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"Some statistical properties of estimates of no-effect concentrations"

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SOME STATISTICAL PROPERTIES OF ESTIMATES OF NO-EFFECT CONCENTRATIONS

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Abstract—No-effect concentrations (NECs) for toxicants are of interest from a biological and a legislation point of view. Using artificial, but typical, examples of the results of a bioassay on survival and two different models for the concentration-effect relationship, we show that the likelihood based confidence set of the NEC as parameter of the hazard model has quite acceptable statistical properties. Contrary to the hazard model, the NEC of the standard log-logistic model did not differ significantly from zero.

Key words—NEC, bioassays, survival, hazard model, log-logistic model, profile likelihood

INTRODUCTION

The aquatic toxicity of chemical compounds with respect to the survival of animals is tested frequently on a routine basis by exposing cohorts of individuals to a set of chosen concentrations during a standardized period. A standard measure to characterize the toxicity is the concentration at which the survival probability is half that of the control, the so-called LC50, here denoted as c_{L50}. However, the concentration that has no effect is of much more practical interest for many purposes. The most frequent choice is the no-observed effect concentration (NOEC), i.e. the highest applied concentration that did not give statistically significant effects compared to the control. It is usually identified on the basis of (mutually dependent) tests against the control (cf. Williams, 1971). An inherent problem to this procedure is that the null-hypothesis states "there is no effect at the applied concentration" (in other words: the concentration is "safe"), so sloppy experimental procedures result in high NOEC's; this is certainly an undesirable coupling. To resolve this problem, the application of "small-effect concentrations" has recently been considered (Pack, 1993), but an inherent problem to this approach is the arbitrariness of a choice for "small". Moreover, the estimated value for a small-effect concentration depends very sensitively on the particular concentration-effect relationship (e.g. the log-logistic one), which has no scientific justification. The basis is purely empirical and weak in the "tails". An attractive alternative is the no-effect concentration (NEC), treated as a model parameter (Kooijman, 1981; Cox, 1987). Here we study to what extent it is model-specific by comparing the extended standard log-logistic model and the hazard model for concentration-effect relationship. Both models are discussed in some detail in Kooijman (1981) and Bedaux and Kooijman (1994), respectively, and the biological backgrounds in Kooijman (1993). Here, we give a short introduction to these models and focus on the typical case where the surviving individuals are counted once only, at the end of the experiment.

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EFFECT MODELS

We choose this observation time as our time unit and consider the survival probability as a function of the concentration only. This removes the dimension time from the parameters of the survival probability. The standard procedure to model bioassays for survival is to assume that the survival probability is independent and identical for each individual in a cohort. This implies that the number of survivors follows a binomial distribution. The log-likelihood function for the experimental result to find $\{n(c_i, t)\}_{i=1}^{k}$ survivors after exposure time t to the compound, which is present in concentration c_i is then

$$\ln l = \sum_{i=1}^{k} n(c_i, 1) \ln q(c_i, 1) + \sum_{i=1}^{k} (n(c_i, 0) - n(c_i, 1)) \ln p(c_i, 1) \quad (1)$$

where $q(c_i, t) \equiv 1 - p(c_i, t)$ denotes the survival probability at time t.

The standard model for survival assumes that an individual dies as soon as the tissue-concentration exceeds a (fixed) threshold value which differs among individuals. These threshold values are conceived as random trials from a log-logistic distribution (see Ashton, 1972), which just has a purely empirical

basis. The standard model is extended by subtracting a no-effect-tissue-concentration from the tissue-concentration. The model has the unrealistic property that a fraction of the exposed individuals survives the toxicant, even if the exposure is very long and the concentration very high.

The hazard model assumes that the hazard rate is proportional to the tissue-concentration minus the no-effect-tissue-concentration (Kooijman, 1993; Bedaux and Kooijman, 1994). In contrast to the standard model, all individuals are treated as being equal in the most simple version of the hazard model, but the killing process itself is stochastic rather than deterministic. Another difference is that the compound will eventually kill all individuals that accumulate the compound above the no-effect-tissue-concentration.

For both models we will assume that the uptake and elimination behaviour of the compound follows simple first order kinetics, while the concentration in the environment remains constant and the initial concentration in the tissue is 0, which is typical for most routine toxicity tests. Extensions of this idea are easy to implement (Kooijman, 1993). We also assume here that the individuals do not grow during exposure.

Extended standard model

The no-effect concentration at the time of observation relates to the (ultimate) no-effect concentration as $c_0 \equiv c_{0,\infty}/1 - \exp\{-k_a\}$, where k_a is the elimination rate. Since the elimination rate does not affect the concentration response relationship in the standard model (at one single observation time), we have no information about k_a, so we can only extract information about c_0 from survival data, but not about $c_{0,\infty}$. The elimination rate does affect the concentration response relationship in hazard model, however. Obviously, information about the elimination rate can be obtained from survival data much more easily (and reliably) if several observation times would have been available that reveal how effects build up in time. For the hazard model, it is thus possible to obtain both c_0 and $c_{0,\infty}$ from a single concentration response relationship.

The extended standard model for $c > c_0$ is

$$q(c) = q_0\left(1 + \left(\frac{c - c_0}{c_{L50} - c_0}\right)^{1/\beta}\right)^{-1} \qquad (2)$$

where q_0 stands for the control survival probability; β is the gradient parameter which directly relates to the (maximum) slope of the graph where the response is plotted against the concentration. The parameter c_{L50} (usually known as LC50) represents the concentration for which the survival probability is half that in the control, so $q(c_{L50}) = q_0/2$. It depends on the exposure time in a way similar to the no-effect concentration: $c_{L50} = c_{L50,\infty}/1 - \exp\{-k_a\}$. We also have the same problem here: If nothing is known about the elimination rate, we have no information about $c_{L50,\infty}$. We need more than one observation time in the standard model to obtain that information. For $c < c_0$, we have $q(c) = q_0$.

Hazard model

The hazard model for $c > c_0$ is

$$q(c) = q_0 \exp\{-k_+ k_a^{-1}[c \exp\{-k_a\} + (c - c_{0,\infty})(k_a - 1 + \ln\{1 - c_{0,\infty}/c\})]\} \qquad (3)$$

where k_+ stands for the killing rate. For $c < c_0$, we have $q(c) = q_0$. This survival probability results from the hazard rate $h(c,t)$ via $q(c) = \exp\{-\int_0^t h(c,\tau)\,d\tau\}$ (by definition), with

$$h(c,t) = h(0,t) + k_+((1 - \exp\{-tk_a\})c - c_{0,\infty})_+ \qquad (4)$$

The index + indicates that negative values between the brackets should be replaced by 0. The first term $h(0,t)$ stands for the hazard rate in the control, which is taken to be constant. So $\exp\{-\int_0^t h(0,\tau)\,d\tau\} = q_0$. The killing rate appears here as a simple proportionality factor for the hazard rate. The term $(1 - \exp\{-tk_a\})c$ in the hazard rate is proportional to the tissue-concentration, from which the no-effect concentration is subtracted. Although the hazard model equation (3) might seem more complex than the extended standard model equation (2) at first sight, the hazard rate of the extended standard model is much more complex than equation (4). It is just a matter of presentation.

Since the information content of a concentration response relationship about the elimination rate is poor, the limiting case for very large and very small elimination rates are of special interest. These limiting cases will be referred to as the exponential and the Weibull model, respectively, and amount to

$$q(c) \overset{k_a \to \infty}{=} q_0 \exp\{-k_+(c - c_0)\} \qquad (5)$$

$$q(c) \overset{k_a \to 0}{=} q_0 \exp\left\{-\frac{1}{2}kc(1 - c_0/c)^2\right\} \qquad (6)$$

The exponential model (5) can be derived from the hazard model (3) in a straightforward way, but the relationship between the Weibull model (6) and the hazard model is more complex. This is because effects relate to tissue-concentrations, while the model is formulated in terms of environment-concentrations. Tissue-concentrations build up linearly in time for $k_a \to 0$, so that $c_{0,\infty} \to 0$ and the waiting time till the tissue-concentration exceeds the no-effect-tissue-concentration is inversely proportional to the environment-concentration. The interpretation of c_0 in (6) is the limit of $c_{0,\infty}/k_a$. It has here the dimension of a concentration because we have chosen the exposure time as unit of time, which is a bit misleading. We also have $k_+ \to \infty$ such that killing acceleration

example	hazard model					extended standard model			
	q_0	c_0	k	$k_\dagger$	k_a	q_0	c_0	c_{L50}	β
1	0.950	1.303	5.143			0.950	0.763	2.045	0.225
2	1.000	0.749	2.510			1.000	0.000	1.858	0.228
3	0.945	1.000		1.407	2.507	0.945	0.981	2.066	4.259
4	1.000	0.654	1.635			1.000	0.000	1.986	0.300

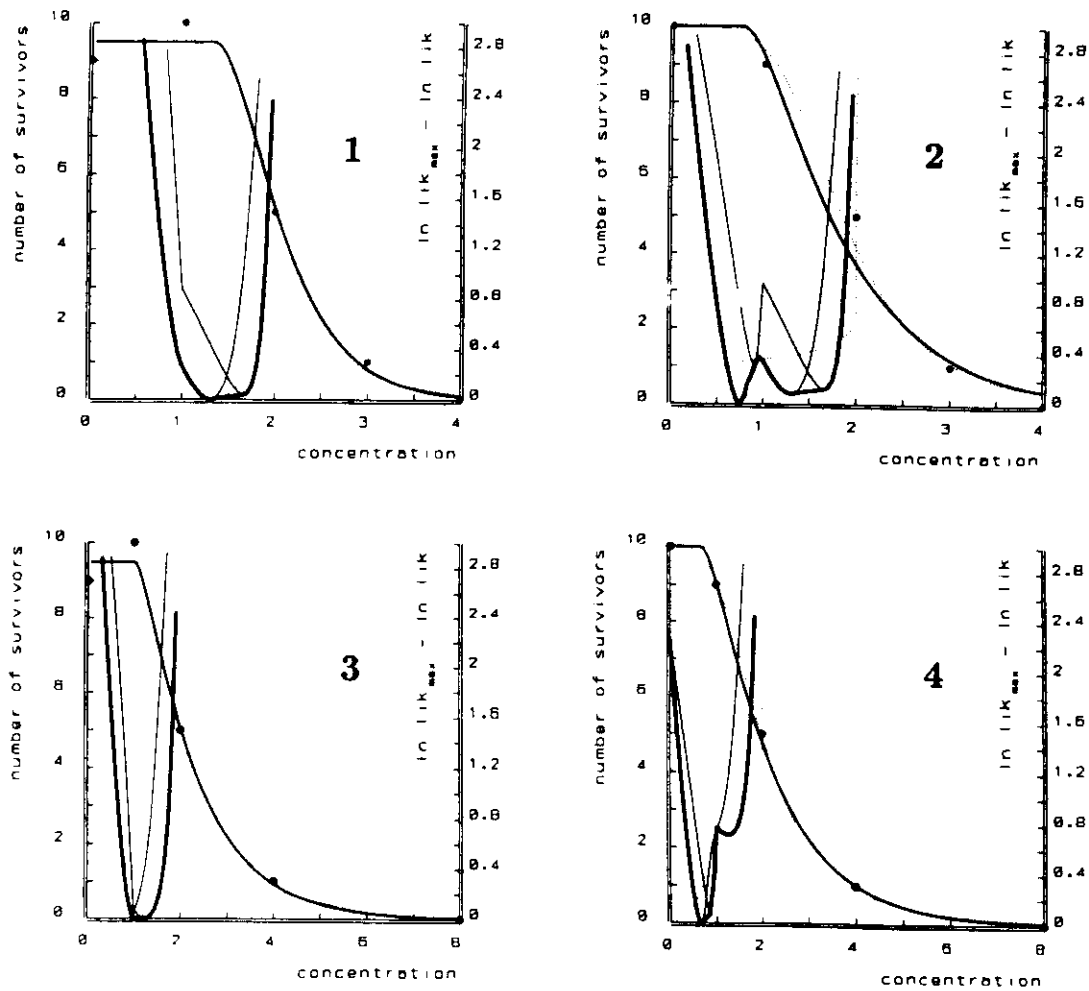

Fig. 1. The five dots in each graph represent numbers of "observed" survivors out of 10. The expected number of survivors is plotted for the hazard model (solid curve) and the extended standard model (dotted curve), based on the maximum likelihood estimates of the parameters, which are given below. The difference between the maximum log-likelihood and the profile log-likelihood is also plotted as a function of the NEC, for both models (dotted and fat curve) as well as for the Weibull (left thin curve) and the exponential model (right thin curve).

Table 1. The chosen concentrations and surviving individuals out of ten

Example	Concentration				
1 and 2	0	1	2	3	4
3 and 4	0	1	2	4	8
Example	**Survivors**				
1 and 3	9	10	5	1	0
2 and 4	10	9	5	1	0

$k \equiv k_t k_a$ remains fixed, which leads to the hazard rate $h(c, t) = h(0,t) + kc(t - c_0/c)_+$ and so to (6).

EXAMPLES

Practice teaches that it is hard to obtain partial effects in more than one or two concentrations, for most compounds. This gives problems to obtain point estimates for c_0. A practical solution to this problem is to choose a variety of values for c_0 and obtain the maximum likelihood estimates for the other parameters via a Newton Raphson procedure, for instance. We then inspect the profile log-likelihoods (see e.g. McCullagh and Nelder, 1991, p. 254; or Carroll *et al.*, 1995), i.e. the log-likelihood function that is maximized with respect to all parameters except c_0, as a function of c_0. The difference of the profile log-likelihoods and the maximum log-likelihood is plotted in Fig. 1. Analyses are given for four artificial but typical examples of experimental results, where $n(c_i, 0) = 10$ has been chosen for all c_i in all examples (see Table 1). The concentrations have a fixed difference in one pair of examples and a fixed factor in the other pair, while the number of survivors have been chosen identically. This is done to investigate the effect of the morphology of the observed concentration response relationship on the estimate for c_0. A second comparison is based on the interchange between the number of survivors in the control and the lowest concentration. This is done to investigate how uncertainty about the cause of death (i.e. control vs. toxicant-induced mortality) affects the estimate for c_0.

CONCLUSIONS

Both the extended standard and the hazard model appear to fit the data well. Since the shape of the concentration response relationship in examples 1 and 2 differs from examples 3 and 4, the fit is not very sensitive to details of the shape. The best fitting hazard model proved to be the Weibull model (6) in three examples (see Fig. 1). The examples being typical, we expect that the tiny differences in goodness of fit cannot be used to choose between the models in practice. This means that scientific arguments must be used for the choice, rather than statistical ones. The hazard model (with the exponential and Weibull model as special cases) has a statistical advantage above the standard one, because it has less parameters, which generally leads to smaller confidence intervals. This is even more obvious if several observation times are considered simultaneously, because the elimination rate then also appears in the extended standard model, while no new parameters show up in the hazard model.

The profile log-likelihood functions are plotted in the same Fig. 1 to compare these functions with the concentration response relationships. The profiles can be used to obtain confidence sets for the no-effect concentration c_0. When the large sample theory for likelihood ratios applies (see Silvey, 1975), the likelihood based α-level confidence set for c_0 is given by $\{c_0 | 2(\ln l(\hat{c}_0) - \ln l(c_0)) \leq \chi_1^2(\alpha)\}$, where $\ln l(c_0)$ denotes the profiles log-likelihood in c_0 and $\chi_1^2(\alpha)$ is a number such that for a random variable z that is χ^2-distributed with 1 degree of freedom we have $\text{Prob}\{z \leq \chi_1^2(\alpha)\} = \alpha$. Likelihood based confidence sets seem to be more robust against deviations from "large samples" than the interval $\{c_0 | (c_0 - \hat{c}_0)^2 \hat{\text{var}}(\hat{c}_0) \leq \chi_1^2(\alpha)\}$ in models like these (Kooijman, 1983; Carroll *et al.*, 1995). Application of this idea in Fig. 1 means that the 90% or 95% confidence set contains the c_0-values for which the log-likelihood is 1.35 or 1.92 lower than the maximum log-likelihood.

Deviations from the large-sample theory can be translated into corrections on the χ^2-values. Figure 1 shows that such corrections would hardly affect the confidence limits of $\hat{c}_0$, since the profile log-likelihood functions are very steep. Practice has little interest in high accuracy at this point. We can conclude that the NEC for the extended standard model is not significantly different from 0, but for the hazard model it is for all examples. This reflects an important structural difference between both models. If the hazard model is acceptable on scientific grounds, it should be preferred above the extended standard model, not because of tiny differences in fit, but because of its structural properties.

The fat curves in Fig. 1 represent the profile log-likelihood functions of the hazard model. They coincide with the profile log-likelihood functions of the Weibull model for the lower concentrations and that of the exponential model for the higher concentrations. The relatively small shift between the profile log-likelihood functions of the Weibull and the exponential model correspond with the change of the elimination rate k_a from 0 to ∞. We conclude that a single observation time for surviving individuals results in poor knowledge about the elimination rate, but this has little effect on the estimate for the no-effect concentration.

Examples 2 and 4 have no deaths in the control and one in the lowest concentration. The profile log-likelihood functions for these examples have two local maxima, which correspond with two possible interpretations for the cause of death: is it control mortality (right local maximum), or toxicant-induced (left local maximum)? The profiles show that the

interpretation in terms of toxicant-induced mortality is more likely in these two examples. Examples 1 and 3 have one death in the control and none in the lowest concentration. Here, we have just one possible interpretation for the cause of death (namely control mortality) and just one local maximum for the profile log-likelihood function. The profile log-likelihood functions thus quantify the probabilities for the alternative causes of death.

The software package DEBtox as provided in Kooijman and Bedaux (1996) can be used to obtain the profile likelihood functions.

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