Wat. Res. Vol. 25, No. 10, pp. 1237–1242, 1991
Printed in Great Britain.

0043-1354/91 $3.00 + 0.00

ESTIMATION OF ECOTOXICOLOGICAL PROTECTION LEVELS FROM NOEC TOXICITY DATA

CONNIE WAGNER[1] and HANS LØKKE[2]*

[1]Laboratory of Environmental Sciences and Ecology, Technical University of Denmark, Building 224, DK-2800 Lyngby and [2]Ministry of the Environment, National Environmental Research Institute, Division of Terrestrial Ecology, Vejlsøvej 25, DK-8600 Silkeborg, Denmark

(First received August 1990; accepted in revised form March 1991)

Abstract—An extrapolation method is presented for the evaluation of the effect of toxic compounds on all species in a community from single species tests on selected species representing the community. The method assumes log-normal distribution of the toxicity data. By extrapolation, a lower statistical tolerance limit is determined so that one can assert with a certain probability that only a certain percentage of all the species in the community are influenced. The method was tested on ecotoxicological data for 11 aquatic species, and it has been compared with two existing methods when used for a low number of species. The first implies unwarranted assumptions and resulted in considerably higher critical concentrations than the present method when used for a low number of species. The second method applies log-logistic confidence limits and yielded critical concentrations which were in agreement with the presented method.

Key words—ecotoxicology, extrapolation, tolerance limit, NOEC, safety factor, log-logistic distribution, log-normal distribution

NOMENCLATURE

α = location parameter of the log-logistic distribution (ln ML^{-3})
β = scatter parameter of the log-logistic distribution (ln ML^{-3})
$\delta = 1 - [\text{degree of confidence}]$
$\delta_1 = P\{\ln \text{NOEC} \leqslant \ln HC_p\}$
$\delta_2 = P\{Z \leqslant \ln HC_p\}$
μ = mean of the normal distribution (ln ML^{-3})
$\hat{\mu}$ = estimate of μ (ln ML^{-3})
σ^2 = variance of the normal distribution (ln $ML^{-3})^2$
σ^2 = estimate of σ^2 (ln $ML^{-3})^2$
C_n = coefficient with value $\ln[(1-\delta_1)^{1/n}/(1-(1-\delta_1))^{1/n}]$
d_m = value such that $P\{S_m > d_m\} = \delta_2$
EC_{50} = effect concentration for 50% of the organisms (ML^{-3})
e_m = left confidence limit. Factor for a fractile in the logistic distribution
$f_m = \sqrt{3}/\pi \cdot e_m \cdot C_1$
HC_p = hazardous concentration for p% of the species (method of Van Straalen and Denneman) (ML^{-3})
HCS = hazardous concentration for the most sensitive of n species in a community (method of Kooijman) (ML^{-3})
k = one-sided tolerance limit factor for a normal distribution
K_p = hazardous concentration for sensitive species in the log-normal distribution model (ML^{-3})
k_p = ln K_p fractile in the standardized normal distribution for ln NOEC (ln ML^{-3})
L = left confidence limit for a fractile of the log-logistic distribution
LC_{50} = lethal concentration for 50% of the organisms (ML^{-3})
m = number of test species (toxicity data)
n = number of community or of ecosystem species
NOEC = no observed effect concentration (ML^{-3})
$p = P\{\ln \text{NOEC} \leqslant \ln K_p\}$
s_m = sample standard deviation of m ln NOEC values (ln ML^{-3})
T = application factor between K_p and $\exp(\bar{x}_m)$
u_p = fractile of the standardized normal distribution
$\bar{x}_m$ = mean of m ln NOECs (ln ML^{-3})
z = estimate for k_p (ln ML^{-3})
Z = the distribution for the estimate for HC_p

INTRODUCTION

An important objective of laboratory testing of the effects of chemical compounds or of complex mixtures of compounds is to assess the impact on natural ecosystems and to calculate risk factors. A simple ecotoxicological risk analysis can be performed by the extrapolation of acute or chronic toxicity data for various test species by regression techniques (Suter *et al.*, 1985; Blanck, 1984; Slooff *et al.*, 1986).

Alternatively, laboratory-to-field extrapolation procedures may use distribution models for sets of acute or chronic toxicity test data (Kooijman, 1987; Van Straalen and Denneman, 1989). In these methods it is assumed that LC_{50} or NOEC values for single test species and for all species in a community are stochastically independent variables with the same log-logistic distribution. Normally, the parameters are not known and they should be estimated. Then, it is not possible to determine, e.g. the confidence limits for the parameters by analytical measures. Therefore, computer simulated correction factors are introduced in the models. Okkerman *et al.*

*Author to whom all correspondence should be addressed.

(1989) compared the method of Van Straalen and Denneman (1989) with the method developed by the EPA (1984) and that proposed by Stephan *et al.* (1985). They found that the latter two were less reliable. The method of the EPA has insufficient scientific basis and the method of Stephan *et al.* uses the triangular distribution implying the assumption that there is a threshold value below which effects will not occur.

However, the log-logistic distribution is very similar to the log-normal distribution. From graphs of the two distributions it can be shown that they are almost identical when choosing the right parameters. Theoretically, the best distribution function should be chosen to fit the data. The normal distribution has for many decades held a central position in statistics. It represents many possibilities for extension of the extrapolation model compared to the logistic model which is used mainly to represent growth curves. The possibility of a simple analytical solution renders the logistic distribution attractive. However, the parameters need estimation just as for the normal distribution. Based on these considerations the authors prefer the log-normal distribution in the extrapolation method and outline an extrapolation method which uses the normal distribution instead of the logistic distribution.

EXTRAPOLATION METHOD BASED ON TOLERANCE LIMITS

As in the models of Kooijman (1987) and Van Straalen and Denneman (1989) we consider a sample of species which is selected from a community, e.g. all animals in an aquatic ecosystem. It is important that the species are representative for the community. For these test species the toxicity of a compound or a mixture of compounds is determined by laboratory methods by use of a defined endpoint. We wish to emphasize that in practice, rather than by a random procedure species are selected for which toxicity data are already available. Besides, the endpoints may vary. Therefore, it is questionable whether a sample of species is independent or represents a natural community without serious bias. However, in the following the NOEC (no observed effect concentration) is used for the calculations assuming a defined endpoint for toxicity. It is assumed that the NOEC values are stochastically independent variables with the same distribution for the single test species as for all community species. The distribution is assumed to be logarithmic normal, i.e. the natural logarithm of NOEC (ln NOEC) is normally distributed with parameters μ and σ^2. The base 10 logarithm can just as well be used.

A hazardous concentration K_p for sensitive species is defined in such a way that NOEC for $(1-p)\cdot 100\%$ of all species in a community is greater than K_p. According to the definition,

$$P\{\ln \text{ NOEC} \leqslant \ln K_p\} = p. \tag{1}$$

Hence, the fractile u_p of the standardized normal distribution is equal to $(\ln K_p - \mu)/\sigma$, where μ and σ^2 are parameters in the distribution for ln NOEC. Let $k_p = \ln K_p$, then

$$k_p = \mu + \sigma \cdot u_p, \tag{2}$$

where k_p is a fractile in the distribution for ln NOEC and u_p is found in statistical tables. K_p then becomes

$$K_p = \exp(\mu + \sigma \cdot u_p). \tag{3}$$

Usually, only the estimates for the parameters are known. They can be found from the following equations where m is the number of test species

$$\hat{\mu} = \bar{x}_m = \sum_{i=1}^{m} x_i/m \tag{4}$$

$$\hat{\sigma}^2 = s_m^2 = \sum_{i=1}^{m} (x_i - \bar{x}_m)^2/(m-1). \tag{5}$$

If μ and σ in equation (2) are substituted by their estimates, the estimate z for k_p is found

$$z = \bar{x}_m + s_m \cdot u_p. \tag{6}$$

In repeated trials z will vary around the fractile k_p and for some trials the estimates will include less than 95% of the species and for other trials more than 95%. When an estimate z is found one would like to know the distribution for the estimates or at least to know the variance for the distribution. For parameters in a distribution the problems are solved by finding a confidence interval, in which the unknown parameter is included with a certain probability. For a fractile in a normal distribution the problem can be solved by finding the statistical tolerance limits. Tolerance limits are often used in statistical quality control. With a lower statistical tolerance limit a concentration can be determined in such a way that with confidence $(1-\delta)$ no more than $p\cdot 100\%$ of all species in a community have a NOEC value smaller than this concentration.

To be reasonably sure that no more than $p\cdot 100\%$ of all species have a NOEC value smaller than K_p, a value k is determined in such a way that with confidence $(1-\delta)$ the concentration $\exp(\bar{x}_m - s_m \cdot k)$ excludes no more than $p\cdot 100\%$ of all species. k can be determined in 3 ways:

(1) exact, resulting in a non-central t-distribution
(2) by using likelihood-theory
(3) approximative, assuming that $(\bar{x}_m - s_m \cdot k)$ is normally distributed.

In the following k is determined by the first method in according with Johnson and Kotz (1970). The fraction of the species having a NOEC value smaller than $\exp(\bar{x}_m - s_m \cdot k)$ is $\leqslant p$ if

$$(\bar{x}_m - s_m \cdot k - \mu)/\sigma \leqslant u_p, \tag{7}$$

where u_p is a fractile in the standardized normal distribution. Equation (7) is defined to be valid with confidence $(1-\delta)$. The parameter k is obtained by

$$k = (1/\sqrt{m}) \cdot t_{1-\delta}(m-1, \sqrt{m}\cdot u_p), \tag{8}$$

where $t_{1-\delta}(m-1, -\sqrt{m}\cdot u_p)$ is a non-central t-distribution with $(m-1)$ degrees of freedom and non-centrality parameter $-\sqrt{m}\cdot u_p$. K_p is redefined by

$$K_p = \exp(\bar{x}_m - s_m \cdot k) = \exp(\bar{x}_m)/T \tag{9}$$

where

$$T = \exp(s_m \cdot k) \tag{10}$$

can be regarded as a safety factor with which the geometric mean of the measured NOEC values should be divided.

When $m \to \infty$ then $k \to -u_p$ and $K_p \to \exp(\mu + \sigma \cdot u_p)$. Therefore, equation (3) is included in equation (9) and consequently, the original definition of K_p is included in the new definition of K_p. The lower statistic tolerance limit K_p is determined so that with the probability $(1-\delta)$ no more than $p\cdot 100\%$ of all species in a community have a NOEC value smaller than K_p.

The non-central t-distribution is given by e.g. Owen (1962). In Table 1 the factor k is calculated for several values of p, δ and m. As a special case the factor k for $p = 0.5$ and $\delta = 0.5$ is estimated by the Student t-distribution. This case is consistent with the method used by EPA (EPA, 1984; Stephan *et al.*, 1985).

Table 1. The factor k is given in equation (8) calculated by the exact method by means of a non-central t-distribution, k is calculated for several values of p, δ and m (Owen, 1962)

	Values of k for $1-\delta = 0.90$ $1-p$						Values of k for $1-\delta = 0.95$ $1-p$				
m	0.900	0.950	0.975	0.990	0.999	m	0.900	0.950	0.975	0.990	0.999
2	10.253	13.090	15.586	18.500	24.582	2	20.581	26.206	31.257	37.094	49.276
3	4.258	5.311	6.244	7.340	9.651	3	6.155	7.656	8.986	10.553	13.857
4	3.188	3.957	4.637	5.438	7.129	4	4.162	5.144	6.015	7.042	9.214
5	2.744	3.401	3.983	4.668	6.113	5	3.413	4.210	4.916	5.749	7.509
6	2.494	3.093	3.621	4.243	5.556	6	3.008	3.711	4.332	5.065	6.614
7	2.333	2.893	3.389	3.972	5.201	7	2.756	3.401	3.971	4.643	6.064
8	2.219	2.754	3.227	3.783	4.955	8	2.582	3.188	3.724	4.355	5.689
9	2.133	2.650	3.106	3.641	4.771	9	2.454	3.032	3.543	4.144	5.414
10	2.066	2.568	3.011	3.532	4.628	10	2.355	2.911	3.403	3.981	5.204
11	2.012	2.503	2.936	3.444	4.515	11	2.275	2.815	3.291	3.852	5.036
12	1.966	2.448	2.872	3.371	4.420	12	2.210	2.736	3.201	3.747	4.900
13	1.928	2.403	2.820	3.310	4.341	13	2.155	2.670	3.125	3.659	4.787
14	1.895	2.363	2.774	3.257	4.274	14	2.108	2.614	3.060	3.585	4.690
15	1.866	2.329	2.735	3.212	4.215	15	2.068	2.566	3.005	3.520	4.607
16	1.842	2.299	2.700	3.172	4.164	16	2.032	2.523	2.956	3.463	4.534
17	1.819	2.272	2.670	3.137	4.118	17	2.002	2.486	2.913	3.414	4.471
18	1.800	2.249	2.643	3.106	4.078	18	1.974	2.453	2.875	3.370	4.415
19	1.781	2.228	2.618	3.078	4.041	19	1.949	2.423	2.840	3.331	4.364
20	1.765	2.208	2.597	3.052	4.009	20	1.926	2.396	2.809	3.295	4.319
21	1.750	2.190	2.575	3.028	3.979	21	1.905	2.371	2.781	3.262	4.276
22	1.736	2.174	2.557	3.007	3.952	22	1.887	2.350	2.756	3.233	4.238
23	1.724	2.159	2.540	2.987	3.927	23	1.869	2.329	2.732	3.206	4.204
24	1.712	2.145	2.525	2.696	3.904	24	1.853	2.309	2.711	3.181	4.171
25	1.702	2.132	2.510	2.952	3.882	25	1.838	2.292	2.691	3.158	4.143
30	1.657	2.080	2.450	2.884	3.794	30	1.778	2.220	2.608	3.064	4.022
35	1.623	2.041	2.406	2.833	3.730	35	1.732	2.166	2.548	2.994	3.934
40	1.598	2.010	2.371	2.793	3.679	40	1.697	2.126	2.501	2.941	3.866
45	1.577	1.986	2.344	2.762	3.638	45	1.669	2.092	2.463	2.897	3.811
50	1.560	1.965	2.320	2.735	3.604	50	1.646	2.065	2.432	2.863	3.766
60	1.532	1.933	2.284	2.694	3.552	60	1.609	2.022	2.384	2.807	3.695
70	1.511	1.909	2.257	2.663	3.513	70	1.581	1.990	2.348	2.766	3.643
80	1.495	1.890	2.235	2.638	3.482	80	1.560	1.965	2.319	2.733	3.601
90	1.481	1.874	2.217	2.618	3.456	90	1.542	1.944	2.295	2.706	3.567
100	1.470	1.861	2.203	2.601	3.435	100	1.527	1.927	2.276	2.684	3.539
120	1.452	1.841	2.179	2.574	3.402	120	1.503	1.899	2.245	2.649	3.495
145	1.436	1.821	2.158	2.550	3.371	145	1.481	1.874	2.217	2.617	3.455
300	1.386	1.765	2.094	2.477	3.280	300	1.417	1.800	2.133	2.522	3.335
500	1.362	1.736	2.062	2.442	3.235	500	1.385	1.763	2.092	2.475	3.277
∞	1.282	1.645	1.960	2.326	3.090	∞	1.282	1.645	1.960	2.326	3.090

ASSESSMENT OF TWO EXISTING EXTRAPOLATION METHODS

The method of Van Straalen and Denneman

The method of Kooijman (1987) defines a hazardous concentration HCS for the most sensitive of n species in a community. On the basis of this method Van Straalen and Denneman (1989) have defined a concentration HC_p in such a way that $\delta_1 \cdot 100\%$ of all species in a community have a NOEC value smaller than HC_p:

$$P\{\ln \text{NOEC} \leqslant \ln HC_p\} = [1 + \exp(\alpha - \ln HC_p)/\beta]^{-1} = \delta_1. \quad (11)$$

This corresponds to equation (1) which determines a fractile of the distribution for ln NOEC values. If α and β are known then HC_p can be calculated from equation (11) for various values of δ_1. Normally, HC_p is calculated from the estimates of α and β. The distribution of the estimate for HC_p is called Z and HC_p is redefined by

$$P\{Z \leqslant \ln HC_p\} = \delta_2. \quad (12)$$

In equation (12) a fractile of the distribution for Z is determined in a similar way as for ln NOEC in equation (11). Since the parameters and even the type of distribution are unknown it is not possible to determine HC_p. However, by computer simulations of the distribution for Z, Kooijman (1987) determines a factor d_m and obtains

$$\ln \text{HCS} = \bar{x}_m - 3 \cdot s_m \cdot d_m \cdot C_n/\pi^2, \quad (13)$$

where

$$C_n = \ln[(1-\delta_1)^{1/n}/(1-(1-\delta_1))^{1/n}], \quad (14)$$

and m is the number of test species, and $\bar{x}_m$ and s_m are the mean and the standard deviation of the ln NOEC values, respectively. Equation (11) is identical with the method of Kooijman for $n = 1$. Consequently, the method of Van Straalen and Denneman is included in the method of Kooijman. The only difference is that Kooijman used LC_{50} values and Van Straalen and Denneman used NOEC values.

The method of Aldenberg et al.

Aldenberg *et al.* (1991) adopted the same basic assumptions for prediction of toxicological protection levels as in Kooijman (1987). They calculated by computer simulation a new factor e_m instead of d_m which was used for the calculation of a concentration L, which they called a left confidence limit. However, according to the definition e_m corresponds to a lower statistic tolerance limit:

$$L = \bar{x}_m - f_m \cdot s_m, \tag{15}$$

where

$$f_m = \sqrt{3}/\pi \cdot e_m \cdot C_1. \tag{16}$$

The value of f_m is given in Aldenberg *et al.* (1991) and the factor is comparable with the factor k for a lower tolerance limit given in Table 1. It is seen that f_m is slightly greater than k. Thus, 10^L which is comparable with the lower tolerance limit K_p is slightly less than K_p.

COMPARISON OF THREE EXTRAPOLATION METHODS

The present method is compared with the methods of Van Straalen and Denneman (1989) and of Aldenberg *et al.* (1991). The data set from Slooff and Canton (1983) given by Okkerman *et al.* (1989) was selected because it has been used for method comparisons by the cited authors. The data set consists of NOEC values which we prefer rather than lethality data for the risk assessment of ecosystems. However, we are aware that small sets of NOEC data may be heterogenous and may not fulfill the ideal criteria on log-normal distribution. Eight chemical compounds were included, potassium bichromate, sodium bromide, tetrapropylene benzene sulphonate, 2,4-dichloroaniline, 4-nitrotoluene, 2,4-dinitro-*o*-cresol, dimethoate, and pentachlorophenol. Eleven different species were used, bacteria (*Pseudomonas fluorescens* and *Microcystis aeruginosa*), algae (*Scenedesmus pannonicus*), plants (*Lemna minor*), crustaceans (*Daphnia magna*), insects (*Culex pipiens*), hydrozoans (*Hydra oligactis*), molluscs (*Lymnaea stagnalis*), fish (*Poecilia reticulata* and *Oryzias latipes*), and amphibians (*Xenopus laevis*). The endpoints were survival, growth, and reproduction. It may be expected that a small data set of NOEC values of the organophosphorus insecticide dimethoate does not fit the log-normal distribution due to the specific acetylcholinesterase inhibition of this compound.

The lower statistic tolerance limit K_p is calculated for protection of 95% of the species ($p = 0.05$), and $\delta = 0.05$ and 0.10, respectively, by the exact method, i.e. by use of equation (9) and Table 1. For $\delta = 0.15$, K_p has been calculated for $p = 0.05$ by an approximative method (Hald, 1952). The results are shown in Table 2.

In Table 3 the HC_p-values from Okkerman *et al.* (1989, Table IV) are given for $\delta_1 = 0.05$ and $\delta_2 = 0.05$. The value 10^L according to Aldenberg *et al.* (1991) is shown for these values of δ_1 and δ_2.

In Tables 2 and 3 K_p and HC_p have also been calculated under the assumption that $\mu = \bar{x}_m$ and $\sigma^2 = s_m$ for 11 species. K_p is calculated from equation (3) and HC_p from equation (11) for $\alpha = \mu$ and $\beta = \sigma \cdot \sqrt{3}/\pi$ (Van Straalen and Denneman, 1989). In this way the fractile K_p in the log-normal distribution can be compared to the fractile HC_p in the log-logistic distribution. The fractile HC_p is equal to the fractile 10^L, because they have the same distribution. The difference between K_p and HC_p is very small in this case. The reason is that the log-normal and the log-logistic distributions are almost identical. Thus, one would expect to get almost the same results when only the estimates for μ and σ^2 are known. Since the HC_p values are slightly greater than the K_p values when μ and σ^2 are known, it is to be expected that

Table 2. K_p calculated by the new method for different numbers of test species (m) for $p = 0.05$, meaning protection of 95% of all species in a community or ecosystem. K_p is calculated for $\delta = 0.05$ and 0.10 by the exact method, and for $\delta = 0.15$ by the approximative method

	$K_2Cr_2O_7$	NaBr	TPBS	2,4-DCA	4-NT	DNOC	DMTH	PCP
m	$p = 0.05$, $\delta = 0.05$							
3	7.5×10^{-9}	4.8×10^{-8}	1.3×10^{-2}	7.1×10^{-9}	8.2×10^{-5}	2.2×10^{-8}	1.7×10^{-14}	4.4×10^{-4}
5	2.8×10^{-4}	3.4×10^{-3}	8.1×10^{-3}	7.3×10^{-5}	6.4×10^{-3}	1.3×10^{-5}	1.9×10^{-6}	2.2×10^{-6}
7	3.3×10^{-3}	3.7×10^{-2}	1.4×10^{-2}	1.8×10^{-3}	3.0×10^{-2}	4.1×10^{-4}	1.0×10^{-4}	5.0×10^{-5}
9	1.1×10^{-2}	1.6×10^{-1}	3.7×10^{-2}	5.6×10^{-3}	7.1×10^{-2}	1.6×10^{-3}	5.9×10^{-4}	1.5×10^{-4}
11	1.3×10^{-2}	2.9×10^{-1}	5.2×10^{-2}	1.0×10^{-2}	1.2×10^{-1}	3.7×10^{-3}	5.7×10^{-4}	4.5×10^{-4}
m	$p = 0.05$, $\delta = 0.10$							
3	2.0×10^{-6}	4.4×10^{-5}	6.1×10^{-2}	1.6×10^{-6}	1.8×10^{-3}	4.9×10^{-6}	2.8×10^{-10}	2.1×10^{-3}
5	1.3×10^{-3}	2.4×10^{-2}	2.3×10^{-2}	4.3×10^{-4}	1.8×10^{-2}	1.1×10^{-4}	2.5×10^{-5}	1.6×10^{-5}
7	7.3×10^{-3}	1.4×10^{-1}	3.1×10^{-2}	4.6×10^{-3}	5.7×10^{-2}	1.3×10^{-3}	4.8×10^{-4}	1.7×10^{-4}
9	1.8×10^{-2}	3.9×10^{-1}	6.3×10^{-2}	1.1×10^{-2}	1.1×10^{-1}	3.5×10^{-3}	1.8×10^{-3}	3.5×10^{-4}
11	2.1×10^{-2}	6.2×10^{-1}	8.3×10^{-2}	1.7×10^{-2}	1.8×10^{-1}	6.8×10^{-3}	1.5×10^{-3}	8.5×10^{-4}
m	$p = 0.05$, $\delta = 0.15$							
5	4.4×10^{-3}	1.2×10^{-1}	5.4×10^{-2}	1.8×10^{-3}	4.3×10^{-2}	5.8×10^{-4}	1.9×10^{-4}	8.2×10^{-5}
7	1.4×10^{-2}	4.0×10^{-1}	5.7×10^{-2}	9.5×10^{-3}	9.5×10^{-2}	3.2×10^{-3}	1.7×10^{-3}	4.4×10^{-4}
9	2.9×10^{-2}	8.3×10^{-1}	9.6×10^{-2}	1.8×10^{-2}	1.6×10^{-1}	6.4×10^{-3}	4.4×10^{-3}	6.8×10^{-4}
11	3.1×10^{-2}	1.1	1.2×10^{-1}	2.6×10^{-2}	2.4×10^{-1}	1.1×10^{-2}	3.4×10^{-3}	1.4×10^{-3}
m	$p = 0.05$							
∞	8.0×10^{-2}	4.9	3.0×10^{-1}	7.3×10^{-2}	4.9×10^{-1}	3.6×10^{-2}	2.3×10^{-2}	5.0×10^{-3}

Table 3. Comparison of HC_p calculated according to Van Straalen and Denneman (1989), 10^L according to Aldenberg *et al.* (1991) and K_p calculated by the present method for $p = \delta_1 = 0.05$, and $\delta = \delta_2 = 0.05$ and different numbers of test species (m)

	m	$K_2Cr_2O_7$	NaBr	TPBS	2,4-DCA	4-NT	DNOC	DMTH	PCP
HC_p	3	4.7×10^{-4}	3.2×10^{-2}	2.8×10^{-1}	2.9×10^{-4}	3.8×10^{-2}	9.8×10^{-4}	3.4×10^{-6}	9.2×10^{-3}
10^L	3	3.5×10^{-9}	1.9×10^{-8}	1.0×10^{-2}	3.4×10^{-9}	5.4×10^{-5}	1.1×10^{-8}	4.5×10^{-15}	3.6×10^{-4}
K_p	3	7.5×10^{-9}	4.8×10^{-8}	1.3×10^{-2}	7.1×10^{-9}	8.2×10^{-5}	2.2×10^{-8}	1.7×10^{-14}	4.4×10^{-4}
HC_p	5	4.5×10^{-3}	1.2×10^{-1}	5.6×10^{-2}	1.9×10^{-3}	4.4×10^{-2}	6.1×10^{-4}	2.0×10^{-4}	8.6×10^{-5}
10^L	5	1.6×10^{-4}	1.6×10^{-3}	5.4×10^{-3}	3.7×10^{-5}	4.3×10^{-3}	5.8×10^{-6}	7.2×10^{-7}	1.0×10^{-6}
K_p	5	2.8×10^{-4}	3.4×10^{-3}	8.1×10^{-3}	7.3×10^{-5}	6.4×10^{-3}	1.3×10^{-5}	1.9×10^{-6}	2.2×10^{-6}
HC_p	7	1.3×10^{-2}	3.7×10^{-1}	5.4×10^{-2}	8.9×10^{-3}	9.1×10^{-2}	3.0×10^{-3}	1.5×10^{-3}	4.0×10^{-4}
10^L	7	2.1×10^{-3}	1.8×10^{-2}	9.2×10^{-3}	1.1×10^{-3}	2.1×10^{-2}	2.3×10^{-4}	4.5×10^{-5}	2.6×10^{-5}
K_p	7	3.3×10^{-3}	3.7×10^{-2}	1.4×10^{-2}	1.8×10^{-3}	3.0×10^{-2}	4.1×10^{-4}	1.0×10^{-4}	5.0×10^{-5}
HC_p	9	2.8×10^{-2}	7.7×10^{-1}	9.2×10^{-2}	1.7×10^{-2}	1.6×10^{-1}	6.1×10^{-3}	4.1×10^{-3}	6.4×10^{-4}
10^L	9	8.6×10^{-3}	1.1×10^{-1}	3.1×10^{-2}	4.4×10^{-3}	6.0×10^{-2}	1.2×10^{-3}	3.9×10^{-4}	1.1×10^{-4}
K_p	9	1.1×10^{-2}	1.6×10^{-1}	3.7×10^{-2}	5.6×10^{-3}	7.1×10^{-2}	1.6×10^{-3}	5.9×10^{-4}	1.5×10^{-4}
HC_p	11	2.9×10^{-2}	1.0	1.1×10^{-1}	2.5×10^{-2}	2.3×10^{-1}	1.0×10^{-2}	3.0×10^{-3}	1.2×10^{-3}
10^L	11	1.0×10^{-2}	2.0×10^{-1}	4.1×10^{-2}	8.0×10^{-3}	1.0×10^{-1}	2.7×10^{-3}	3.5×10^{-4}	3.2×10^{-4}
K_p	11	1.3×10^{-2}	2.9×10^{-1}	5.2×10^{-2}	1.0×10^{-2}	1.2×10^{-1}	3.7×10^{-3}	5.7×10^{-4}	4.5×10^{-4}
	m	$p = \delta_1 = 0.05$							
HC_p	∞	8.2×10^{-2}	5.2	3.1×10^{-1}	7.5×10^{-2}	5.0×10^{-1}	3.8×10^{-2}	2.5×10^{-2}	5.3×10^{-3}
10^L	∞	8.2×10^{-2}	4.9	3.1×10^{-1}	7.5×10^{-2}	5.0×10^{-1}	3.8×10^{-2}	3.5×10^{-2}	5.3×10^{-3}
K_p	∞	8.0×10^{-2}	4.9	3.0×10^{-1}	7.3×10^{-2}	4.9×10^{-1}	3.6×10^{-2}	2.3×10^{-2}	5.0×10^{-3}

the 10^L values are slightly greater than the corresponding K_p values when only the estimates are known. Actually, the opposite is found.

From Table 3 it is seen that the HC_p values calculated by means of Kooijman's factor d_m for $\delta_2 = 0.05$ correspond to the K_p values for $\delta = 0.15$. The 10^L values for $\delta_2 = 0.05$ are smaller than the K_p values for $\delta = 0.05$. The general trend is that K_p, HC_p and 10^L are increasing when the number of test species is increasing. The calculations based on three species only, give scattered results. For six of eight compounds the values of K_p, HC_p and 10^L based on three test species follow the general trend. For two of the compounds (tetrapropylene benzene sulphonate and pentachlorophenol) it is the contrary for both K_p, HC_p and 10^L. Therefore, it is recommended at least five test species be used to be reasonably sure of determining limits which are not too high.

DISCUSSION

The three described extrapolation methods are all very sensitive to variations in the data for the different test species. If the species represent a small part of the distribution curves only, the variance between species is underestimated, and the protection concentration will be too high. Therefore, there is a need for procedures testing if the selected test species represent the ecosystem.

The method of Van Straalen and Denneman (1989) resulted in higher protection concentrations (HC_p) than the other methods. The explanation is found by looking at the quite different criteria proposed by the authors. The number of tested species (m) and the probability (δ_2) of obtaining too high estimates for HCS or HC_p, control the correction factor d_m of the methods of Kooijman (1987) and of Van Straalen and Denneman (1989), respectively. No interpretation of the calculation is given by Kooijman who just states that HCS is determined for fixed values of m, n, δ_1 and δ_2. In fact, Kooijman determines a concentration which is smaller than the estimate for the fractile HCS with confidence $(1 - \delta_2)$. We wish to know something about the fractile HCS, but nothing is really said about it. The estimate for HCS is greater than the fractile HCS in roughly 50% of all events.

Since the method of Kooijman includes that of Van Straalen and Denneman it is expected that the factor d_m could be used in both methods. But by the derivation of d_m it is assumed, that C_n is $\geqslant 10$, that means very far to the left on the log-logistic distribution curve. Therefore, the HCS values are much lower than the HC_p values. In Van Straalen and Denneman (1989) very low values for C_n are used, e.g. if $\delta_1 = 0.05$ then $C_n = C_1 = 2.94$.

The factor d_m in Kooijman (1987) is only dependent on m and δ_2. Compared with a lower tolerance limit it can be seen from equation (8) that the factor k is dependent of m, p and δ. This corresponds to the fact that d_m depends on three factors, m, δ_1 and δ_2. Aldenberg *et al.* (1991) determine e_m for one value of δ_1 and different values of δ_2. Thus, e_m depends on three factors, m, δ_1 and δ_2, as well. Calculations suggest that the d_m values are too low when used in the method of Van Straalen and Denneman, and that d_m for $C_n < 10$ depends on m, δ_1 and δ_2. The result is that HC_p is estimated too high when d_m is used. This is confirmed by Aldenberg *et al.* (1991) who found that in the method of Van Straalen and Denneman (1989) the value of HC_p is overestimated when using Kooijman's factor d_m.

The present method deals with these difficulties by using the log-normal distribution and tolerance limits. Aldenberg *et al.* (1991) have solved the problems by proposing a correction factor e_m which was calibrated based on randomly generated standard logistic data. The resulting confidence limits of Aldenberg *et al.* are smaller, but within a factor of two from the tolerance limits calculated by the present method at the same level of significance. The exponential expressions used in both methods may create the observed differences because of small

differences in the k factors, respectively e_m. Thus, the method of Aldenberg *et al.* (1991) based on the log-logistic distribution is apparently in excellent agreement with the present method based on the log-normal distribution.

The three described extrapolation methods all deal with the effects of chemical compounds on large groups of species in ecosystems. However, the models do not account for other aspects such as the interaction between species or the modifying influence of the non-biological components of the environment on the exposure and on the biological effect. Furthermore, the toxicological endpoints used for calculations are heterogenous, e.g. lethality, growth rate and hatching. Neither, is it justified to use large taxonomic distances ranging from bacteria and plants to insects and fishes. A large variation of endpoints and taxa in combination with a limited number of test species may cause serious errors in the extrapolation procedure because of wrong assumptions of the distribution model or the lack of representativeness of the selected taxa. Therefore, we recommend the usage of short taxonomic distances and homogeneous endpoints. Additionally, the distribution function of the test data should be controlled.

The extrapolation methods can be employed in the environmental standard setting of specific discharges of wastewater. Data from laboratory toxicity testing may be sufficient for the estimation of the potential effects on the environment if they are based on random sampling of the effluent. However, if the extrapolation method is used for setting maximum acceptable concentrations of single compounds, the combined effect of all compounds present in the environment should be accounted for. This may be done by an addition model summing the critical concentrations of the single compounds.

The use of extrapolation methods for regulatory purposes has recently been considered by The Netherlands Ministry of Housing, Physical Planning and Environment, and OECD (Balk, 1990; OECD, 1990). The statistical extrapolation methods utilize all toxicity data of a data set, and may produce reliable results if sufficient data are available, and if standards are set for the quality of data. The numerical values of p and δ should be chosen on the basis of investigations on several data bases. We stress the need for validation of the methods at ecosystem level before they are implemented for regulatory purposes.

Acknowledgements—We are indebted to Dr Henrik Spliid, the Institute of Mathematical Statistics and Operations Research, the Technical University of Denmark, for his useful comments on the paper. The work described in this paper was funded by the Danish National Agency of Environmental Protection.

REFERENCES

Aldenberg T. and Slob W. (1991) Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. National Institute of Public Health and Environmental Protection (RIVM), The Netherlands. Report No. 719102002.

Balk F. (1990) Ecotoxicological effect assessment: extrapolation from single species toxicity data. BKH, Consulting Engineers Bongaerts, Kuyper and Huiswaard B. V., The Hague, The Netherlands, Report.

Blank H. (1984) Species dependent variation among aquatic organisms in their sensitivity to chemicals. *Ecol. Bull.* **36,** 107–119.

EPA (1984) Estimating "concern levels" for concentrations of chemical substances in the environment. Environmental Effect Branch, Health and Environmental Review Division, U.S. Environmental Protection Agency.

Hald A. (1952) *Statistical Theory with Engineering Applications*. Wiley, New York.

Johnson N. L. and Kotz S. (1970) *Continuous Univariate Distributions*, Vol. 1. Wiley, New York.

Kooijman S. A. L. M. (1987) A safety factor for LC_{50} values allowing for differences in sensitivity among species. *Wat. Res.* **21,** 269–276.

OECD (1990) OECD Workshop on Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment, 10–12 December 1990, Arlington, Va, U.S.A.

Okkerman P. C., van der Plassche E. J., Slooff W., Van Leeuwen C. J. and Canton J. H. (1991) Ecotoxicological effects assessment: a comparison of several extrapolation procedures. *Ecotoxical. envir. Safety* **21,** 182–193.

Owen D. B. (1962) *Handbook of Statistical Tables*. Addison–Wesley, Mass.

Slooff W. and Canton J. H. (1983) Comparison of the susceptibility of 11 freshwater species to 8 chemical compounds. II (semi)chronic toxicity tests. *Aquat. Toxicol.* **4,** 271–282.

Slooff W., Van Oers J. A. M. and De Zwart D. (1986) Margins of uncertainty in ecotoxicological hazard assessment. *Envir. Toxicol Chem.* **5,** 841–852.

Stephan C. E., Mount D. I., Hansen D. J., Gentile J. H., Chapman G. A. and Brungs W. A. (1985) Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency, PB85-227049.

Suter G. W. II, Barnthouse L. W., Breck J. E., Gardner R. H. and O'Neill R. V. (1985) Extrapolation for the laboratory to the field: how uncertain are you? In *Aquatic Toxicology and Hazard Assessment* (Edited by Cardwell R. D., Purdy R. and Bahner R. C.), pp. 400–413. Am. Soc. for Testing and Materials, Philadelphia, Pa.

Van Straalen N. M. and Denneman G. A. J. (1989) Ecotoxicological evaluation of soil quality criteria. *Ecotoxicol. envir. Safety* **18,** 241–251.