B-Tox: A Bayesian Facelift for Ecotoxicology

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Abstract:
Despite more than 20 years of severe criticism, the No Observed Effect Concentration (NOEC) is the most widely used measure of toxicity in ecotoxicology. Coupled with the equally problematic concept of a Species Sensitivity Distribution (SSD), the NOEC is used to determine a 'safe' concentration for toxicants in animals and the receiving environment. These 'safe' concentrations are used in regulatory contexts as well as underpinning critical design decisions associated with major infrastructure projects such as de-salination plants, off-shore oil rigs and ocean outfalls. The core of the criticisms of contemporary ecotoxicological practice focus on the inadequacies and inappropriate use of classical modes of statistical inference. This paper describes an alternative Bayesian framework for estimating the No Effect Concentration (NEC) and inference for the Hazardous Concentration (HC*x*).

Key Words: dose-response modeling, hazardous concentration, NOEC, NEC, SSD

1. Introduction

Ecotoxicology is a multidisciplinary field of study that is primarily concerned with the effects of natural and synthetic chemicals on the living components of an ecosystem. Early studies focussed on the effects of chemicals on humans and in 1939 the American Conference of Governmental Industrial Hygienists published the first maximum allowable concentrations for chemical exposures (by humans). It wasn't until 1962 and the publication of Rachel Carson's *Silent Spring* that the wider impact of chemicals in the environment was more fully appreciated. Since that time a vast literature has accumulated in which the theoretical, computational, statistical, and socio-economic aspects associated with the identification of 'safe' concentrations have been discussed.

A key feature of modern ecotoxicology is its high reliance on 'classical' modes of statistical inference. As noted by Fox (2010) the integration of statistics and (eco)toxicology has been more by osmosis than by design. The Frequentist tools of T-tests, ANOVA, and multiple comparison techniques are de facto standards for ecotoxicological analysis.

The starting point in the identification of a 'safe' concentration for a chemical in the environment is the quantification of its toxicity to each of a small number of species selected from the ecosystem under consideration. Although there are many measures of toxicity, one of the most common is the no observed effect concentration or NOEC. This measure continues to be widely used by governments and regulatory agencies around the world despite more than 20 years of severe criticism and even calls for it to be banned (Laskowski 1995, Kooijman 1996, Warne and van Dam 2008). 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).

Notwithstanding these concerns, ecotoxicologists use a small sample of NOECs (typically fewer than 10) and a process of 'statistical extrapolation' to infer a concentration that is harmful to some arbitrarily small (*x%*) of *all* species in an ecosystem. The resulting (hazardous) concentration is referred to as the HC_x and is the basis of setting 'safe' concentrations in both regulatory and non-regulatory contexts by governments and jurisdictions around the world including Europe, Australia, New Zealand, and Canada. The inferential step from sample to population is via an equally contentious concept called the species sensitivity distribution or SSD. The SSD is a theoretical *cdf* fitted to the small sample of NOECs.

While it is not the purpose of the present paper to review the many issues surrounding the use of NOECs and SSDs, readers requiring more information will find the collection of papers in Posthuma et al. (2002) a useful starting point. A good review of the statistical issues associated with ecotoxicological risk assessment is provided by Van der Hoeven (2004) while more recently Fox (2006, 2008, 2009) provided commentary on the use of statistical methods in ecological risk assessment.

In this paper we describe an alternative to NOEC-based inference for ecotoxicology. Unlike NOECs which are not underpinned by any model, we commence with a plausible dose-response model and use Bayesian techniques to estimate its parameters. One of these parameters is the true *no effect concentration* or NEC. The approach has a number of attractive features, not least of which is the ability to incorporate expert opinion in the form of prior densities for each of the model parameters and the explicit representation of uncertainty in the estimated NEC.

2. Bayesian Inference for the HC_x

A serious limitation of current ecotoxicological practice is the inability to represent uncertainty in the NOEC. This is a consequence of the definition of a NOEC – it is the largest concentration used in a dose-response experiment for which the mean response is statistically indistinguishable from the mean response for the 'control' dose (usually zero). While a number of statistical tests are available for this purpose, perhaps the most common is Dunnett's test for comparing all treatments with a control (Dunnett, 1955). It is common practice in dose-response modeling to use a geometric progression of concentrations where, for example, a doubling of successive concentrations is used. Because the NOEC is one of the *fixed* concentrations employed in the dose-response experiment, it is readily appreciated that the NOEC could differ from the true NEC by the same factor of two and furthermore the quality of this estimator (in terms of precision and bias) is indeterminate.

In the following sections we describe the Bayesian estimation of the no effect concentration. We then illustrate how the posterior distribution for a sample of NECs

estimated in this way can be incorporated in the fitting of an SSD to provide statements of uncertainty in the final HC_x.

2.1 Estimation of the no effect concentration

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. 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 $I(z) = \begin{cases} 1 & z > 0 \\ 0 & z \le 0 \end{cases}$; $E\left[Y_i \middle| x_i\right]$ denoting 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.

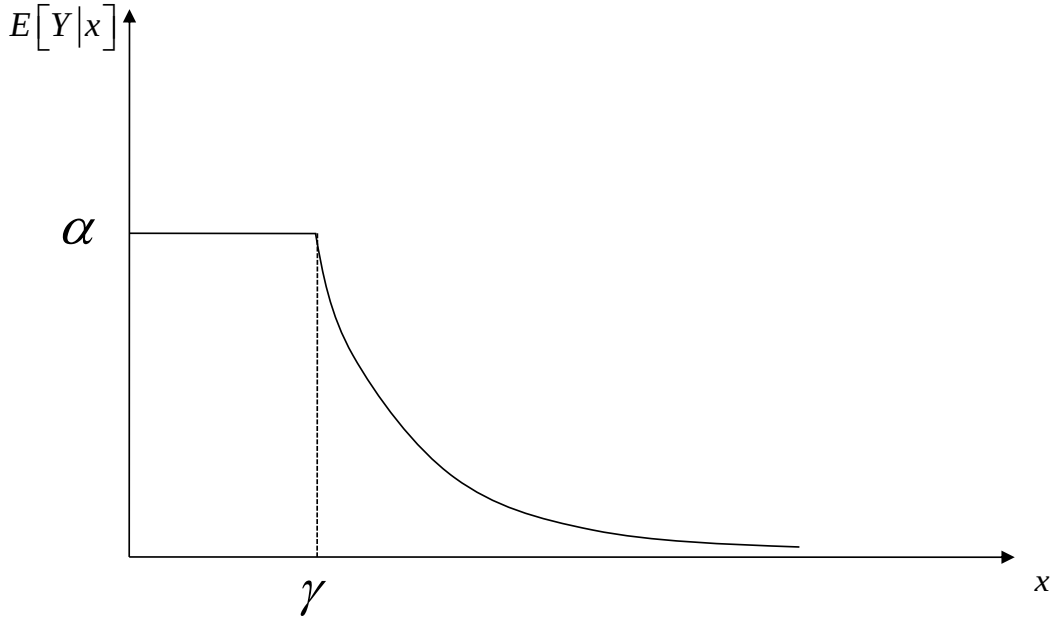

Figure 1: Form of the effects threshold model with exponential decay. γ is the true no effect concentration (NEC).

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 specification of prior distributions affords the analyst with an opportunity to inject formally elicited expert opinion about each of the model parameters and when incorporated with the dose-response data MCMC techniques can be used to obtain the empirical posterior densities. The steps are readily programmed using the OpenBUGS software tool (available at http://www.openbugs.info/w/) as illustrated in the following example.

2.1.1 *Example – Desalination Plant toxicity investigations*

The Olympic Dam mine at Roxby Downs (South Australia) is the world's largest known uranium deposit. As part of a planned mine expansion, a desalination plant is proposed to be built on the coast some 280km to the south at Point Lowly to supply water to the mine (Figure 2). The mine owners commissioned a number of scientific studies to investigate the toxicity of the desalination plant's brine discharge to a sample of marine species found at Point Lowly. Detailed results can be found in Appendix O10 of the environmental impact statement (EIS) available at http://www.bhpbilliton.com/bb/odxEis/downloads/appendices.jsp

Reproduced in Table 1 are results for the 96 hour survival test for the tiger prawn *Penaeus monodon*.

Table 1: Number of prawns surviving (out of 10) after 96 hour exposure to varying effluent concentrations for each of four replicates.

	Effluent concentration (%)					
rep	**0**	**2.1**	**4.1**	**8.3**	**16.5**	**33**
1	10	6	8	10	8	0
2	6	8	8	8	6	4
3	10	10	10	10	6	2
4	10	10	8	10	6	2

As previously mentioned, conventional ecotoxicological practice would proceed as follows: (i) convert the data in Table 1 to proportions and possibly apply the arcsine transformation to restore a greater degree of normality; (ii) pool the data across replicates

and analyze using a one-way ANOVA model with concentration as the single factor; (iii) assuming a significant result at the previous step, use a multiple comparison procedure such as Dunnett's test to determine the NOEC as described above.

Figure 2: Location of Olympic Dam minesite at Roxby Downs and proposed pipeline to Point Lowly on the South Australian coast. (*Google Earth image*).

The results of Dunnett's test are shown in Figure 3. It is apparent from an inspection of the confidence intervals in Figure 3 that the largest concentration for which the mean response is not significantly different from the mean response of the control group is 16.5. Thus the estimated NOEC is 16.5%.

The OpenBUGS implementation of our Bayesian approach is requires the specification of prior densities for the parameters of equation (2). We are reasonably confident that α is somewhere between 0.7 and 1 and have chosen a uniform distribution on this interval.

```
Dunnett's comparisons with a control

Family error rate = 0.05
Individual error rate = 0.0129

Critical value = 2.76

Control = level (0) of concen

Intervals for treatment mean minus control mean

Level    Lower   Center    Upper  --------+---------+---------+---------+-
 2.1   -0.3411  -0.0500   0.2411                   (--------*-------)
 4.1   -0.3411  -0.0500   0.2411                   (--------*-------)
 8.3   -0.2411   0.0500   0.3411                      (-------*--------)
16.5   -0.5411  -0.2500   0.0411               (-------*-------)
33.0   -0.9911  -0.7000  -0.4089  (-------*-------)
                                 --------+---------+---------+---------+-
                                      -0.70     -0.35      0.00      0.35
```

Figure 3: Results of Dunnett's test applied to data of Table 1 (converted to proportions).

Past experience with similar analyses suggests that the parameter β is between 0 and 1 and we have chosen the beta(2,3) density to reflect this belief (Figure 4a). Finally, we are confident that the true NEC is somewhere between 5 and 20 and we have chosen a gamma density with shape parameter 17.0156 and scale parameter 0.6465 (Figure 4b).

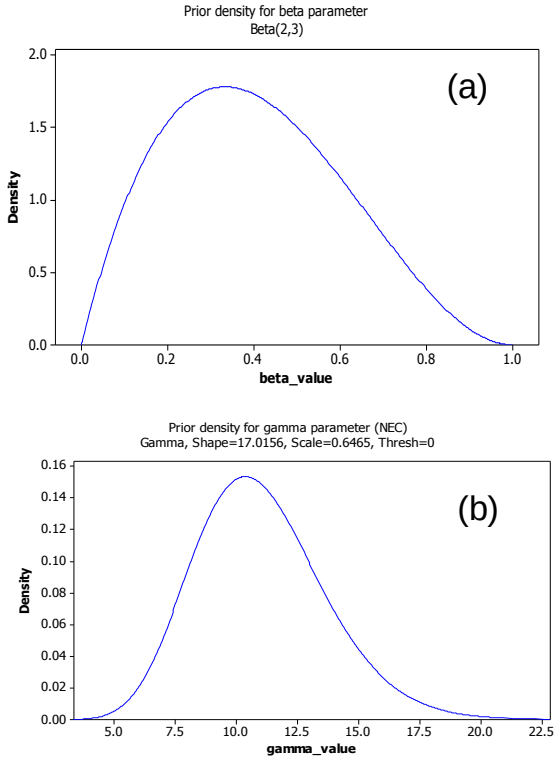

Figure 4: Prior density for β parameter (a) and γ parameter (b) in model given by equation (2).

The complete OpenBUGS code and data for this example are given in Figure 5. An important feature of the approach is that the response variable (number of prawns surviving at each concentration) is more aptly modeled as a binomial random variable.

```
model
        {

# NOEC as given in EIS
NOEC<-16.5

# specify model priors

alpha~dunif(0.7,1.0)
beta~dbeta(2,3)
gamma~dgamma(17.0156,1.5469)

# read in data

for (i in 1:24)
        {

# theta is true proportion at each dose - given by equation (2) in text

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

# response is r (number of prawns surviving) - assumed to be binomial

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

#  estimate proportion of prawns that would survive a concentration equal to the EIS NOEC

p_NOEC<-alpha*exp(-beta*(NOEC-gamma)*step((NOEC-gamma)))

}

# data
x[] r[] N[]
0.0     10      10
2.1     6       10
4.1     8       10
8.3     10      10
16.5    8       10
33.0    0       10
0.0     6       10
2.1     8       10
4.1     8       10
8.3     8       10
16.5    6       10
33.0    4       10
0.0     10      10
2.1     10      10
4.1     10      10
8.3     10      10
16.5    6       10
33.0    2       10
0.0     10      10
2.1     10      10
4.1     8       10
8.3     10      10
16.5    6       10
33.0    2       10
END
```

Figure 4: OpenBUGS code for Bayesian estimation of the NEC for desalination plant example.

Prior to gathering information on the empirical posterior densities for each of the model parameters, we used a 'burn-in' run of 10,000 iterations. The model was then run for a further 100,000 iterations with sampling of the output every 20th. iteration (to reduce autocorrelation in the sampled output). Figure 5 shows the empirical posterior densities and Table 2 summarizes the results of these 5,000 estimates.

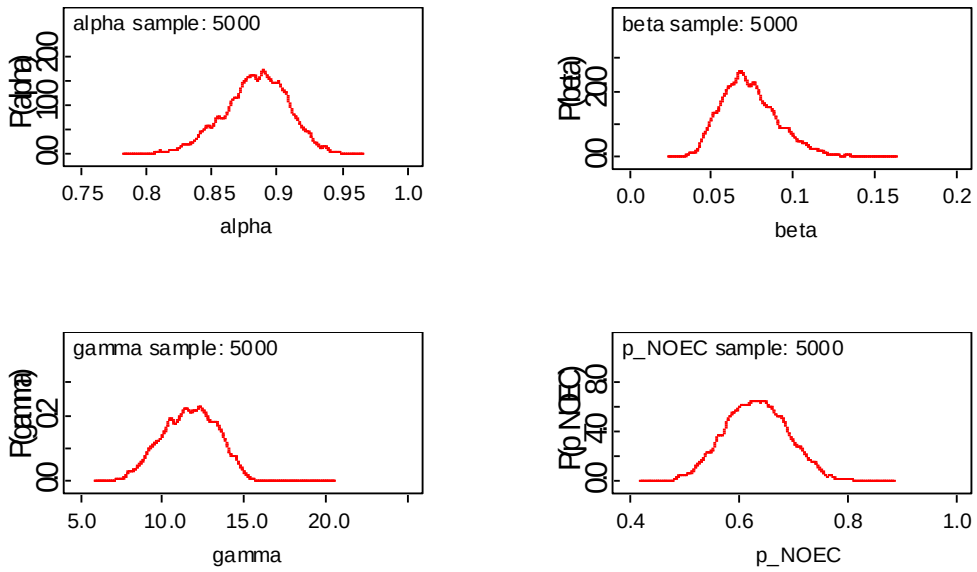

Figure 5: Empirical posterior densities for model parameters α, β, γ and probability of prawn survival at concentrations ≥ NOEC.

Table 2: Summary statistics for posterior distributions of model parameters and probability of prawn survival at concentrations ≥ NOEC.

	mean	sd	MC_error	val2.5pc	median	val97.5pc
alpha	0.8834	0.02478	3.31E-04	0.8307	0.8849	0.9275
beta	0.07356	0.01783	2.63E-04	0.04496	0.07134	0.114
gamma	11.65	1.679	0.02331	8.352	11.7	14.62
p_NOEC	0.6321	0.05917	8.91E-04	0.5206	0.6316	0.7459

Our point estimate of the NEC is 11.7 with an associated 95% credibility interval of 8.35 to 14.62. We note that this credibility does not include the previously estimated NOEC of 16.5. It is also readily determined from the OpenBUGS output that the probability P[NEC>=16.5] is 0.00085 which suggests that a NEC of 16.5 is extremely implausible. Finally, we can use the fitted model to estimate the prawn survival at a concentration equal to the reported NOEC or conversely, the mortality at this concentration. From Figure 6 we see that this latter figure is about 36%.

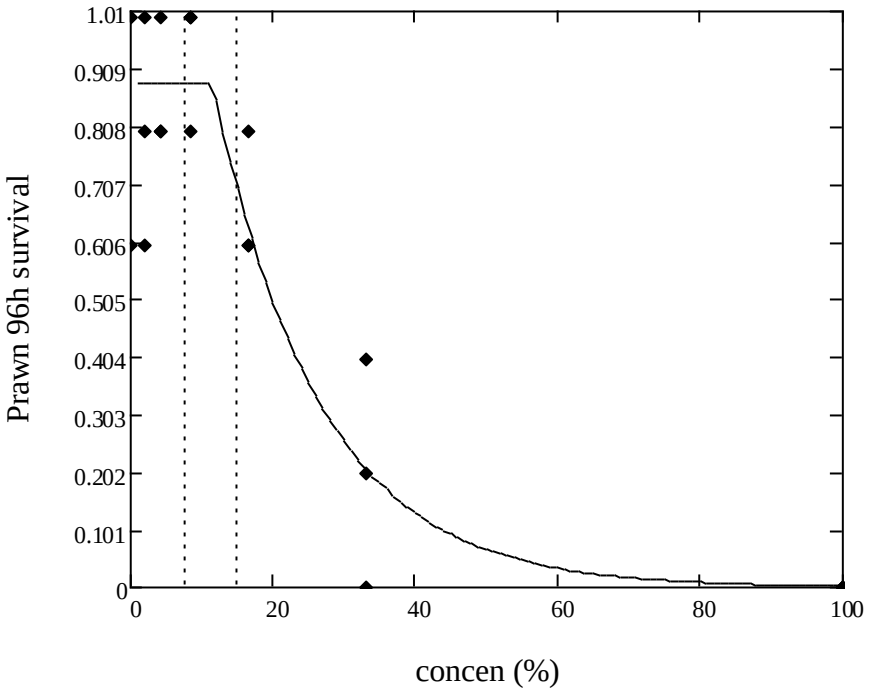

Figure 6: Prawn survival data (as proportions) plotted against effluent concentration (%) (solid diamonds). Solid line is fitted dose-response model using Bayesian point estimates of model parameters. Dashed vertical lines depict limits of 95% credibility interval for the NEC.

2.2 Estimating the uncertainty in the HC_x

We next turn our attention to estimating the uncertainty in the all important HC_x – the concentration for which it is claimed (*100-x)%* of *all* species will be protected provided the environmental concentration of the contaminant does not exceed HC_x. Space limitations preclude a comprehensive treatment of this topic and therefore only an outline is provided here.

The idea is to use the empirical posterior densities for the γ parameter (the NEC) for a number (*k*) of species using the methods outlined in this paper. By randomly sampling from each of these posterior densities, we obtain one realisation of *k* estimated NECs (Figure 7a). To this sample of *k* estimated NECs we fit a theoretical SSD (as is conventionally done) from which the x^{th} percentile is determined – where *x* is some arbitrarily, pre-determined small value (typically 5% or 1%). This procedure is repeated for each *k*-sample of NECs (Figure 7b). By repeating the process many times (eg 10,000 – 100,000) we can obtain the empirical density of the HC_x distribution (Figure 7c) and use this to obtain point and interval estimates for the true value. This procedure represents an alternative, and we believe, superior approach to quantifying the uncertainty in the estimated HC_x . Current practice fits one SSD to a single sample of *k* NOECs and estimates the uncertainty in the single HC_x estimate either by appealing to the asymptotic properties of the SSD parameter estimates or bootstrapping.

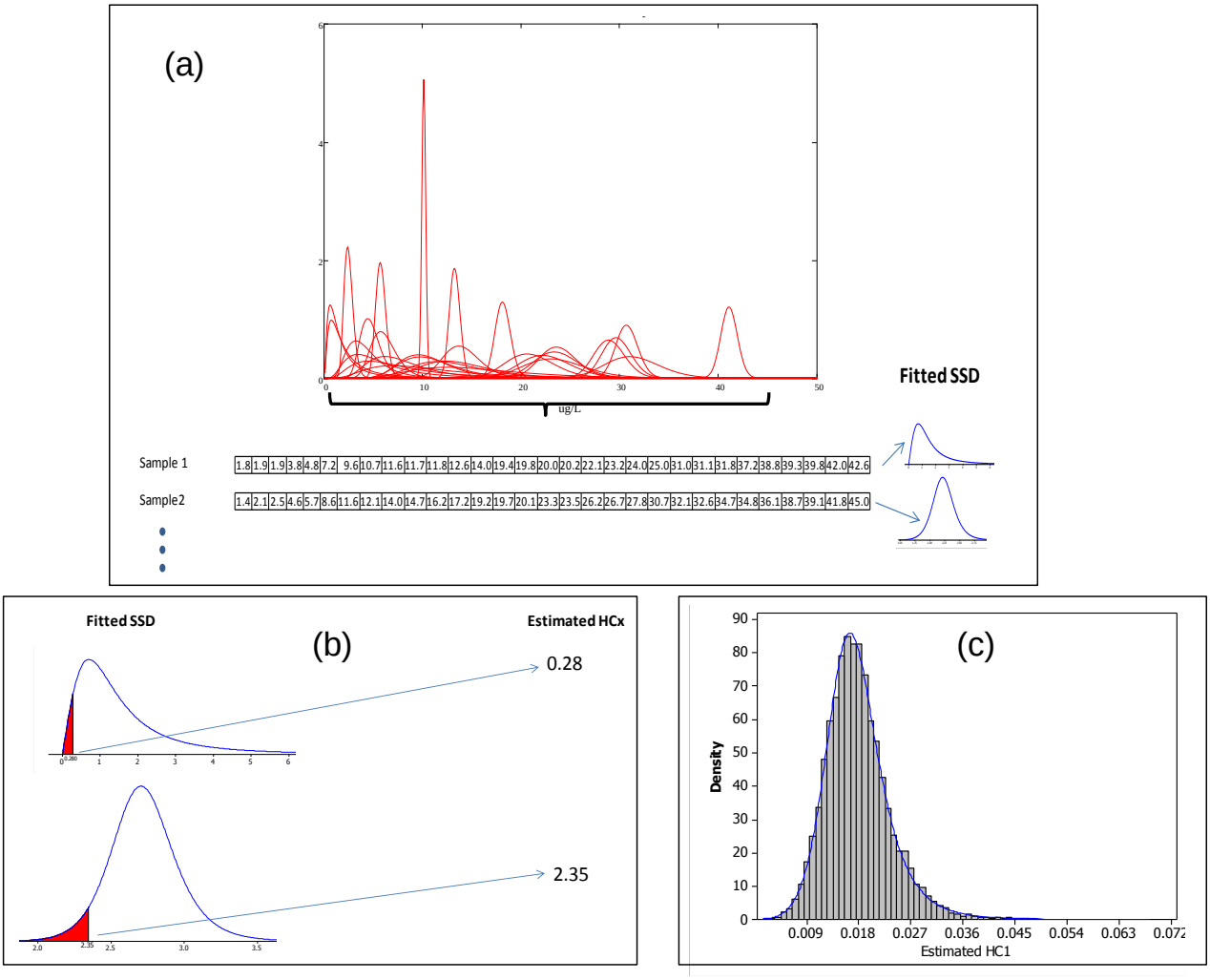

Figure 7: Illustration depicting procedure for obtaining point and interval estimates for the HC_x. (a) Red curves are *posterior* densities for the NECs of a sample of k species. Random samples are repeatedly drawn from these k distributions; (b) a theoretical probability model (the SSD) is fitted to each sample of k NECs and the HC_x determined for each. The procedure is repeated many times resulting in (c) the empirical density for HC_x.

3. Conclusions

In this paper we have described a general Bayesian framework for identifying critical threshold concentrations for ecosystem protection. Starting with a flexible dose-response model, we use Bayesian parameter estimation techniques to derive relevant posterior distributions. Unlike conventional practice, our approach provides a comprehensive assessment of the uncertainty in estimated measures of toxicity for individual species. Furthermore, the utilisation of the posterior densities in the fitting of species sensitivity distributions admits both point *and* interval estimates of the HC_x – the concentration affecting x% of all species.

In view of the flexibility and robustness of the proposed approach, coupled with the ability to incorporate expert knowledge about key biological processes, we contend that this alternative paradigm may alleviate, if not remove some of the long-standing problems associated with the use of traditional modes of statistical inference for ecosystem protection.

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