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"No-effect concentrations in algal growth inhibition tests"

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NO-EFFECT CONCENTRATIONS IN ALGAL GROWTH INHIBITION TESTS

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Abstract—We propose three simple models for effects of chemical compounds on the growth of batch cultures of algae that allow the estimation of the no-effect concentration. The growth model assumes that the costs for growth is proportional to the concentration that exceeds the no-effect level. The hazard model assumes that the hazard rate is proportional to the concentration that exceeds the no-effect level. The adaptation model is similar to the hazard model, but the effects only occur at the start. The no-effect concentrations of the three models turn out to be very similar. Copyright © 1996 Elsevier Science Ltd

Key words—NOEC, EC50, NEC, growth rate, killing rate, adaptation

INTRODUCTION

The effects of chemical compounds on aquatic biological systems are tested routinely with a set of simple toxicity tests, where groups of individuals for a single species, usually originating from a laboratory culture, are exposed to a set of concentrations of a chemical during some standardized period. The No-Observed Effect Concentration (NOEC) is defined as the highest tested concentration that gives no significant deviation from a control without the chemical (Bartlett *et al.*, 1974, Bringmann and Kühn, 1980). The usefulness of this frequently used statistic suffers from lack of knowledge about the power of the statistical test that is used. This power also depends, of course, on the probability of an error of the first kind (usually taken to be 5%), which is rather arbitrary. In addition, the NOEC is highly dependent on the test design, since it can only assume values of tested concentrations. A compromise exists between the number of different concentrations that are used in the test and the number of replicates per concentration. Precision increases with the number of different concentrations and the power increases with the number of replicas. In the environmental risk assessment of chemicals, the prediction of environmental no-effect concentrations relies heavily on laboratory test derived NOEC values. However, most standard ecotoxicity tests were originally designed for determining EC50 values.

In an attempt to address the problems inherent to the NOEC, it is current practice to derive PNECs (Predicted Environmental No-Effect Concentrations) from EC50 data using a scheme of fixed application factors. The draft March 1995 version of the EU technical guidance document for environmental risk assessment of new and existing substances suggests factors of 10, 50, 100 and 1000, depending on the amount and quality of the data available. Because dose-response slopes can be very compound-specific, some workers proposed EC5, EC10 or other "small"-effect values. Such an approach is difficult to apply to risk assessment, because of a lack of consensus about the precise definition of "small". The smaller the effect size in descriptive models the larger the confidence interval and the more the estimate becomes dependent on the specific model that has been used to describe the results. Since the empirical log-logistic model does not have a scientific basis, this is quite an obstacle. This problem becomes less important for the larger effect sizes, such as the EC15 or EC20. The problem then becomes how "small" effects in the laboratory translate into effects outdoors and how the effects of emissions for various compounds in a certain area combine when each is allowed to have a "small" effect.

In this article we discuss the applicability of a No-Effect Concentration (NEC) in algal growth inhibition tests. There are at least four internationally accepted standard test descriptions, i.e. the nearly identical OECD guideline 201 (OECD, 1984), the International Standard ISO 8692 (ISO, 1989) and the EU Guideline C3 (EU, 1992) covering the freshwater environment, and the International Standard, ISO 10253 (ISO, 1994) covering the marine environment. The assessment of the NOEC value in these methods is, in lack of better, only superficially defined, and mostly left to the judgement of the particular scientist

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whether a standard statistical test should be applied or not. The weakness of this approach has been recognized by the working groups developing these methods. The NOEC value will depend on the variation among replicate test vessels and the deviations of treated algal populations from the control. Relatively high NOEC values may therefore result from a badly performed test. The NEC does not suffer from the statistical problems of the NOEC, because the null hypothesis is that the NEC equals zero. A poor power results in an inability to reject the null hypothesis, and leads to the conclusion that the tested compound requires further research.

The present article is one of a series (Bedaux & Kooijman, 1994; Kooijman & Bedaux, 1996, 1996a, 1996b) that deals with similar NECs in the other OECD toxicity test methods. All models behind these analyses assume that the effect size on the various physiological target processes is proportional to the concentration of the compound that exceeds a no-effect concentration in the organisms. The Dynamic Energy Budgets (DEB) theory is used to identify the target processes. This theory is described in Kooijman (1993). The choice of a linear relationship between effect size and the tissue-concentration relates to the idea of a Taylor-approximation to the 'real' effect size. So the actual effect size might be a complex function of the tissue-concentration, but we use only the first term of its Taylor approximation. For highly non-linear relationships, this only works for small effect sizes. The inclusion of more terms of the Taylor-approximation hardly makes sense in the light of the concept that physiological processes can be ordered with respect to sensitivity for a particular compound. At low concentrations only the most sensitive process is affected, but at high concentrations many processes are affected. It will be very difficult to make reliable models for large effects. Since risk assessment requires knowledge of small effects, not of large effects, the situation that large effects are possibly not well captured by model predictions is hardly relevant.

To simplify the reasoning, we assume that the elimination rate is small with respect to the inverse of the interdivision interval, so the intra-cellular concentration is almost instantaneously in equilibrium with the environment-concentration, which makes sense for minute algal cells. As long as the aqueous bioavailable concentration of the test compound remains approximately constant and is not reduced by sorption or other elimination mechanisms, the toxic dose can be regarded constant throughout the test. So the focus is on population growth at a constant environment-concentration of test compound. Irrespective of the physiological complexity of the cell cycle, the fact that the daughter cells repeat the physiological behaviour of the mother cell implies that the population will grow exponentially in cell numbers as long as the environment is constant.

MODELS

A summary of the algal growth inhibition test according to the standard test methods is as follows: Batch cultures of one of the recommended algal species (i.e. the fresh water green alga *Selenastrum capricornutum* or *Scenedesmus subspicatus*, or the marine diatom *Skeletonema costatum* or *Phaeodactylum tricornutum*) are started with a fixed cell density of 10^4 cells ml^{-1} in the prescribed media with different additions of test compound, usually below the solubility in water. Although the guideline prescribes concentrations in terms of effect sizes, these choices are less important for the analyses that we propose here. Temperature and light intensity are constant during exposure, but a precise temperature and light intensity are not prescribed, only an allowable range. The test has been designed so that ideally exponential growth in the control cultures can be sustained for the entire duration of the test, which has been fixed at 72 h. It is specified as a validity criterion that the growth rate must be high enough to allow the biomass in the control cultures to increase by at least a factor 16, which corresponds to a minimum growth rate of $0.92\ d^{-1}$. The growth rates normally obtained for the standard test species *S. capricornutum* and *S. subspicatus* may vary from about 1.2 to $2.0\ d^{-1}$, increasing with light intensity and temperature (Hanstveit, 1982, 1991). The mean growth rates for *S. costatum* and *P. tricornutum* are $2.40\ d^{-1}$ and $1.73\ d^{-1}$, respectively, determined in an international ring test (Hanstveit, 1991). The corresponding biomass increase during 3 d amounts to a factor 37 and 400, respectively. In practice a 72 h exponential growth is only achieved with *S. capricornutum* and *P. tricornutum*. The other species grow approximately logistically, because of the large cell volume of *S. subspicatus* (resulting in a large inoculated biomass) or to the high growth rate of *S. costatum*. The guidelines, however, allow for the use of the exponential growth phase for the evaluation of effects. The biomass is usually measured by electronic particle counting, by spectrophotometry (optical density) or by fluorometry (in vivo chlorophyll fluorescence), which implies that both the living and the dead cells contribute (dead cells somewhat less than living cells with fluorometry). Formally, the biomass (i.e. dry weight or total cell volume) is the proper measure for the algal growth. The cell density (i.e. cell numbers per volume) may be used as long as they relate to the biomass (which is not the case when cells form chains).

We assume here that the cell number in the control grows exponentially, that is

$$\frac{\mathrm{d}}{\mathrm{d}t}N = \dot{\mu}_0 N$$

$$N(t) = N(0)\exp\{\dot{\mu}_0 t\} \qquad (1)$$

where $\dot{\mu}_0$ is the control population growth (dimension time^{-1}). We consider three different effects.

Growth model

The cost of growth in terms of nutrients or energy is inversely proportional to the population growth rate. The linear effect model as mentioned in the introduction amounts to the assumption that the costs for growth are linear in the intracellular concentration of test compound. For tiny organisms such as unicellular algae, these costs are thus linear in the environment-concentration. This leads to

$$\frac{\mathrm{d}}{\mathrm{d}t}N = \dot{\mu}_c N$$

$$N(t,c) = N(0,c)\exp\{\dot{\mu}_c t\} \text{ with}$$

$$\dot{\mu}_c = \dot{\mu}_0(1 + c_G^{-1}(c - c_0)_+)^{-1} \quad (2)$$

where c is the concentration of test compound in the environment, c_0 is the NEC and c_G is the "tolerance concentration" which just serves as a proportionality constant. It is so named because the less toxic the compound, the higher its value. Note that an interpretation of this parameter is c_G = EC50–NEC, where the EC50 is the concentration that causes a reduction of the population growth rate by a factor of two. The concept EC50 is very familiar in the analysis of toxicity tests. Despite the simple relationship with the tolerance concentration, we will not use the parameter EC50. The first reason is that the combination EC50 and NEC behaves worse than the combination c_G and c_0 in a statistical sense, because their estimates have a higher (negative) correlation coefficient. The second reason is that the relationship between EC50 and the tolerance concentration is less simple in other models (see the adaptation model). The notation $(c - c_0)_+$ indicates that we replace negative values of $c - c_0$ by zero. We assume that no death occurs if the compound affects the energetics of the cells.

Hazard model

The second mechanism of toxic effect is via the hazard rate that is assumed to be proportional to the intra-cellular concentration that exceeds the no-effect concentration. The surviving cells grow at the same rate as those in the control. The change in the numbers of living and dead cells becomes

$$\frac{\mathrm{d}}{\mathrm{d}t}N_1 = \dot{\mu}_c N_1 = (\dot{\mu}_0 - \dot{k}_+(c - c_0)_+)N_1$$

$$\frac{\mathrm{d}}{\mathrm{d}t}N_0 = \dot{k}_+(c - c_0)_+ N_1$$

where we have no dead cells at the start of the experiment, so $N_0(0) = 0$. The parameter $\dot{k}_+$ just serves as a proportionality constant and is called the "killing rate". The total (living plus dead) number of cells amounts to

$$N(t,c) = N(0,c)\left(\frac{\dot{\mu}_0}{\dot{\mu}_c}\exp\{\dot{\mu}_c t\} + 1 - \frac{\dot{\mu}_0}{\dot{\mu}_c}\right) \quad (3)$$

Ignoring cell lysis during the 3 d of the test, the counted number of cells corresponds with the total number of cells. Notice that the total number of cells does not grow exponentially if effects on the hazard rate occurs.

Adaptation model

The third mechanism of toxic effects is via the change from the control situation of the stock culture to the experimental test condition. The effect is the same as in the hazard model, but it occurs only during a short (fixed) period of exposure. If the cells survive this transition, they are not affected by the compound, so the resistant cells are selected. The survival probability then amounts to $\mathscr{F} = \exp\{-c_H^{-1}(c - c_0)_+\}$ and the total (living plus dead) number of cells to

$$N(t,c) = N(0,c)(\mathscr{F}\exp\{\dot{\mu}_0 t\} + 1 - \mathscr{F}) \quad (4)$$

where "tolerance concentration" c_H just serves as proportionality constant. It is inverse to the product of the killing rate and the length of the sensitive period. The survival probability $\mathscr{F}$ can be interpreted as the fraction of resistant cells in the control culture.

STATISTICS

The number of cells in any experimental unit is assumed to be normally distributed with a mean that behaves as explained in the model section and a variance that is (about) proportional to the squared mean. So the coefficient of variation is assumed to be constant. This depends, however, on the accuracy and the method for measurement of the biomass. A constant variance, independent from the mean, is an attractive alternative. The most straightforward estimation criterion is the maximum likelihood method. To find the parameter estimates, we have to evaluate the matrix of second derivatives of the cell numbers to the three parameters: $\dot{\mu}_0$, c_0 and c_G, c_H or $\dot{k}_+$. Since the formulae become lengthy, a less elegant but useful alternative is to apply weighted non-linear regression, where the weight coefficients are taken inverse to the squared observed cell numbers. This gives the additional advantage that we can give less weight to cultures that show a large effect. The applicability of the likelihood method should be tested, however, if the weight coefficients affect the results substantially. If the (control) cultures grow too fast (depending on the algal species) the last data point will show a deviation from exponential growth, because the cultures become nutrient limited. This will happen if the light intensity and temperature are both approaching the upper limits of the prescribed ranges. The weight of such a deviating data point can also be reduced for biological reasons. (One should always be extremely careful not to exclude data points because the model does not fit.)

The profile ln likelihood (cf. McCullagh and Nelder, 1989) for c_0 is $l(c_0) = \Sigma_{ij} w_{ij} \ln \hat{\sigma}_0/\hat{\sigma}_1$, where $\hat{\sigma}_0$

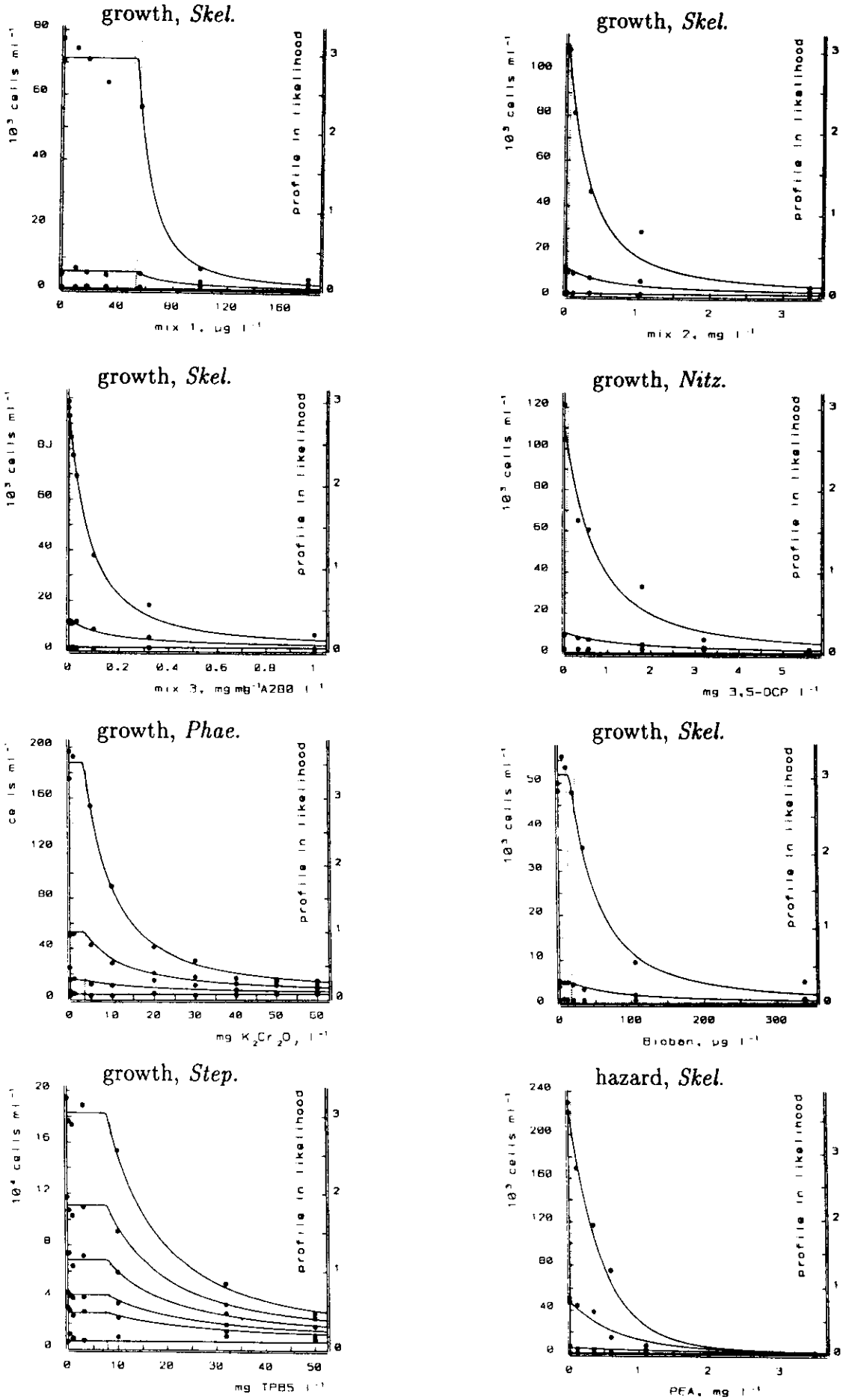

Fig. 1(a) *See caption opposite*

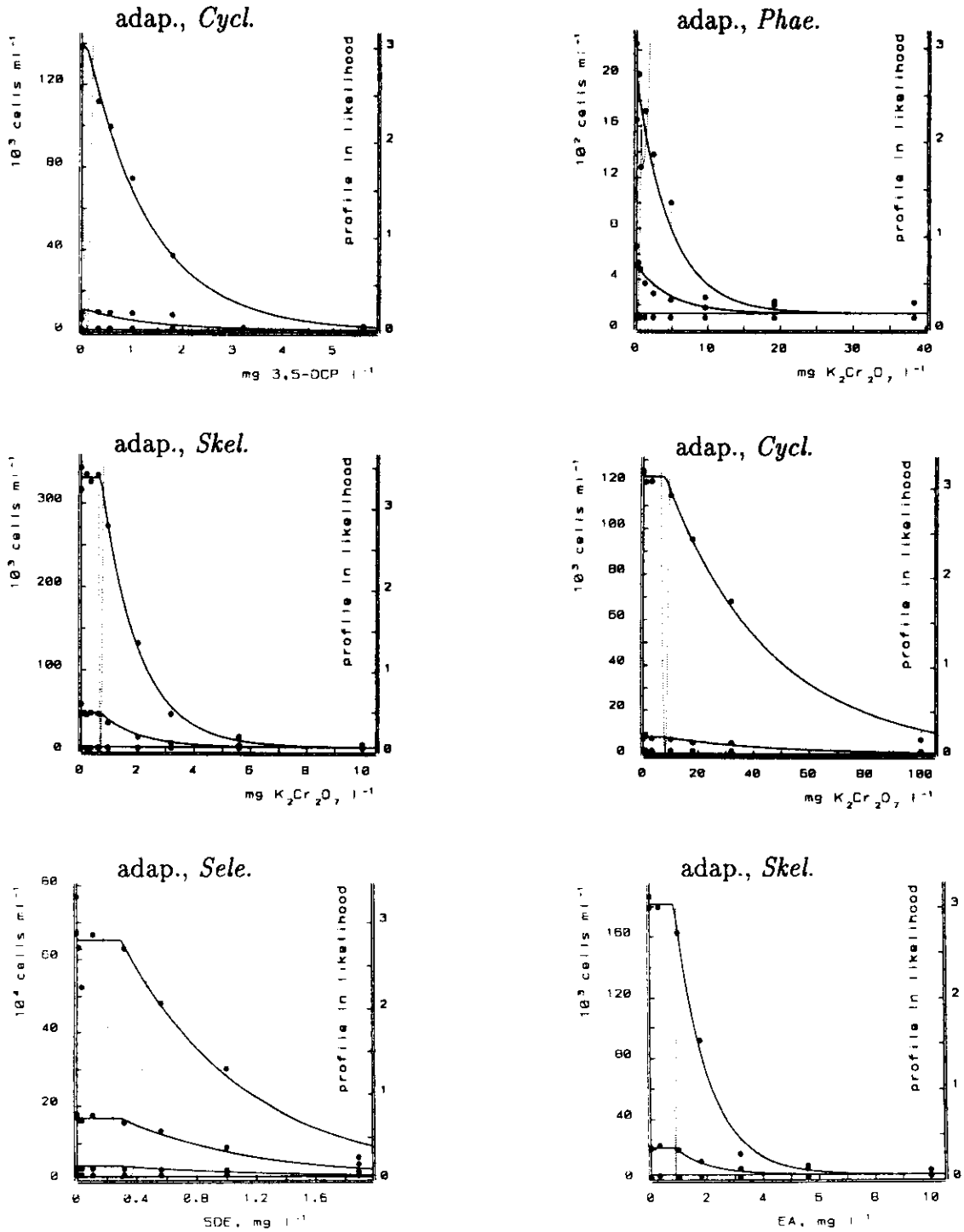

Fig. 1. The best fitting of the three models (the growth, adaptation or hazard model) is shown in these examples, together with the profile ln likelihood function for the no-effect concentration c_0. The various curves in each figure corresponds with an observation time. Observations at times at which the control deviates from exponential growth have not been included. The abbreviations for the compounds and algal species are given in the legends to Table 1.

stands for the estimated standard deviation, given the value for c_0, and $\hat{\sigma}_1$ stands for the estimated standard deviation given the maximum likelihood estimation of c_0. The estimated variance, i.e. the squared standard deviation, is $\hat{\sigma}^2 = \Sigma_{ij}^{-1} w_{ij}$ $\Sigma_{ij} w_{ij} (N_{ij} - N(t_i, c_j))^2$. The factor $\Sigma_{ij} w_{ij}$ stands for the sum of all weight coefficients, where the summation is over all time points and concentrations, including the control. The confidence set for c_0 can be obtained from the profile ln likelihood, where we use the property that two times the profile ln likelihood at any given value for c_0 under the null hypothesis that this is the correct value, as asymptotically χ^2 distributed with one degree of freedom. The α-level confidence set for c_0 is then given by $\{c_0 | l(c_0) \leqslant \chi_1^2(\alpha)/2\}$ where $\chi_1^2(\alpha)$ is a number such that $\int_0^{\chi_1^2(\alpha)} (\pi x)^{-1/2} \exp\{-x\}\, dx = \alpha$ (cf. Silvey, 1975; Kooijman, 1983).

EXAMPLES

Figure 1 shows the experimental results of 14 algal growth inhibition tests for a variety of compounds and algal species. The parameters of the three models are given in Table 1.

The 99% confidence interval for the no-effect concentration c_0 is approximately the point estimate plus and minus 2.56 times the standard deviation. So for the growth model of the test with TPBS we obtain a confidence interval for c_0 of $\{6.86, 8.77\}$ mg l^{-1} (see Table 1). A χ^2-distributed variable with one degree of freedom exceeds the value 6.635 with a probability of 1%. The 99% confidence interval can be read from Fig. 1 by looking at the values of c_0 for which the profile ln likelihood is below $6.635/2 = 3.317$. This gives a very similar confidence interval, i.e. $\{6.75, 8.7\}$ mg l^{-1}. This illustrates the applicability of the large sample theory for the likelihood ratio test: the shape of the ln likelihood function is in most cases perfectly parabolic. In two other cases, we see that the profile ln likelihood function has two local minima and, in the test with ethoxylated alcohol, we see an odd behaviour of this function because of the stimulatory effect of the compound at low concentrations, which is usually referred to as hormesis (Stebbing, 1982). This little understood phenomenon must be left unexplained.

In many cases the three models all fit well to the same data; they would be hard to tell apart graphically. The no-effect concentration proves to be very insensitive for the choice of model. (This is in contrast with EC-small values, see Introduction.) In a few cases the mean deviation $\hat{\sigma}$ differs by a factor of two between the models; here we can choose between the different modes of action of the compound on the basis of fit. The maximum deviation occurs in the test for potassium dichromate with *Cyclotella*. The fact that the c_0 differs here by a factor of three is of no problem because we should select the value of the best fitting model. The NEC differs significantly from zero

Table 1. Parameter estimates and standard deviations of the examples given in Fig. 1. The three rows for each compound/species combination correspond with the growth, hazard and adaptation model, respectively. The units of the parameters are: N_0, $\hat{\sigma}$: units of cells density (given for each example); $\hat{\mu}$: d^{-1}; c_0, c_G, c_H: units of compound concentration (given for each example); $\hat{k}_t$: (units of compound conc. × d)$^{-1}$ Compounds: 3,5-DCP = 3,5-dichlorophenol, TPBS = Tetrapropylenebenzene sulphonate, PEA = polyethylene amine, SDE = substituted diphenoxyethane, EA = ethoxylated alcohol, Mix 1,2 = mixture of organic N,S-compounds, Mix 3 = mixture of nonionic surfactants. Species: *Cycl.* = *Cyclotella operculata*, *Phae.* = *Phaeodactylum tricornutum*, *Skel* = *Skeletonema costatum*, *Nitz.* = *Nitzschia palea*, *Step.* = *Stepanodiscus hantzschii*, *Scen.* = *Scenedesmus subspicatus*, *Sele.* = *Selenastrum capricornutum*.

Comp/spec.	N_0	s.d.	$\hat{\mu}_0$	s.d.	c_0	s.d.	$c_G/\hat{k}_t/c_H$	s.d.	$\hat{\sigma}$
PEA, mg l^{-1}	1.04	0.273	1.93	0.095	0.0191	0.021	1.93	0.222	6.95
Skel., 10^3 cells ml^{-1}	1.10	0.212	1.91	0.070	0	0.020	0.943	0.062	5.32
	1.42	0.449	1.78	0.115	0.146	0.055	0.390	0.070	8.92
3,5-DCP, mg l^{-1}	0.766	0.352	2.88	0.267	0	0.091	3.75	0.880	4.49
Nitz., 10^3 cells ml^{-1}	0.768	0.393	2.86	0.298	0	0.102	0.731	0.137	5.17
	0.910	0.496	2.76	0.316	0	0.106	1.05	0.186	5.40
3,5-DCP, mg l^{-1}	0.646	0.312	2.89	0.261	0.167	0.050	5.12	0.798	4.66
Cycl., 10^3 cells ml^{-1}	0.824	0.239	2.76	0.157	0.113	0.043	0.947	0.036	3.29
	1.11	0.304	2.60	0.149	0.101	0.043	1.27	0.077	3.23
$K_2Cr_2O_7$, mg l^{-1}	1.10	0.238	1.44	0.123	0	0.355	11.7	2.85	1.50
Phae., 10^2 cells ml^{-1}	1.09	0.218	1.44	0.106	0	0.421	0.138	0.026	1.43
	1.29	0.232	1.35	0.094	0	0.423	5.45	0.914	1.37
$K_2Cr_2O_7$, mg l^{-1}	4.355	0.337	1.26	0.027	3.48	0.237	26.1	1.85	3.68
Phae., cell ml^{-1}	4.30	0.375	1.26	0.030	2.44	0.413	0.0418	$2.8\,10^{-3}$	4.25
	5.24	0.548	1.19	0.036	1.80	0.597	12.5	0.930	5.29
$K_2Cr_2O_7$, mg l^{-2}	5.66	0.685	2.10	0.063	0.777	0.031	3.46	0.307	7.21
Skel., 10^3 cells ml^{-1}	5.67	0.573	2.10	0.053	0.710	0.036	0.553	0.0311	6.17
	6.59	0.511	2.022	0.040	0.683	0.031	1.33	0.0547	4.90
$K_2Cr_2O_7$, $\mu g\,l^{-1}$	0.800	0.372	2.72	0.254	24.8	4.83	59.9	40.2	5.16
Cycl., 10^3 cells ml^{-1}	0.739	0.151	2.78	0.112	10.5	1.17	0.0200	$1.6\,10^{-3}$	2.23
	0.851	0.123	2.71	0.079	7.96	0.57	38.1	1.51	1.61
SDE, mg l^{-1}	0.980	0.195	1.43	0.069	0.410	0.055	2.13	0.435	3.21
Sele., 10^4 cells ml^{-1}	0.962	0.184	1.43	0.067	0.364	0.066	0.637	0.106	3.11
	1.057	0.199	1.40	0.065	0.295	0.040	0.823	0.092	3.09
Bioban, $\mu g\,l^{-1}$	0.336	0.087	2.72	0.140	16.1	1.43	20.3	25.0	1.46
Skel., 10^3 cells ml^{-1}	0.341	0.093	2.71	0.147	14.8	1.88	0.0132	$1.4\,10^{-3}$	1.55
	0.369	0.105	2.67	0.154	14.4	2.03	52.9	5.31	1.61
TPBS, mg l^{-1}	0.655	0.036	0.481	$8.6\,10^{-3}$	7.82	0.365	36.2	3.07	0.412
Step., 10^4 cells ml^{-1}	0.633	0.037	0.487	$9.3\,10^{-3}$	6.78	0.559	0.0123	$8.3\,10^{-4}$	0.447
	0.696	0.038	0.472	$8.5\,10^{-3}$	6.08	0.610	20.5	1.11	0.416
EA, mg l^{-1}	2.68	0.581	2.01	0.104	0.923	0.032	3.45	0.413	5.18
Skel., 10^3 cells ml^{-1}	2.88	0.403	1.97	0.067	0.895	0.029	0.566	0.0334	3.58
	3.46	0.427	1.88	0.059	0.886	0.029	1.16	0.0575	3.29
Mix 1, $\mu g\,l^{-1}$	0.494	0.147	2.62	0.157	53.2	0.87	56.7	15.2	2.17
Skel., 10^3 cells ml^{-1}	0.495	0.148	2.62	0.158	51.5	1.16	0.0346	$6.1\,10^{-3}$	2.21
	0.514	0.160	2.60	0.165	51.1	1.25	20.7	3.34	2.28
Mix 2, mg l^{-1}	1.59	0.131	2.07	0.042	0	$1.3\,10^{-3}$	0.363	0.018	1.35
Skel., 10^3 cells ml^{-1}	1.61	0.229	2.06	0.073	0	$2.8\,10^{-3}$	6.09	0.435	2.39
	1.78	0.308	2.01	0.088	0	$3.5\,10^{-3}$	0.115	9.41	2.88
Mix 3, mg l^{-1}	1.39	0.201	2.10	0.070	0.0228	$8.0\,10^{-3}$	1.39	0.101	2.51
Skel., 10^3 cells ml^{-1}	1.36	0.278	2.11	0.099	0.0075	0.012	1.508	0.109	3.58
	1.50	0.351	2.06	0.112	0	0.015	0.435	0.032	4.02

in 10 out of 14 cases. The cases where the NEC does not differ from zero indicate that the experiment should be repeated with an adjusted concentration series.

DISCUSSION

Our method shows that simple mechanistic models can be used successfully to describe the results of algae inhibition growth tests. It has less parameters than the standard analysis, which relates the population growth rate to the concentration of compound according to the log-logistic model (cf. Kooijman *et al.*, 1983). Independent from and inconsistent with this model, a no-observed effect concentration (NOEC) is usually identified for risk assessment purposes. This implies that four rather than three parameters are usually estimated: the control population growth rate, the EC50, the gradient parameter, and the NOEC. The proposed method replaces the EC50 plus gradient parameter by either the tolerance concentration or the killing rate, and the NOEC by the NEC. The latter is now a real parameter with a confidence interval, not just one of the tested concentrations.

Our method avoids the complexities inherent to NOEC and EC-"small" values. The parameter c_0 does not suffer from the statistical problems of the NOEC. It seems not to be very sensitive to error in the identification for the specific mode of action for the compound. This is of importance because routine toxicity tests are not very suitable for this purpose. It would help, for instance, to distinguish the living from the dead cells, but this requires extra effort. Our method also avoids the complexities that are inherent to small effect concentrations. Similar conclusions apply to other standard routine toxicity tests, such as the chronic reproduction *Daphnia* test. The combined evidence supports a rejection of the conventional NOEC/EC50 based analysis in favour of the NEC based analysis with specific effects to the various biological endpoints. The examples presented in this article have been analyzed earlier with the method described in Kooijman *et al.* (1983), supplemented with NOEC "estimates". Application of the new method shows that the estimated NECs correspond well with the NOECs. The control population growth rates tend to be slightly lower than estimated with use of a logistic growth model. This is partly because of the problem of detecting deviations from exponential growth, but also in the estimation of the carrying capacity of the logistic growth model.

An additional advantage of our mechanistic approach is that assumptions about the kinetics of the compounds that prove to be too simple can easily be replaced by more complex (and hopefully more appropriate) ones for scientific purposes. This obviously requires a more elaborate experimental setup. Being process-oriented, the analysis can be extended to include the effects of degradation and metabolic transformation. Such an effort is essential to evaluate the consequences of emissions in the environment. This consistency between models for risk assessment and for scientific purposes is essential if we take risk assessment seriously.

The observation that the three different models frequently fit well to the same data set, invites an attempt to covert the three toxicity measures (c_G, c_H, $\dot{k}_t$) into each other. We can do so by equating the EC50 for biomass at the moment at which the control population exceeds n times the inoculated value. The test requires that $n \geqslant 15$. Simple mathematics reveals the following relationships

$$\dot{k}_t c_G = (1 - x)\dot{\mu}_0\left(\frac{\ln n}{\ln 2} - 1\right) \text{ and}$$

$$\frac{c_G}{c_H} = \left(\frac{\ln n}{\ln 2} - 1\right)\ln\frac{n-1}{n/2-1}$$

where x is the solution of $x \ln n = \ln\{1 + x(n/2 - 1)\}$. For $n = 15$ this simplifies to $\dot{k}_t c_G = 1.233\dot{\mu}_0$ and $c_G = 2.86c_H$. This exercise also shows that the EC50 for biomass itself is totally useless to characterize the effects of compounds because this measure depends on the length of the test (choice for n) and on growth conditions (value for $\dot{\mu}_0$, which depends on media, light and temperature). For further discussion of these points see Nyholm (1985). Nonetheless, it is frequently used and current standard protocols prescribe that this figure be reported along with an EC50 for the population growth rate.

The software package DEBtox, as provided in Kooijman & Bedaux (1996), can be used to do all computations for the application of the models that are discussed in this article.

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REFERENCES

Bedaux, J. J. M. and Kooijman, S. A. L. M. (1994) Statistical analysis of bioassays, based on hazard modeling. *Environ. Ecol. Stat.* **1**, 303–314.

Bartlett, L., Rabe, F. W. and Funk, W. H. (1974) Effects of copper, zinc and cadmium on *Selenastrum capricornutum*. *Wat. Res.* **8**, 179–185.

Bringmann, G. and Kühn, R. (1980) Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication inhibition test. *Wat. Res.* **14**, 231–241.

EU 1992. EU Guideline No C.3. *Growth inhibition test with algae. Off. J. Eur. Comm.* L 383A, 179–186.

Hanstveit, A. O. 1982. *Evaluation of the results of the third ISO-interlaboratory study with an algal toxicity test.* ISO Document ISO/TC147/SC5/WG5/N64; TNO-Report CL82/128, TNO-Environmental Sciences, Delft.

Hanstveit, A. O. 1991. *The results of an international ring test of the marine algal growth inhibition test according to ISO/DP 10253.* TNO-Report R91/236. TNO-Environmental Sciences, Delft.

ISO 1989. International Standard, ISO 8692: 1989 (E). Water Quality—Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*, Genève.

ISO 1994. Draft International Standard, ISO/DIS 10253: Water quality—Marine algae growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*, Genève.

Kooijman, S. A. L. M. (1983) Statistical aspects of the determination of mortality rates in bioassays. *Wat. Res.* **17,** 749–759.

Kooijman, S. A. L. M. (1993) *Dynamic Energy Budgets in Biological Systems. Theory and applications in ecotoxcology.* Cambridge University Press, pp. 350.

Kooijman, S. A. L. M. and Bedaux, J. J. M. (1996) Some statistical properties of estimates of no-effect concentrations. Wat. Res. **30,** 1724–1728.

Kooijman, S. A. L. M. and Bedaux, J. J. M. (1996a) Analysis of toxicity tests on *Daphnia* survival and reproduction. *Wat. Res.* **30,** 1711–1723.

Kooijamn, S. A. L. M. and Beduaus, J. J. M. (1966b) Analysis of toxicity tests on fish growth. *Wat. Res.* **30,** 1633–1644.

Kooijman, S. A. L. M. and Bedaux, J. J. M. (Eds) (1996) *The analysis of aquatic toxicity data.* Amsterdam: VU University Press.

Kooijman, S. A. L. M., Hanstveit, A. O. and Oldersma, H. (1983) Parametric analyses of population growth in bioassays. *Wat. Res.* **17,** 727–738.

McCullagh, P. and Nelder, J. A. (1989) *Generalized linear models.* Monographs on Statistics and Applied Probability 37. Chapman & Hall, pp. 511.

Nyholm, N. 1985. Response variable in algal growth inhibition tests—Biomass or growth rate? *Wat. Res.* **19,** 273–279.

OECD 1984. OECD Guideline for Testing of Chemicals 201: *Alga, growth inhibition test.*, Paris.

Silvey, S. D. (1975) *Statistical inference.* Monographs on Statistics and Applied Probability 7. Chapman and Hall, pp. 192.

Stebbing, A. R. D. (1982) Hormesis—the stimulation of growth by low levels of inhibitors. *Sci. Total Environ.* **22,** 213–234.