation 2 and non-informative gamma priors for the precision terms $1/\sigma_i^2$. The prior distributions assigned to the model terms in equation 2 are listed in Table 3. We have chosen informative priors that reflect an 'educated' guess (informed by an inspection of a plot of the data) as to the likely position of the NEC. In essence, this corresponds to what is known as 'empirical Bayes'.

< *Insert Table 3 here* >

The WinBUGS code for this model is given in the Appendix. The results of 50,000 MCMC simulations collated after an initial 'burn-in' of 10,000 runs are shown in Table 4. The posterior cumulative distribution functions for the fitted NECs are shown in Figure 5.

These distributions reflect the variation in estimated NECs that arise from the variation in responses from individual test organisms.

< Insert Table 4 here >

< Insert Figure 5 here >

It is evident from Figure 5 that the probability of an effect at a concentration of 22.5(%) is 50% for *Lemna* and 100% for the other three species.

A comparison of our estimated NECs with the NOECs and IC_{10} values reported by Hogan et al. (2008) is given in Table 5.

< Insert Table 5 here >

We see from Table 5 that there is reasonable agreement between all three estimates for *Chlorella* and *Hydra* however there are significant discrepancies among the estimates for *Lemna* and *Moinodaphnia*. In particular, our estimated NEC for *Lemna* is an order of magnitude greater than the IC_{10} reported by Hogan et al. (2008). The difference is an artifact of the two very different methods used to obtain the respective estimates. Our NEC is an estimate of a parameter in a dose-response model. According to Hogan et al. (2008), the IC_{10} "involves fitting straight lines between each successive concentration … then interpolating the relevant 'effect' or 'inhibition' size of interest". Thus the IC_{10} is an empirical estimate that neither accommodates data variability nor is predicated on any plausible model of the response-generating mechanism. The impact of these differences on the fitted SSD and estimated HC_1 is examined next.

Estimation of the HC_1

In Australia, the recommended procedure for estimating the HC_x is embedded in the software tool known as BurrliOz which is distributed with the national water quality guidelines document (ANZECC/ARMCANZ 2000). The procedure is a generalization of the method described by Aldenberg and Slob (1993). Using the BurrliOz software with the data in Table 5 we examined the differences in the estimated HC_1. Following Hogan et al. (2008) we used a concentration of 50 (%) for the fifth species (*Mogurnda*) for the NEC, NOEC, and IC_{10}. The resulting HC_1 values are, respectively: 0.0015%; 0.04%; and 0.35% corresponding to dilutions of: 1:66,666; 1:2,500; and 1:285. Hogan et al. (2008) rounded up the last ratio and recommended that a dilution of 1:300 "would be expected to ensure the appropriate level of protection for the downstream aquatic ecosystem". The large discrepancy among the three estimates of the HC_1 suggests that a 1:300 dilution possibly underestimates the actual dilution required to achieve the desired level of protection. Interestingly, if we follow Aldenberg and Slob (1993) and fit a two-parameter log-logistic distributions to the data of Table 5 we get HC_1 estimates of 0.031% using NEC values; 0.048% using NOEC data; and 0.083% using IC_{10} values, corresponding respectively to dilutions of: 1: 3,225; 1:2,082; and 1:1,204. While no general conclusions can be drawn from this result, the Aldenberg and Slob (1993) method has resulted in a more consistent set of estimates for HC_1. In any event, the conclusions are the same – the IC_{10} data have resulted in a significantly larger 'safe' concentration and the dilution required is greater than 1:300.

Finally, and to conclude this analysis, we interrogate the posterior distributions for the estimated NECs to explore the range of uncertainty that is possible when the HC_1 is

estimated from the fitted SSD. To this end, we randomly generated approximately 10,000 sequences of NEC quadruples from the posterior distributions of $\{\hat{\gamma}_1, \hat{\gamma}_2, \hat{\gamma}_3, \hat{\gamma}_4\}$ and, for reasons outlined above, fixed the fifth NEC at 50 (%). A two-parameter logistic distribution was fitted to each realization of NEC parameters from which the HC_1 was obtained. The histogram of the resulting collection of HC_1 estimates is shown in Figure 6 together with a fitted log-logistic probability model. The range of HC_1 values is from 0.0018% to 0.0716% with a median value of 0.0172%. The discrepancy between this median and the value of 0.031% given above is due to the fact that the latter was the result of fitting a *single* distribution to *n=5* median NECs and estimating the HC_1 whereas the present result is obtained by fitting distributions to individual realizations of the NECs and calculating the median of the resulting collection of $n \simeq 10,000$ estimated HC_1 values. The latter approach is considered to be superior since it utilizes information about the distributional properties of the individual NECs and, provided sufficient simulations are performed, will comprehensively explore the joint parameter space for $\{\gamma_1, \gamma_2, \gamma_3, \gamma_4\}$. The median concentration of 0.0172% for this method corresponds to a 1:5,813 dilution and, conceptually, is equivalent to a 99:50 trigger value (ANZECC/ARMCANZ 2000). On the basis of this analysis and the previous results we are reasonably confident in asserting that a 1:300 dilution as recommended by Hogan et al. (2008) is too liberal by a factor of about 20.

< Insert Figure 6 here >

DISCUSSION

Over the past decade deterministic methods of risk assessment such as the *hazard quotient* (HQ) approach have given way to probabilistic methods. Thus for example, in

328 Australia and elsewhere, the use of species sensitivity distributions (SSDs) is the preferred
329 method for establishing concentration thresholds (or 'trigger values') for chemical
330 contaminants in water bodies (ANZECC/ARMCANZ, 2000). While probabilistic
331 ecological risk assessments (PERAs) are underpinned by a more comprehensive treatment
332 of uncertainty than their deterministic counterparts, they are not without limitations and a
333 number of concerns have been identified (Fox 1999, 2006, Newman et al. 2000, Isnard et
334 al. 2001, Pires et al. 2002, Verdonck et al. 2003). As noted by Fox (2001), conventional
335 methods of inference via ANOVA, t-tests, regression and related techniques do a superb
336 job when the attendant assumptions have been reasonably met. The problem is that
337 environmental data are notoriously 'messy'. Data collection tends to be opportunistic
338 resulting in samples that are anything but random. The ubiquitous normal distribution
339 assumption underpinning conventional statistical analyses is important, but not as critical
340 as some often overlooked requirements such as homogeneous error variance and
341 independence. Environmental data often exhibit many, if not all of the attributes that render
342 them particularly unsuited to conventional modes of analysis. Among these are: small
343 sample sizes; discreteness; over-dispersion; non-stationarity in space and time;
344 heterogeneous error structures; lack of independence; and extreme skewness / kurtosis. It is
345 not surprising therefore that the NOEC – a quantity determined as the result of a
346 significance test applied to a small sample of non-randomly selected non-normal data, is
347 potentially fatally flawed as an estimator of a no effect concentration.

348 In a recent and timely article, Newman (2008) critically reviewed the role of
349 classical statistical inference in environmental toxicology and chemistry. His survey of 10
350 randomly selected articles published between 1996 and 2006 from 10 "representative

journals with good impact factors" found none had made any use of Bayesian methods. This is an astonishing result and highlights the need for greater education and awareness of this important statistical paradigm. Indeed, Newman's second recommendation is that "the teaching of statistics to environmental science students should shift away from a traditional emphasis on hypothesis testing to a more flexible approach embracing other valuable vantages, especially the Bayesian and information theory-based vantages" (emphasis added).

CONCLUSIONS

In this paper we have described a general Bayesian framework for identifying critical threshold concentrations for ecosystem protection. We have developed the models and provided sufficient mathematical and computational detail with the use of realistic examples to help facilitate the integration of Bayesian methods into the environmental toxicologists' toolkit of statistical techniques. We believe that our approach for estimating a NEC is superior to current NOEC-based methods by virtue of the following: (i) the NEC is estimated as a parameter of a general dose-response model as distinct from the NOEC which is constrained to be one of the test concentrations; (ii) statements of *precision* can be attached to the estimated NEC whereas they are inadmissible for NOECs; (iii) the Bayesian framework provides an opportunity for the researcher to inject personal belief in the form of a *prior* probability distributions for the NEC and other model parameters; (iii) both discrete and continuous probability models for the response-generating mechanism are readily handled thus removing the constraint of assumed normality as is the case with the procedure used to derive NOECs. To this end, it is hoped that this alternative paradigm

373 may alleviate, if not remove some of the long-standing problems associated with the use of
374 traditional modes of statistical inference for ecosystem protection.

375 Finally, it should be appreciated that whatever *inputs* (NEC, NOEC, IC_x, EC_x, etc.)
376 are used in the development of a SSD, some fundamental issues concerning probabilistic
377 ecological risk assessment (PERA) remain. As has been demonstrated in this paper, it is
378 still possible to obtain an unrealistically low HC_x from a SSD fitted to a collection of
379 NECs. That our analysis of the Hogan et al. (2008) data resulted in a dilution that would
380 yield a toxicant concentration that was below background levels should not be viewed as a
381 failure of our Bayesian NEC-based approach. This situation is not uncommon with PERAs
382 and is the reason the Australian government attached very modest confidence levels
383 (typically 50%) to trigger values obtained using its probabilistic method
384 (ANZECC/ARMCANZ, 2000). The problem lies with the invariably small data sets, the
385 non-random selection of test species, and an unfounded belief that the limited class of
386 probability models used in such exercises is capable of adequately describing behavior in
387 the extreme tails of an SSD. This remains an area for on-going research and development.

388

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APPENDIX: WINBUGS CODE FOR EXAMPLES IN TEXT

Daphnia

```
model
      {

alpha~dgamma(0.0001,0.0001)
beta~dgamma(0.0001,0.0001)
gamma~dgamma(0.0001,0.0001)
for (i in 1:6)
      {

      theta[i]<-alpha*exp(-beta*(x[i]-gamma)*step((x[i]-gamma)))

            r[i]~dbin(theta[i],N[i])
      }
}

x[] r[] N[]
0.05 77 80
0.17 79 80
0.28 76 80
0.52 76 80
0.87 71 80
1.14 0 80
END

list(alpha=1,beta=1,gamma=0.1)
```

Mine Pit waters : single NEC

```
model
      {

                  gamma~dunif(0,100)

for (j in 1:4)
      {

            alpha[j]~dnorm(0,0.000001)
            beta[j]~dnorm(0,200)C(0,)

            tau[j]~dgamma(0.0001,0.0001)
            sigma[j]<-sqrt(1/tau[j])
}

      for (i in 1:117)

      {

      mu[i]<-alpha[species[i]]*exp(-beta[species[i]]*(x[i]-
gamma)*step((x[i]-gamma)))

            Y[i]~dnorm(mu[i],tau[species[i]])
      }


}

      # Input data

      x[] Y[] species[]
      0 1.4383 1
      0 1.3913 1
      0 1.4779 1
      0.3 1.3379 1
      .
      .
      .
      30 0 4
      END

# Initial values

list(gamma=10)

alpha[] beta[] tau[]
1 0.065 0.1
1 0.065 0.1
1 0.065 0.1
1 0.065 0.1
END
```

Mine Pit waters : multiple NECs

```
model
      {

               gamma[1]~dlnorm(2.124,1.708)
            gamma[2]~dlnorm(3.201,1.582)
            gamma[3]~dlnorm(1.221,0.437)
            gamma[4]~dlnorm(-1.278,0.404)


#           theta[1:5]~ddirch(a[1:5])

 theta[1]<-0.004
  theta[2]<-0.004
  theta[3]<-0.004
  theta[4]<-0.004


NEC<-10


for (j in 1:4)
      {

            alpha[j]~dnorm(0,0.000001)
            beta[j]~dnorm(0,200)C(0,)

            tau[j]~dgamma(0.0001,0.0001)
            sigma[j]<-sqrt(1/tau[j])
}


      for (i in 1:117)

      {

      mu[i]<-alpha[species[i]]*exp(-beta[species[i]]*(x[i]-
gamma[species[i]])*step((x[i]-gamma[species[i]])))

            Y[i]~dnorm(mu[i],tau[species[i]])
      }

for (j in 1:4)
      {
       ynew[j]<-culmative(gamma[j],NEC)
      }

      s<-inprod(theta[1:4],ynew[1:4])


}
```

```
# Initial values

alpha[] beta[] tau[] gamma[]
1 0.065 0.1 10
1 0.065 0.1 3
1 0.065 0.1 3
1 0.065 0.1 0.3
END


      # Input data

      x[] Y[] species[]
      0 1.4383 1
      0 1.3913 1
      0 1.4779 1
      0.3 1.3379 1
      .
      .
      .
      30 0 4
      END

      a[]
      0.05
      0.125
      0.025
      0.3
      4.5
      END
```

639 **Table 1. *Daphnia magna d*ata taken from Biesinger et al. (1982) showing numbers surviving y_i**
640 **after 21 days out of an initial n_i at various concentrations of three compounds of mercury.**

Toxicant	Hg concentration (ug/L)	ni	ri
Mecuric chloride	0.05*	180	171
	0.43	80	65
	0.91	80	73
	1.82	180	160
	3.53	180	108
	5.31	20	0
Methyl mecuric chloride	0.05*	80	77
	0.17	80	79
	0.28	80	76
	0.52	80	76
	0.87	80	71
	1.14	80	0
Phenyl mecuric acetate	0.05*	35	33
	0.35	15	15
	0.54	35	30
	1.12	35	33
	1.90	35	26
	3.00	35	1

* original concentration reported as < 0.1

641

642 **Table 2. Parameter estimates and selected percentiles of the posterior distribution for model**
643 **given by equation 4.**

Toxicant	Parameter	mean	standard deviation	Percentiles P2.5	P50	P97.5
mercuric chloride	alpha	0.90	0.01	0.87	0.90	0.92
	beta	1.94	1.13	0.52	1.56	4.54
	gamma	3.23	0.23	2.66	3.27	3.45
methyl mercuric chloride	alpha	0.95	0.01	0.92	0.95	0.97
	beta	1188	3139	17	107	12350
	gamma	0.95	0.08	0.01	0.86	1.11
phenyl mercuric acetate	alpha	0.93	0.03	0.87	0.93	0.97
	beta	2.58	1.33	1.20	2.26	5.97
	gamma	1.82	0.20	1.57	1.79	2.59

644

Table 3. Prior distributions used for the Ranger mine RP 2 waters example. Parameters relate to equation 2 in text. *N(·,·)* denotes a normal distribution*; N(·,·)|(0,∞)* denotes the half-normal distribution; and LN*(·,·)* denotes the lognormal distribution.

	Species			
Parameter	*Chlorella*	*Lemna*	*Hydra*	*Moinodaphnia*
α	$N\left(0,10^6\right)$	$N\left(0,10^6\right)$	$N\left(0,10^6\right)$	$N\left(0,10^6\right)$
β	$N\left(0,1/200\right)\left\|(0,\infty)\right.$	$N\left(0,1/200\right)\left\|(0,\infty)\right.$	$N\left(0,1/200\right)\left\|(0,\infty)\right.$	$N\left(0,1/200\right)\left\|(0,\infty)\right.$
γ	$LN\left(2.124,1.708\right)$	$LN\left(3.201,1.582\right)$	$LN\left(1.221,0.437\right)$	$LN\left(-1.278,0.404\right)$

650 **Table 4. Parameter estimates and selected percentiles of the posterior distribution based on**
651 **50,000 MCMC simulations for Ranger mine RP 2 waters data. Individual NECs identified as**
652 **gamma parameter for each species. Values represent concentrations of pit-waters (%).**

			Percentiles		
Parameter	**mean**	**standard deviation**	**P2.5**	**P50**	**P97.5**
γ_1	8.603	2.466	5.685	8.12	16.66
γ_2	24.95	13.08	4.775	22.61	53.29
γ_3	2.778	0.6317	1.459	2.752	4.15
γ_4	0.193	0.1565	0.01122	0.1498	0.581

653

654

655 **Table 5. 'No effect estimates' for Ranger mine RP 2 waters data using: (a) model-based NEC of**
656 **this paper; (b) NOECs and IC_{10} estimates as given by Hogan et al. (2008). Figures in parentheses**
657 **are: (a) 95% credibility interval; and (b) 95% confidence interval.**

658

Species	NEC (a)	NOEC (b)	IC10 (b)
Chlorella	8.12 (5.68,16.66)	10	7.5 (0,16)
Lemna	22.61 (4.78,53.29)	3	2.1 (0.4,13)
Hydra	2.752 (1.46,4.15)	3	3.5 (0,4.3)
Moinodaphnia	0.15 (0.01,0.58)	0.3	0.6 (0.2,0.8)

659

660

Figure Legends

Figure 1. Dose-response model given by equation 2.

Figure 2. Graphical representation of dose-response model for *Daphnia* example.

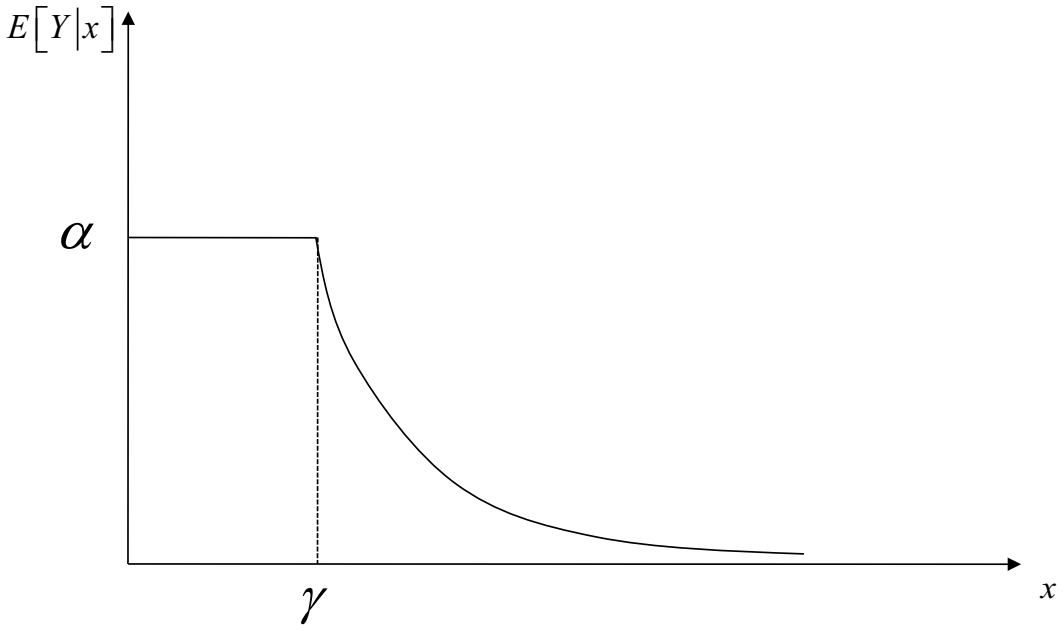
Figure 3. Sample posterior distributions for model parameters for phenyl mercuric acetate: (a) distribution of α parameter ; (b) distribution of α parameter; and (c) distribution of α parameter.

Figure 4. Sample data (solid diamonds) and fitted model (solid line) for phenyl mercuric acetate. Vertical dashed lines indicate 95% credibility interval for NEC.

Figure 5. Posterior distributions for gamma parameters (NEC) for Ranger Mine pit water example based on 50,000 MCMC iterations.

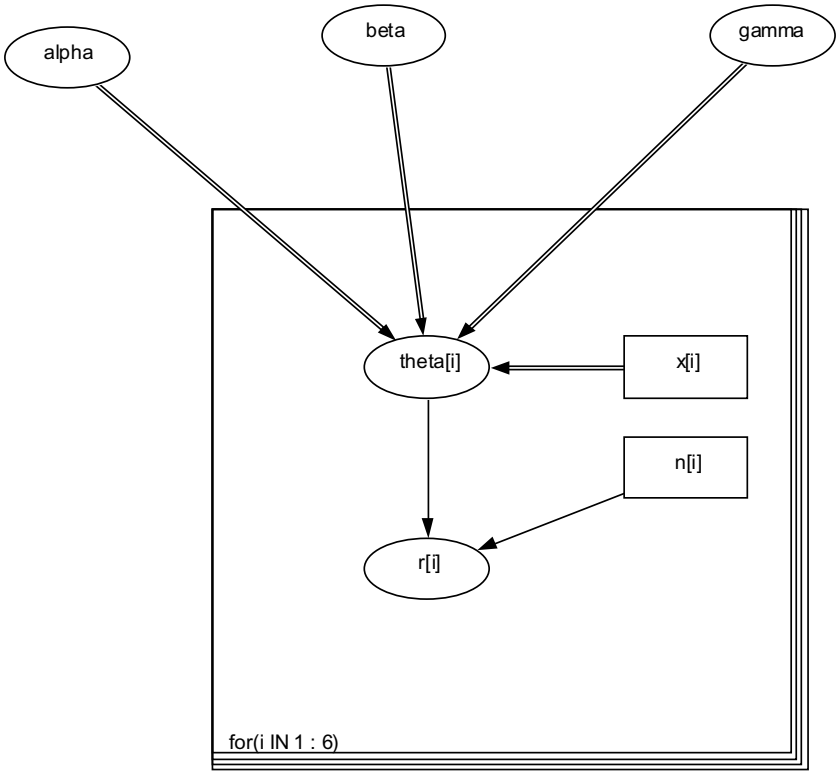
Figure 6. Histogram of 10,000 estimated HC_1 values for Ranger Mine pit water example (rectangles) and fitted 3-parameter lognormal distribution (solid line).

679 **Figure 1.**
680



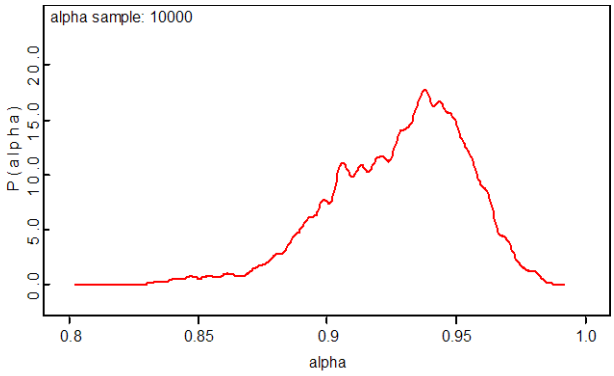
681
682

Figure 2.

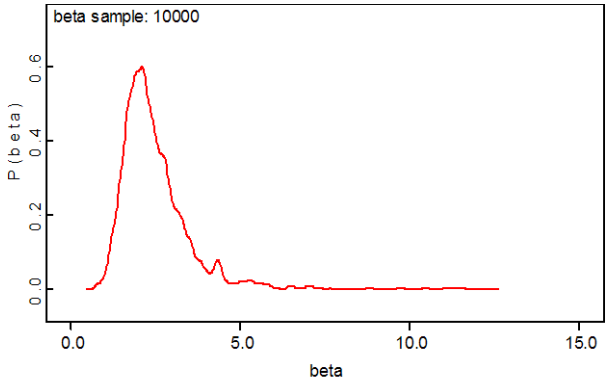


688 **Figure 3.**

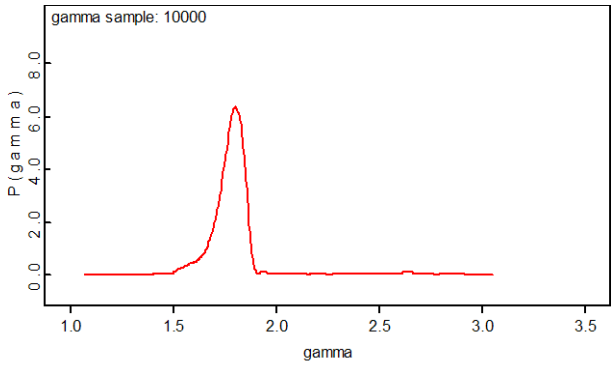
(a)



(b)

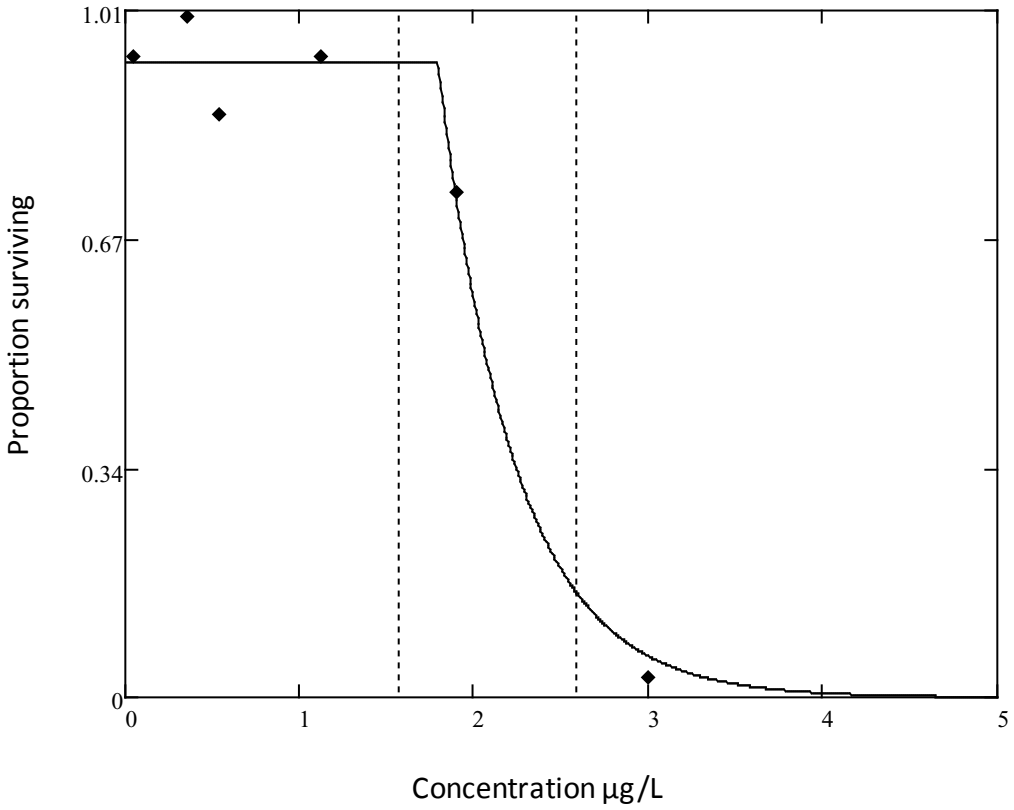


(c)



689
690

691 **Figure 4.**
692



693

Figure 5

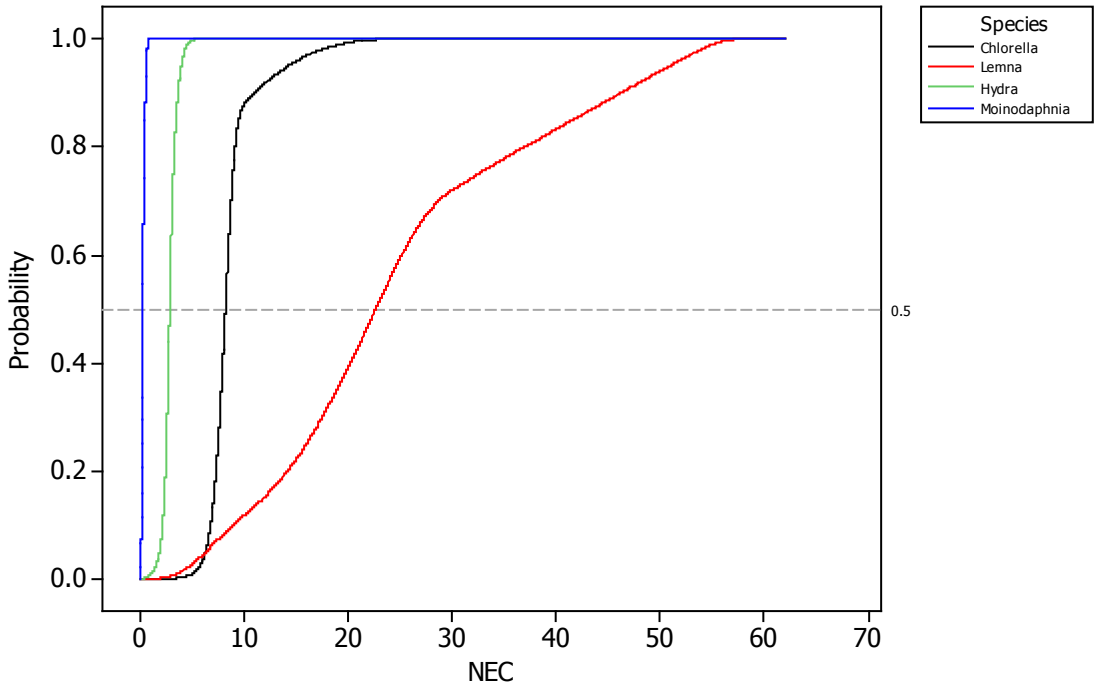
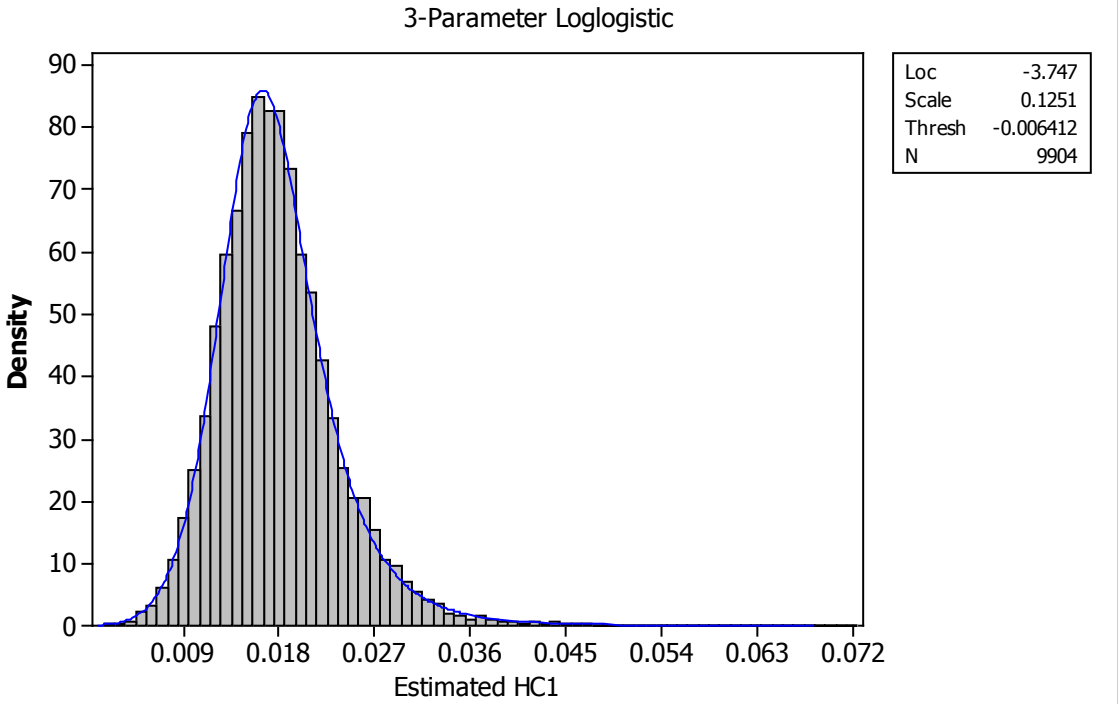

Species
Chlorella
Lemna
Hydra
Moinodaphnia
Probability
1.0
0.8
0.6
0.4
0.2
0.0
0.5
0
10
20
30
40
50
60
70
NEC

Figure 6


3-Parameter Loglogistic
Loc -3.747
Scale 0.1251
Thresh -0.006412
N 9904
Density
0
10
20
30
40
50
60
70
80
90
0.009
0.018
0.027
0.036
0.045
0.054
0.063
0.072
Estimated HC1