# Comparison of Population Level and Individual Level Endpoints To Evaluate Ecological Risk of Chemicals 

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S Supporting Information


#### Abstract

Ecological risk assessments (ERA) of chemicals are often based on mortality and reproduction of individuals. To protect populations, fixed safety factors are applied to the data. However, the relationship between individuals and populations cannot easily be described by predefined numbers. The use of population models may reduce uncertainty and, hence, the risk for erroneous assessments. However, introducing models also introduces additional complexity. Therefore, it is desirable to keep the models as simple as possible. The objective of the present study was to determine whether simple risk equations or matrix models can improve ERA compared to traditional endpoints. To examine this, complex models that included environmental stochasticity and density dependence were used to simulate population level risk based on doseresponse data for five chemicals. The risk, measured as probability for pseudo extinction and recovery time, was then  compared to risk estimates based on individual level data (acute and chronic), risk equations, and simple matrix models. The results showed that the simple matrix models reduced uncertainty by more than $88 \%$ and $76 \%$ compared to acute and chronic data, respectively. Also the simple risk equation reduced uncertainty considerably ( $80 \%$ and $61 \%$ compared to acute and chronic data, respectively).


## INTRODUCTION

Ecological risk assessment (ERA) is a scientific process to estimate the probability for ecological impact following a certain activity, such as the release of a chemical. ${ }^{1}$ Presently, ERAs of chemicals are mainly based on observations of effects in individual organisms. ${ }^{2}$ For example, the two most commonly used metrics to estimate ecological risk for fish populations are acute LC50 (the Lethal Concentration that kills $50 \%$ of the fish) and chronic NOEC for reproduction (No Observed Effect Concentration, the highest tested concentration without a statistical effect). However, the environmental protection goals are mainly defined on the population level, ${ }^{3}$ such as population size, sustainable harvest, and risk for extinction. To be useful for decision makers, the observations on individuals have to be extrapolated to the population level. The most frequently used method for extrapolation is fixed safety factors, ${ }^{2,4}$ which are set to reduce ecological risk to acceptable levels. However, it has been shown that current extrapolations with fixed safety factors involve a large degree of uncertainty, and can lead to both overand underprotective risk assessments. ${ }^{5-7}$

To reduce uncertainty, it has been suggested that population models should be used more frequently in ERA..$^{8-11}$ Population models provide a link between the individual and the
population based on mathematical equations. ${ }^{12}$ This means that the same kind of tests that are used to determine LC50 and NOEC can be used to provide the input for population models. The models can be used to predict the population level responses, which in turn can be used to guide the decision-making process. ${ }^{13}$ A shift from studying the effects of chemicals on individual organisms to higher levels of organization is also gaining momentum among regulatory authorities. ${ }^{14}$ However, before population models are used routinely in ERA, it should be evaluated if they actually provide better information for risk management.

Population models range in complexity, from simple differential equations that only predict population size ${ }^{15}$ to advanced models where population structure, resource limitations, and natural environmental variability (stochasticity) are considered. ${ }^{16}$ More advanced models provide a higher level of ecological realism, but also require more data for the parametrization. To estimate how environmental stochasticity

[^0]A


$=8$| $\mathrm{F}_{\mathrm{O}+}$ | $\mathrm{F}_{1+}$ | $\mathrm{F}_{2+}$ | $\mathrm{F}_{3+}$ | $\ldots$ | $\ldots$ | $F_{\max -1}$ | $F_{\max }$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{S}_{0+}$ |  |  |  |  |  |  |  |
|  | $\mathrm{S}_{1+}$ |  |  |  |  |  |  |
|  |  | $\mathrm{S}_{2+}$ |  |  |  |  |  |
|  |  |  | $\mathrm{S}_{3+}$ |  |  |  |  |
|  |  |  |  | $\ldots$ | $\ldots$ |  |  |
|  |  |  |  |  | $S_{\max -1}$ | $\left(S_{\max }\right)$ |  |







Figure 1. (A) The Leslie matrix $(M)$ includes fertility values $(F)$ in the first row and survival rates $(S)$ in the subdiagonal. In the perch model, no maximum age was used. Therefore, an additionl survival rate was added in the lower right corner ( $S_{\max }$ ), reflecting the continuous survival probability of the oldest (and largest) perch. The population vector ( $N$ ) consists of the number of individuals in the different age classes ( $n$ ). By multiplying $M$ with $N$ of time $t, N$ of time $t+1$ is given. (B) Toxic impact on annual survival ( $\left(\operatorname{Imp} p_{\mathrm{S}}\right)$ and fertility $\left(\operatorname{Imp}_{\mathrm{F}}\right)$ after exposure to five chemicals. The impacts are presented as proportions of control ( $1=$ undisturbed).
and population density affect the population, observations over a substantial period of time are often needed. Furthermore, setting up, using, and interpreting such models require expertise in population modeling. A more realistic alternative for ERA may be to use simpler models, where environmental variability and density dependence are ignored. Such models require significantly less data for the parametrization, but may still represent a clear improvement compared to the traditional LC50 and NOEC. An example of models that may be useful in ERA are matrix models, which are stage structured models based on simple demographic data (survival and reproduction). ${ }^{17}$ Matrix models have a long history of use in conservation biology ${ }^{12,18}$ and have been used to make successful recommendations on how best to protect threatened and endangered species. ${ }^{19}$ Matrix population models have also been used to evaluate the effects of toxic chemicals in a number of studies. ${ }^{20-24}$ Furthermore, computer software are available for matrix population models so that they can be used by risk assessors without an extensive mathematical background. ${ }^{13}$ Hanson and Stark ${ }^{25}$ presented an even simpler method to estimate population level risk, using simple linear risk equations that were derived from population models. Using such equations would require no understanding of population models, and no specific computer software, but may still provide an improved estimate of population level risk.

In the present paper, ERA based on simple risk equations and matrix models was compared to ERA based on traditional LC50 and NOEC. This was done by comparing the resulting predicted no effect concentrations (PNEC) to the probability for pseudo extinction and the recovery time for two fish species that were simulated using complex models that included environmental stochasticity and density dependence. Doseresponse relationships for five chemicals were created from data presented in previously published papers. The two (models of) fish species and the five dose-response curves should be seen as random replicates to test the hypothesis that different ERA
methods provide different risk estimates and different levels of uncertainty. The results were evaluated to see how informative the different methods for ERA (LC50, NOEC, risk equations, and matrix models) are for risk managers. However, because of the method used, the study does not provide specific risk estimates for the two species that were simulated using the complex population models. The examined data are only valuable as a basis for comparing the different PNECs.

## METHODS

Population Models. The present study was based on matrix population models for the fish species eelpout (Zoarces viviparous) and perch (Perca fluviatilis). The models were age structured and included density dependence and environmental stochasticity. The data that were used to parametrize the models were taken from the Program for Integrated Fish Monitoring, which is funded by the Swedish Environmental Protection Agency. ${ }^{26}$ Both models were based on females only, assuming that males are not limiting for the population growth. Age-specific vital rates (survival and fertility) were estimated from the data and inserted into a Leslie Matrix ( $M$ ). The survival rate is the proportion of a given age class that survive for one time step and the fertility value is the average number of surviving descendants from a female of a specific age class during one time step. Fertility is the product of fecundity (number of fry/eggs) and survival to first age class (survival of recruits, $S_{\text {rec }}$ ). Fertility values for all age classes are inserted in the top row of $M$, and the survival rates are inserted in the subdiagonal. By multiplying $M$ with the population vector $(N)$ of time $t, N$ of time $t+1$ is given (Figure 1A). From $N$, the number of individuals ( $n$ ) in each age class is given.

The population model for eelpout was parametrized from 10 years of catch data from the Swedish reference site Kvädöfjärden. A detailed description of the model parametrization is given in Hanson et al. ${ }^{27}$ The eelpouts were caught and counted in November each year. From this data set, a population model

Table 1. Data on Five Chemicals Used to Retrieve LC50 and NOEC Values and to Construct Dose-Response Curves: LC50 and Reproductive NOEC Values, Species Tested, Duration of the Test, and the Reference Used (Dose-Response Curves are Shown in Figure 1B)

| chemical | LC50 | NOEC | unit | species | duration (days) | reference |
| :--- | :---: | :---: | :--- | :--- | :---: | ---: |
| 2,3,7,8-tetrachlorodibenzo- - -dioxin (TCDD) | 3545 | $0.18^{a}$ | $\mathrm{ng} / \mathrm{kg}^{b}$ | Oncorhynchus mykiss | 342 | 34 |
| 4-nonylphenol (4-NP) | 221 | 6 | $\mu \mathrm{~g} / \mathrm{L}$ | Oncorhynchus mykiss | 51 |  |
| Bis(tributyltin) oxide (TBT) | 14.7 | 0.66 | $\mu \mathrm{~g} / \mathrm{L}$ | Cyprinodon variegatus | 133 | 35 |
| Mercuric chloride (MC) | 168 | 0.5 | $\mu \mathrm{~g} / \mathrm{L}$ | Pimephales promelas | 36 |  |
| Zinc pyrithione (ZnP) | 5.31 | 1.22 | $\mu \mathrm{~g} / \mathrm{L}$ | Pimephales promelas | 60 | 28 |

${ }^{a}$ For TCDD, the lowest tested concentration ( $1.8 \mathrm{ng} / \mathrm{kg}$ ) had a small effect on reproduction and NOEC could not be determined. Adding one more concentration at the lower end of the scale is, therefore, a realistic estimate of NOEC. ${ }^{b}$ For TCDD, the exposure was dietary. This means that the fish were exposed to doses rather than concentrations. For simplicity, the terms LC50 and NOEC will still be used, although the correct terms would be LD50 (Lethal Dose) and NOEL (No Observed Effect Level).
with a projection interval of one year was created, starting and ending in November. The females were grouped into the age classes $0+, 1+, \ldots, 8+$, where age $0+$ represents the young of the year (born in January), $1+$ represents those that were born one year earlier, etc. Survival rates and fertility values were set up in a $9 \times 9$ Leslie matrix. Survival rates for age classes $2+$ and older were estimated directly from the catch data. To estimate survival for recruits, $0+$ and $1+$, it was assumed that survival increased linearly from birth until age $2+$. Eelpout is a viviparous species (gives birth to live young), and the data included information about fecundity in terms of the number of fry in the ovaries in November. The age-specific fertility was estimated based on fecundity, the proportion of mature females in the age class, and $S_{\text {rec. }}$. Based on the data, environmental stochasticity was introduced by letting survival rates and fertility values vary between years. In addition to the previously described model, ${ }^{27}$ density dependence was added. The process for determining density dependence is described in 28 . The density dependence was set to affect fecundity using the Beverton-Holt model. ${ }^{17}$

The population model for perch was parametrized from a 17 -year data set of catches from Kvädöfjärden. The parameterization of the perch model is described in detail by Hanson. ${ }^{29}$ The data were based on catches made in August each year. Also in this case, the projection interval was, thus, one year (August to August). Based on the catch data, 13 age classes were used in the model, rendering a $13 \times 13$ Leslie matrix. The age classes are referred to as $0+, 1+, \ldots, 12+$, where $0+$ is the young of the year (born in May), $1+$ were born one year earlier, etc. No maximum age was used (i.e., no age where survival is zero). Therefore, age class $12+$ included all females of age 12 and older. The mean survival rate of adults was estimated from catch curves as suggested by Ricker. ${ }^{30}$ For juvenile age classes, there were not enough data to estimate survival. Therefore, survival was assumed to increase linearly from birth to age class $3+$. Fertility was based on the length of the females according to studies by Mann ${ }^{31}$ and Heibo and Vollestad. ${ }^{32}$ Because length increased with age, this rendered age-specific fecundity values. Environmental stochasticity was introduced into the models by allowing $S_{\mathrm{rec}} S$, and length growth to vary randomly between years. As a result of this, mean length and fecundity at a given age varies between years. The magnitudes of the variations were adjusted to fit the data using maximum likelihood. ${ }^{29}$

Toxicity Data. For the present study, two different types of data were needed. Dose-response relationships for annual survival and fertility were needed for the models (including the risk simulations), and traditional data ( 96 h LC50 and chronic NOEC) were needed for the traditional risk ratios. For the hypothesis that was examined, it could be argued that the
chemicals should represent a random selection of chemicals that are typically assessed for ecological risk. In principle, the data could be constructed rather than being derived from real toxicity tests (as long as the same data are applied to all methods). However, without a foundation in real biological data, it would be difficult to set the impacts on survival and reproduction in a realistic relation to each other. Furthermore, the endpoint NOEC cannot easily be derived from constructed dose-response curves, as it depends on the actual concentrations that are used in the toxicity test (one of several drawbacks with the endpoint ${ }^{33}$ ). Toxicity data from constructed dose-response curves would, thus, not fully capture the limitations of NOEC. Therefore, the dose-response curves that were used in the present study were based on previously published studies that examined toxic effects on survival and reproduction for fish. These studies had not been designed to provide the specific types of data that were needed for the present study (annual survival and fertility, LC50 and NOEC). However, the aim of the present study was to compare different methods for ERA, and these somewhat rough dose-response estimates serve well as examples of typical dose-response data that are used in ERA.

Data from five different chemicals were used to determine LC50 and NOEC, and to construct dose-response relationships (Table 1, Figure 1B). Dose-response curves were created by fitting the logistic function to the different sets of data, where survival and fertility were presented as proportions of control. The logistic function was in the form $\operatorname{Imp}=1 /$ $\left(1+\mathrm{e}^{-(\alpha+\beta \times C)}\right)$, where Imp is the proportional survival or fertility after impact ( $I m p_{S}$ and $I m p_{\mathrm{F}}$, respectively), C is the chemical concentration (or dose), and $\alpha$ and $\beta$ are model parameters. The parameters were adjusted to fit the logistic function to the data using the least-squares method. The data were based on studies that ranged from 28 to 342 d , and for two of the chemicals (4-nonylphenol and mercuric chloride), LC50 based on observations after 96 h were also available. However, the times of interest in the present study are 96 h (for LC50) and 365 d (for the models). Therefore, extrapolations between times were necessary. All extrapolations of survival rates between time spans were based on the assumption of equal survival throughout the period.

In the model, fertility is the number of descendants from a female that survive during one time step. When there is a higher chemical concentration, fecundity, hatchability, and survival of recruits are reduced. However, there is also a reduction in survival of adults, which means that there is an increased probability that the female will die before the fry are born or the eggs are laid. To include this effect in the model, observed effects on reproduction were combined with the effects on
survival for the remaining part of the year. The logistic doseeffect functions are shown in Figure 1B.

Simulated Population Level Risk. Risk is often defined as a negative event (hazard) multiplied by the probability that it will occur. However, the need for simplistic measures of risk in ERA means that probability often is ignored. A good example of a measure of population level risk is the probability of pseudo extinction. This is defined as the probability that a population will fall below a predefined population size at any point within a certain time frame. In the present study, the probability for pseudo extinction was estimated from the stochastic models. This was done to get a measure of the ecological risk at different concentrations of the five chemicals. The probability for pseudo extinction was modeled for a time span of 25 years, and the limit for pseudo extinction was set so that the risk to fall below it would be $5 \%$ for an undisturbed population. This limit has no ecological justification, but is in agreement with the common use of $\alpha=0.05$ in hypothesis testing statistics. This level was determined from 10000 simulation runs. The toxic effects were introduced in the models by multiplying the fertility values (first row in $M$ ) with $\operatorname{Imp}_{\mathrm{F}}$, and the survival rates (all other rows in $M$ ) with $\operatorname{Imp} p_{\mathrm{S}}$ (Figure 1). Also this simulation was run 10000 times, allowing for good estimates of the probability for pseudo extinction at different concentrations.

Stressors other than chemicals may add to the population level risk. Those stressors may be natural as well as anthropogenic. A population that is already stressed by chemicals may have a reduced ability to recover from other types of stress. A measure of population level risk that accounts for this is the population recovery potential. ${ }^{39,40}$ In the present study, recovery potential was examined by model projections. The recovery potential was measured as the time it takes for the population to grow from $10 \%$ to $90 \%$ of the carrying capacity. To include probability in the measure, the results are presented as mean $\pm$ standard deviation (SD).

Individual Level Endpoints for ERA. From the toxicity data described above, PNEC was derived from LC50 and NOEC in accordance with the EU technical guidance document in support of the directive on new notified substances (93/67/ EEC), the directive on biocidal products ( $98 / 8 / E C$ ), and the regulation of existing substances (1488/94). ${ }^{2}$ This was done by dividing the ecotoxicological endpoints with an assessment factor. The assessment factor is larger when the data are further from the protection goals. For example, when only data on acute mortality are available, the LC50 is divided by 1000 to determine the PNEC. The use of a factor 1000 is assumed to be protective and is used to ensure that chemicals with a potential to harm the environment will not slip through the ERA process based on only acute data. ${ }^{2}$ If there is evidence that shows that the uncertainty is lower (e.g., by data from similar compounds or a wide selection of species), a lower assessment factor can be used. However, the assessment factor for acute data can never be lower than $100 .{ }^{2}$ When chronic data are available, lower assessment factors can be used to determine the PNEC. If data from only one long-term study is available, the NOEC is divided by 100 to determine the PNEC. If data are available from at least three species, representing different trophic levels, the assessment factor 10 is used. In the present paper, both lower and higher PNECs were presented for ERAs based on LC50 and NOEC. The different PNECs derived from acute data will be referred to as PNEC $_{\text {acute } 1000}$ and PNEC $_{\text {acute } 100}$, for LC50s divided by assessment factors of 1000 and 100, respectively. For chronic data, the two levels will be referred to as $\mathrm{PNEC}_{\text {chronic } 100}$ and $\mathrm{PNEC}_{\text {chronic 10 }}$, for
the NOEC divided by assessment factors 100 and 10 , respectively.

Population Level Endpoints for ERA. The most commonly used endpoint for assessing the ecological risk on the population level is the population growth rate $(\lambda) .^{41-44}$ If $\lambda>1$, the population is growing, and if $\lambda<1$, the population is declining. The $\lambda$-value can easily be obtained from matrix models as the dominant eigenvalue of the Leslie matrix. In many demographic studies of toxic impact, the emphasis has been to determine the concentration where $\lambda=1$, which is seen as a limit for what the population can tolerate without risking extinction. By combining the deterministic models and the dose response data, the concentration where $\lambda=1$ can be determined. This represents the concentration where a population in a constant, average environment would have a stable population size. As there is always some degree of uncertainty in the models and the toxicity data, a safety factor must be used to keep the ecological risk acceptable. In the present study, this was done by dividing the concentration where $\lambda=1$ by an assessment factor of $3(\mathrm{AF}=3)$. This factor is just used as an example in the present study to allow a comparison to PNECs based on traditional risk ratios. The resulting predicted no effect concentration will henceforth be referred to as $\mathrm{PNEC}_{\text {model elpout }}$ and $\mathrm{PNEC}_{\text {model perch }}$.

Deterministic population models, without density dependence, are clear simplifications of the complexity of natural populations. However, they still require data for parametrization, and a risk assessor that is able to set up, use, and interpret the models. Hanson and Stark ${ }^{25}$ presented a simpler method to determine if $\lambda$ is above or below 1 . The method is based on simple linear equations that are derived from five population models for fish. Two linear equations were presented by Hanson and Stark: ${ }^{25}$ one that was set to be protective of all five population models and one that was set to represent the average. In the present study, the protective equation was used to estimate a safe concentration (eq 1).

$$
\begin{equation*}
13.07-2.86 \times S_{\text {tox }}-F_{\text {tox }}>0 \tag{1}
\end{equation*}
$$

Here, $S_{\text {tox }}$ and $F_{\text {tox }}$ are the percent reduction in survival and fertility, respectively. As for the deterministic model, the resulting concentration was divided by $\mathrm{AF}=3$. The resulting concentration will be referred to as $\mathrm{PNEC}_{\text {equation }}$.

Comparison of the different PNECs. To evaluate how well the different PNEC values predict what will happen to the population, they were all compared to the concentration where the probability for pseudo extinction had doubled from $5 \%$ to $10 \%$ according to the simulations of the two species. This concentration will henceforth be referred to as $C_{10 \%}$. This means that for each set of data, ten PNEC/ $\mathrm{C}_{10 \%}$ ratios were retrieved (two species $\times$ five dose-response relationships). To get an estimate of the remaining uncertainty for each of the PNECs, the coefficient of variance ( $\mathrm{CV}=$ standard deviation/mean) was calculated from the ratios. A high CV means that the variation in margin of safety to $C_{10 \%}$ also is high.

It should be noticed that these simulations are not "true" effects for the two species that were used to parametrize the models. However, the aim of the present study was not to predict effect for these particular species, it is to compare different methods for ERA. Simulations of "constructed" species serve well to examine this hypothesis as long as they are realistic estimates of the behavior of real fish populations. Considering the quality of the long-term data sets that were used to parametrize the models,


Figure 2. Probability of pseudo extinction. The curves show the probability for pseudo extinction as a function of chemical concentration for eelpout (left) and perch (right). The vertical lines show the different PNECs (predicted no effect concentration). The values for the different PNECs can be found in Table S1.
we think that that these simulations represent trustworthy estimates of population level risk for two "random" fish species.

## RESULTS AND DISCUSSION

Table S1, Supporting Information, shows all the different PNECs as derived from LC50, NOEC, the risk equation, and
matrix models. Furthermore, the probability for pseudo extinction and the time for recovery at the different PNECs are shown. The probability for pseudo extinction and time to recovery are shown as a function of chemical concentration in Figure 2 and Figure 3, respectively. The vertical lines show the different PNECs that are presented in Table S1.


Figure 3. Mean recovery time. The solid curves show the mean recovery time as a function of chemical concentration for eelpout (left) and perch (right). The dotted curves show the time in which there is a $95 \%$ probability for recovery. The vertical lines show the different PNECs (predicted no effect concentrations). The values for the different PNECs can be found in Table S1.

The minimum level of data that is needed for ERA in the EU is acute data for fish, daphnia, and algae. Assuming that fish is the most sensitive organism, the maximum acceptable concentration in the environment would be $\mathrm{PNEC}_{\text {acute } 1000 \text {. At this }}$ level, the probability for pseudo extinction varied between $5 \%$ and $65 \%$ (Table S1, Supporting Information, and Figure 2) and
the time to recovery varied about 5-fold (Table S1 and Figure 3). In other words acute LC50 is not a very precise measure of risk. Looking closer at the results, the highest risks occurred for TCDD and MC. The reason for this is that reproduction is affected at relatively low concentrations (compared to survival) for these chemicals (Figure 1). Therefore, the risk will be

Table 2. Ratios between the Different PNECs and the Concentration Where the Probability for Pseudo Extinction is 10\% $\left(C_{10 \%}\right)$; A Ratio Higher than One Means That the PNEC Results in More than $10 \%$ Probability for Pseudo Extinction (Average Ratio for Each PNEC and the Coefficient of Variation (CV) are Also Shown)

|  | $\mathrm{PNEC}_{\text {acute }} 1000 / \mathrm{C}_{10 \%}$ | $\mathrm{PNEC}_{\text {acute }} 100 / \mathrm{C}_{10 \%}$ | $\mathrm{PNEC}_{\text {chronic } 100} / \mathrm{C}_{10 \%}$ | $\mathrm{PNEC}_{\text {chronic }}{ }_{10} / \mathrm{C}_{10 \%}$ | $\mathrm{PNEC}_{\text {equation }} / \mathrm{C}_{10 \%}$ | $\mathrm{PNEC}_{\text {model }} / \mathrm{C}_{10 \%}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| EP, TCDD | 1.50 | 15.04 | 0.001 | 0.008 | 0.57 | 0.87 |
| EP, TBT | 0.59 | 5.85 | 0.26 | 2.63 | 0.52 | 0.86 |
| EP, MC | 7.02 | 70.23 | 0.21 | 2.09 | 0.82 | 1.34 |
| EP, 4-NP | 0.32 | 3.20 | 0.09 | 0.87 | 0.63 | 0.96 |
| EP, ZnP | 0.12 | 1.19 | 0.27 | 2.72 | 0.33 | 0.94 |
| perch, TCDD | 1.50 | 14.96 | 0.001 | 0.008 | 0.56 | 1.33 |
| perch, TBT | 0.58 | 5.77 | 0.26 | 2.59 | 0.51 | 1.11 |
| perch, MC | 4.43 | 44.28 | 0.13 | 1.32 | 0.52 | 1.26 |
| perch, 4-NP | 0.25 | 2.54 | 0.07 | 0.69 | 0.50 | 0.97 |
| perch, ZnP | 0.10 | 1.04 | 0.24 | 2.39 | 0.29 | 1.07 |
| average | 1.65 | 16.51 | 0.15 | 1.53 | 0.52 | 1.07 |
| CV | 1.40 | 1.40 | 0.71 | 0.71 | 0.28 | 0.17 |

underestimated when effects on reproduction are ignored (i.e., when only LC50 is used). To reduce the frequency of underprotective risk assessments, higher assessment factors could be used. However, this would also increase the proportion of overprotective risk assessments. For example, there was almost no population level effect at the $\mathrm{PNEC}_{\text {acute } 1000}$ for ZnP . Using a higher assessment factor would make the risk assessment for ZnP even more overprotective. Overprotective risk assessments have economical as well as environmental costs as potentially beneficial chemicals are unnecessarily restricted, thus taking resources from populations and ecosystems that are truly in danger.

When the lower assessment factor for acute data was used, the probability for pseudo extinction was higher than $90 \%$ and the time for recovery was more than 100 years for three chemicals. This would suggest that the lower assessment factor leads to underprotective risk assessments. However, according to the EU guidance document, ${ }^{2}$ the lower assessment factor can only be used if there is available evidence that shows that the uncertainty is lower. Such evidence was not examined in the present study, and it may not be valid to use $\mathrm{AF}=100$ for these specific chemicals. Therefore, it cannot be concluded that $\mathrm{PNEC}_{\text {acute }} 100$ is underprotective. It is quite possible that in cases where evidence exists to show that uncertainty is lower, $\mathrm{AF}=$ 100 would not be underprotective.

When chronic NOEC and AF $=100$ were used to determine PNEC, almost no increase in risk was seen. When chronic NOEC and AF = 10 were used, the probability for pseudo extinction ranged from about $5 \%$ to $35 \%$ and the time to recovery varied about 2 -fold. The variation was, therefore, much smaller than for the LC50. This could suggest that NOEC, despite its shortcomings, is a better measure of risk than LC50. However, it could also mean that NOEC results in overprotective risk assessments.

For PNECs that were based on the risk equation of the matrix models, there was always a small increase in risk, but never a large increase in risk. This means that the clearly overand underprotective assessments were avoided.

A better way to quantify uncertainty is to examine how large the margin of safety is to the concentration where the effect is unacceptable. In the present study, the concentration where the probability for pseudo extinction was $10 \%$, i.e. approximately doubled from the undisturbed population, was used as an example of a level that can be considered as the acceptable limit. Table 2 shows the ratio between the different PNECs and
$\mathrm{C}_{10 \%}$. When acute LC50 data and AF $=1000$ were used, the mean PNEC/C ${ }_{10 \%}$ ratio was 1.65 . This means that PNEC $_{\text {acute }} 1000$ was, on average, $65 \%$ higher than the true acceptable concentration, rendering underprotective results. Looking at the five chemicals individually shows that the $\mathrm{PNEC} / \mathrm{C}_{10 \%}$ ratio ranged from 0.10 to 7.02 . This means that the risk assessment could end up with a PNEC that was only $10 \%$ of the true acceptable concentration, just as well as more than seven times higher than the true acceptable concentration. This large variation resulted in a CV of 1.40 (i.e., the standard deviation of the five PNEC/ $\mathrm{C}_{10 \%}$ ratios was $40 \%$ larger than the average ratio). When chronic data (NOEC) were used, the uncertainty was reduced by about $50 \%(C V=0.71)$. The simple risk equations and matrix models further reduced the uncertainty by $61 \%$ and $76 \%$, respectively ( $\mathrm{CV}=0.28$ and $\mathrm{CV}=0.17$ ).

Note that the assessment factor that is used does not affect the uncertainty, only the relative probability for over- versus underprotective assessments. Adjusting the assessment factors could, thus, reduce the proportion of underprotective assessments, but only at the expense of more overprotective assessments, and vice versa. For example, if $\mathrm{AF}=7023$ were used on LC50, the range of PNEC/ $C_{10 \%}$ ratios would be $0.014-1.00$. This means that underprotective PNECs are avoided, and that the most overprotective PNEC would be $98.6 \%$ lower than the true acceptable limit. If, instead, the AF for the matrix model were adjusted in a similar way ( $\mathrm{AF}=4.02$ ), the range of $\operatorname{PNEC} / \mathrm{C}_{10 \%}$ ratios would be $0.64-1.00$. In this case, the most overprotective PNEC would only be $36 \%$ lower than the true acceptable limit.

It could be argued that the best predictions of ecological risk that were presented in the present study were those that were used to define the ecological risk, i.e., the complex matrix models that included environmental stochasticity and density dependence. However, these models were all parametrized from large data sets that allowed estimates of natural variation between years, density dependence, age specific fertility etc. It cannot be expected that adequate data are available to set up stochastic models in most ERAs of chemicals. Furthermore, even if data may be available, it cannot be expected that the risk assessor has the expertise in population modeling that is needed to parametrize and use the models, and interpret the results. Therefore, it is essential to evaluate if simpler models can be used. In the present study, it was clearly seen that simple risk equations or deterministic matrix models reduced uncertainty in ERA considerably compared to traditional endpoints.

More complex models, such as those that include environmental stochasticity and density dependence, may have a role at higher tiers in ERA.

## - ASSOCIATED CONTENT

## (5) Supporting Information

Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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## Notes

The authors declare no competing financial interest.

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