On the Ecological Risk Assessment of Endocrine Disrupting Chemicals

Yoshinari Tanaka[1,2]

1 Institute of Environmental Science and Technology, Yokohama National University, Tokiwadai 79-7, Yokohama 245-0067, JAPAN
2 CREST, Japan Science and Technology Corporation

Key Words: ecological risk, population-level effect, extinction risk, population growth rate, endocrine disrupting chemicals.

Abstract

Analytical methods and experimental designs for ecological risk estimation of endocrine disrupting chemicals are shortly reviewed and proposed. Fish reproduction tests, which preferably include early life or transgenerational exposure, are proposed as the best empirical basis for risk estimation of freshwater ecosystems.

Introduction

Hazards by endocrine disrupting chemicals (EDCs) onto human health and/or natural ecosystems are abruptly drawing public attention (Coborne *et al.* 1996). Typical EDCs are characterized by their irreversible adverse effects to reproduction systems of organisms, which primary structure is formed by hormonal controls during developmental process in early life stages. Since the early developmental process is sensitive to endocrine disruption, EDCs may have detrimental effects to reproduction at extremely low concentrations (Jobling *et al.* 1996; Gray and Metcalfe 1997).

Although a couple of omens implying impacts of EDCs to wild lives are reported, real quantitative hazards of EDCs to natural populations of organisms or ecosystems are not well understood. As well as direct demonstration for impacts of EDCs to natural populations in fields, ecological risk assessment based on toxicological experiments and exposure measurements or predictions in fields are required for designing an optimal policy coping with chemical pollution.

It is noticeably hard to define endpoints of EDCs since biological responses to EDCs vary according to levels of responses to be measured and are quite different in quality and quantity from responses to pollutant chemicals other than EDCs (European Community 1997). Responses at the individual level may include higher mortality,

biased sex ratio, infertility of males and/or females, and anatomical and/or behavioral anomaly. Most responses to EDCs are connected to reproduction while early life mortality is believed to be an important response to many pollutant chemicals other than EDCs. For ecological risk evaluation these individual-level responses may be readily summarized into the effect on population growth rate as a summary index of population-level effects. The most favorable merit in summarizing responses into population growth rate is feasibility of comparing qualitatively different responses with a common criteria. Simple descriptions of individual-level or tissue-level responses such as vitelogenin production and development of testis-ova or other abnormal sexual organs cannot lead to quantitative risk evaluation.

Since natural ecosystems consist of many populations of interacting species, extinction of populations is one of the most ultimate criteria of ecological risk estimation (Ginzburg *et al.* 1982). Mean time to extinction is also utilized for risk estimation in biological conservation (Primack 1993). The population vulnerability analysis (PVA) is essentially based on extinction probability for a specific duration predicted from long-term simulation of population dynamics (Soule 1987; Primack 1993). Mean time to extinction or extinction probability is closely associated with population growth rate. We review the framework of ecological risk evaluation based on mean time to extinction with special reference to EDCs.

Ecological Risk and Mean Extinction Time of Populations

Analytical solutions for mean extinction time (MET) or extinction probability for a specific duration have been investigated by employing the diffusion approximation or the branching process theory (Lande 1993; Foley 1994; reviewed by Iwasa 1997; Matsuda 1997). Extinction is induced by several factors, i.e. environmental stochasticity, demographic stochasticity, genetic factors (inbreeding depression and accumulation of new deleterious mutations), catastrophic events. Even focusing on a single factor predictions of MET vary noticeably between theoretical models based on somewhat different assumptions and/or approaches. Nonetheless, major properties of the dependence of the factors on demographic and environmental parameters are fairly compatible between models. Lande (1998) has summarized the theoretically deduced relationships between MET and demographic and environmental parameters as a "scaling law" (Table 1). The environmental stochasticity is a major factor inducing extinction of relatively large populations. The other three factors govern extinction of small or declining populations, which are essentially at a final phase of extinction. These factors are not negligible when evaluating ecological risk of chemical pollutants for endangered species or populations. Nonetheless, comprehensive ecological risk

estimation should be based on moderately large populations because most populations in nature are not endangered. Therefore, the environmental stochasticity may be the primary factor of extinction when we evaluate extinction risk of chemical pollutants.

Table 1. The Scaling Law of Extinction Factors

Extinction Risk Factors	Proportional Scaling of MET
Environmental Stochasticity	$N^{2\bar{r}/V_e-1}$
Demographic Stochasticity	$(1/N)e^{2N\bar{r}/V_e}$
Declining Populations	$-(\ln N)/\bar{r}$

N: population size (carrying capacity), $\bar{r}$: intrinsic rate of natural increase, V_e: environmental variance of r

According to the scaling law, MET is roughly proportional to a power of population size: $\bar{T} \propto N^{2r/V_e-1}$, where $\bar{T}$ is MET, N the population size, r the intrinsic rate of natural increase, and V_e is the environmental variance of r. Thus MET decreases geometrically with the relative magnitude of the mean population growth rate to the environmental variance of growth rate, r/V_e. Transforming both sides into logarithm, we get $\log\bar{T} = C + (s-1)\log N$, where C is a constant and $s = 2r/V_e$. Provided that there are initial (hypothetical equilibrium) values of population size (carrying capacity) and population growth rate relative to environmental variance, small changes in $\log\bar{T}$ can be expanded into bivariate Taylor series around the equilibrium values of s and $\log N$: $\Delta\log\bar{T} \approx \Delta s \times \log\tilde{N} + \Delta\log N \times (\tilde{s}-1) + \Delta s \times \Delta\log N$, where Δs and $\Delta\log N$ are small deviations from the equilibrium values. Pollutants' adverse effects on MET may be decomposed into the two fractions if they are small. Some ecotoxicological experiments have shown that pollutant chemicals primarily reduce the population growth rate although a few experiments have suggested significant effects of chemicals on the carrying capacity as well. In nature population size is limited by predation or other environmental effects rather than conspecific density effects. Then the major population-level effect of pollutant chemicals are likely to reduce the s-values. Thus the simplest form of extinction risk due to pollutant chemicals is $\Delta\log\bar{T} \approx \Delta s \times \log\tilde{N}$.
A loss of log MET is roughly estimated by changes in the relative magnitude of the population growth rate and the environmental variance.

Time-Independent Models

If exposure concentrations of pollutant chemicals in natural environments are constant or stationary over time, time-independent approaches are straightforwardly

applied for extinction risk assessment. The time-independent approach may be fairly relevant for ecological risk assessment of EDCs because exposure regime of many EDCs such as bispnenol-A, nonylphenol, and TBT is stationary rather than fluctuating or pulsatory over time. There are two major theoretical approaches to estimate MET risk based on toxicological and exposure data. One is to determine population growth rate (intrinsic rate of natural increase) from static life table data provided under several exposure concentrations. The other is to calculate population growth rate as the largest eigenvalues of Leslie matrix with vital rates varying due to exposure concentrations (e.g. RAMAS series of simulation models).

Effects of pollutant chemicals on intrinsic rate of natural increase are estimated from life table evaluation tests. Fig. 1 is results of a life table test held by the author for p-nonylphenol on *Daphnia galeata*. Responses in the growth rate are fit by a power function, i.e. $r(x) = r_{\max}\left\{1-(x/\alpha)^{\beta}\right\}$ where x is an exposure concentration, $r_{\max}$ is maximum growth rate without exposure, and α and β are parameters to be estimated.

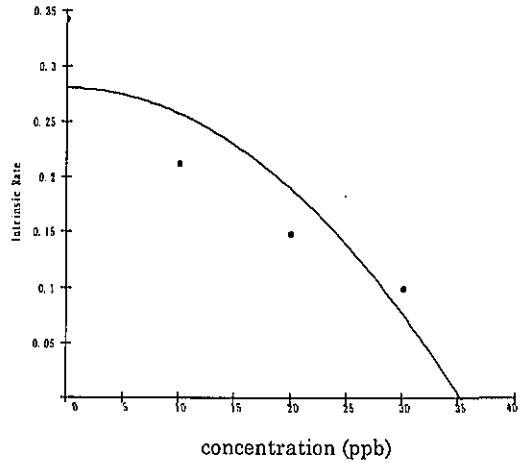

Fig. 1 Response of intrinsic rate of natural increase of *D. galeata* to p-nonylphenol exposure

The dose response function p-nonylphenol on *D. galeata* was evaluated as $\alpha = 35.2$ (ppb) if $\beta = 2$. Tanaka (1998) and Tanaka and Nakanishi (1998) analyzed published life table evaluation data and concluded that β-values are roughly 2 on average among many chemicals and species and α-values are partly predictable from acute LC_{50}s (a linear regression: $\ln\alpha \cong 0.8\ln[LC_{50}]$). Substituting the acute-r regression into the quadratic dose-response function, we get $r \cong r_{\max}\left(1-h^2[LC_{50}]^{0.4}\right)$, where h is proportional exposure concentration relative to acute LC_{50}s. If environmental variance of population growth rate does not change due to chemical exposure, the above equation is equivalent to $\Delta s \cong -\tilde{s} \times h^2[LC_{50}]^{0.4}$, where $\tilde{s}$ is equilibrium s value prior to chemical exposure. Therefore, it is implied that an extinction risk due to chemical exposure is $\Delta \log T \cong -\log \tilde{N} \times \tilde{s} \times h^2[LC_{50}]^{0.4}$ if there is no changes of equilibrium

population size due to chemical exposure.

Many test species, in particular fishes, are not suitable for complete life cycle experiments mostly due to long life span and large labor needed for rearing the animals for a long period. Partial life cycle test, which examines semi-chronic toxicity for survival and reproduction in each (preferably early) life stage, is the best alternative. The most standard method to simulate population dynamics of such stage-structured populations is Leslie matrix model. The population numbers of each life stage are denoted as a vector n of population size, $\mathbf{n} = \begin{pmatrix} n_1 & n_2 & n_3 & n_4 & n_5 \end{pmatrix}^{\mathrm{T}}$ where n_i is population size of the i-th life stage and T is matrix transpose. Population dynamics is expressed as a recurrence equation,

$$\mathbf{n}(t+1) = \mathbf{L}(t)\mathbf{n}(t)$$

where t is time in generation. If only the last life stage reproduces, the projection matrix L has elements (vital rates) as follows,

$$\mathbf{L} = \begin{bmatrix} p_1(1-s_1) & 0 & 0 & 0 & f_5 \\ p_1 s_1 & p_2(1-s_2) & 0 & 0 & 0 \\ 0 & p_2 s_2 & p_3(1-s_3) & 0 & 0 \\ 0 & 0 & p_3 s_3 & p_4(1-s_4) & 0 \\ 0 & 0 & 0 & p_4 s_4 & p_5 \end{bmatrix},$$

where p_i is probability of survival per unit time of the i-th stage, s_i is a proportion of survived individuals in the i-th stage to enter the next stage per unit time, and f_i is per capita reproduction of i-th stage. If some elements depend on time, L comes to be time-dependent.

We assume that exposure of chemicals reduces each element independently and the reductions are at least partly estimated from chronic tests of each life stage. If we denote responses of p_i and f_i to exposure concentration z as $x_i(z)$ and $y_i(z)$, the projection matrix under exposure effect becomes

$$\mathbf{L}(z) = \begin{bmatrix} p_1(1-s_1)(1-x_1(z)) & 0 & 0 & 0 & f_5(1-y_5(z)) \\ p_1 s_1(1-x_1(z)) & p_2(1-s_2)(1-x_2(z)) & 0 & 0 & 0 \\ 0 & p_2 s_2(1-x_2(z)) & p_3(1-s_3)(1-x_3(z)) & 0 & 0 \\ 0 & 0 & p_3 s_3(1-x_3(z)) & p_4(1-s_4)(1-x_4(z)) & 0 \\ 0 & 0 & 0 & p_4 s_4(1-x_4(z)) & p_5(1-x_5(z)) \end{bmatrix}.$$

The response functions to toxicants' effects, $x_i(z)$ and $y_i(z)$, are essentially equivalent to dose-response functions estimated from toxicity experiments. Cumulative normal distribution, logistic function, and Weibul model are the most common dose-response functions.

If the projection matrix and exposure concentration are constant over time, a population increases or decreases at a rate equivalent to the largest eigenvalue λ of the projection matrix, which is determined from a characteristic equation, $|\mathbf{L}-\lambda\mathbf{I}|=0$. The intrinsic rate of natural increase is approximately equal to $\ln\lambda$. Then extinction risk of a stage-structured data is estimated from $\Delta r=\Delta(\ln\lambda)$. Fig. 2 illustrates how the largest eigenvalue of a carp population is reduced by diazinon exposure.

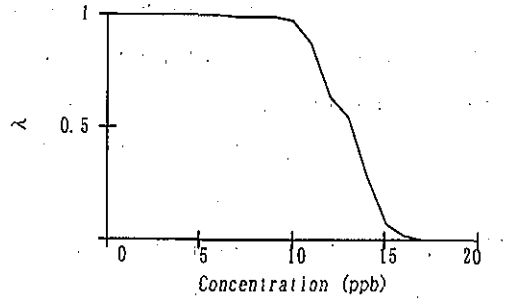

Fig. 2 Changes of largest eigenvalues of Projection matrix of a hypothetical carp population under diazinon exposure. The horizontal axis denotes concentration of diazinon.

In actual environments exposure concentration of pollutant chemicals fluctuates over time and space in many cases. If magnitudes of the fluctuation are small so that the basic assumption of the diffusion approximation is not violated, extinction risk induced by the perturbation of concentration may be incorporated into the time-independent model by calculating variances of population growth rate by the perturbation. Thus total environmental variance of population growth rate is decomposed into a part attributable to background environmental variance v_{env} and a part due to chemical exposure v_{chem}, i.e. $v_{total}=v_{env}+v_{chem}$. Practical methods to estimate v_{env} from long-term population dynamics data is described by Iwasa (1998) and Hakoyama and Iwasa (1998). The variance due to chemical exposure v_{chem} may be estimated from a variance of concentration of a chemical $\mathrm{var}(x)$ using $v_{chem}\cong\left(\partial r/\partial x\right)^2\mathrm{var}(x)$ and $\partial r/\partial x=-r_{\max}\left(2x/\alpha^2\right)$ if responses of population growth rate to exposure concentration are approximated by the quadratic function.

Time-Dependent Models

If fluctuation of exposure concentration or/and environmental factors is so large that the assumption of small perturbation needed for the diffusion approximation is violated, direct numerical simulation using Leslie matrix model is preferred by its minimum mathematical complexities. Concentrations of most agricultural chemicals in nature fluctuate largely and seasonally because many of them are sprayed in a restricted season. Fig. 3 presents a dynamics of diazinon concentration in a natural river stream (Sakura River) exemplifying a large seasonal fluctuation of measured concentration.

The figure also presents an example of simulated population dynamics for a hypothetical carp (*Cyprinus carpio*) population under diazinon exposure.

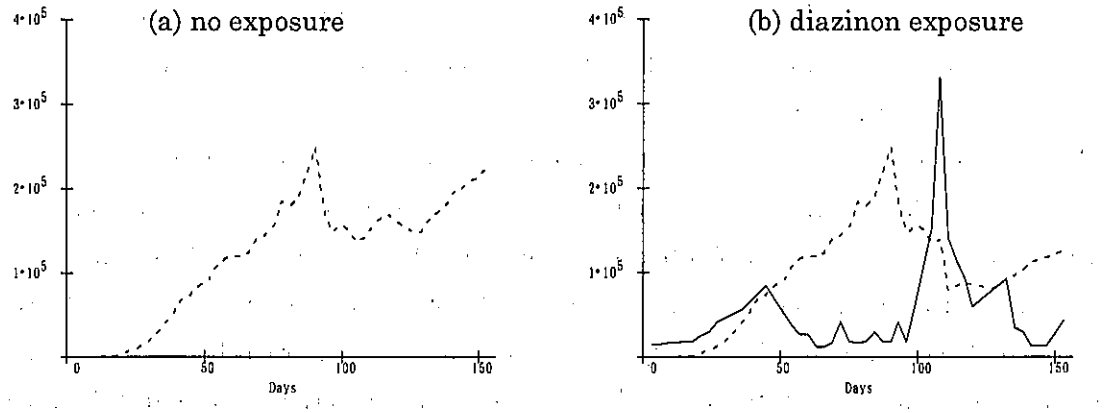

Fig. 3 Measured diazinon exposure dynamics (the line) and simulated population dynamics (the broken lines) of a hypothetical carp population.

In applying the matrix model to simulate population dynamics under exposure of EDCs, we must take into account a fact that low-level EDC exposure to individuals at an early developmental stage can have irreversible adverse effect on reproduction of the same individuals when they are grown to adults through disrupting endocrine systems controlling early development of sexual organs (Jobling et al. 1996; Gray and Metcalfe 1997). Hence there is a time lag between actual exposure and expression of the adverse effects to reproduction, which time-dependent models for EDCs should incorporate.

Under EDC exposure, a vital rate a_{ij} (ij-element of L matrix) is modified by present exposure (z_t: exposure concentration at time t [present time]), and past exposure (δ unit time ago) mediated by the developmental effects of EDCs. If we write effects to present vital rates of exposure at δ unit time before as $x_{ij}(z_{t-\delta}, \delta)$, a time-dependent version of the projection matrix is

$$\mathbf{L}(t, \mathbf{z}) = \begin{bmatrix} & \vdots & \\ \cdots & a_{ij} \times \left(1 - x_{ij}(z_t)\right) \times \left(1 - e^{-\int x_{ij}(z_{t-\delta}, \delta) d\delta}\right) & \cdots \\ & \vdots & \end{bmatrix}.$$

The irreversible developmental effect is dependent upon life stages at which the vital rate is measured and the attributable exposure is achieved, $x_{ij}(\cdot, \delta)$, and exposure concentration at δ unit time before, $z_{t-\delta}$.

Proposed Research for Risk Evaluation of EDCs

To evaluate population-level effect or preferably extinction risk of EDCs is important

for ecological risk assessment of EDCs because rational comparison of hazard causing from the qualitatively different mechanism, i.e. endocrine disruption, of EDCs to other chemicals is only feasible through quantifying the hazards in terms of population-level effect or population extinction. MET analysis is the ultimate goal of investigations on ecological impact due to EDCs.

Although studies concerning biological indicators or endocrinological mechanisms are important for developing comprehensive screening protocols and biological concept of EDCs, it is essential to quantify effects of EDCs on vital rates (survival rate, fecundity, and male fertility) for quantitative ecological risk estimation. In vivo fish reproduction test is the best candidate that is expected to provide fine empirical basis (Arcand-Hoy and Benson 1998). we propose a scheme of fish reproduction test using Japanese medaka (*Oryzias latipes*). The major properties are (1) transgenerational effect of EDCs is to be assayed since parental fish are exposed by chemicals, (2) early stages (egg and larvae) are under exposed, and (3) reproduction of both sexes (female fecundity and male fertility) are assayed separately. These data will provide effects of EDCs on vital rates and population growth rate. Although decomposition between the early developmental effects including the transgenerational effect and acute effects that are not associated with endocrine disruption is incomplete, these experiments will make ecological risk estimation based on MET feasible for populations under constant exposure.

References

Arcand-Hoy and Benson (1998) Env.Toxicol.Chem. 17, 49-57.

Colborne, T., D. Dumanoski, and J. P. Myers (1996). *Our Stolen Future.* Pergamon.

European Community 1997. *European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife.* Workshop Publication EUR 17549. Paris.

Ginzburg, L. R., L. B. Slobodokin, K. Johnson and A. G. Bindman (1982) *Risk Analysis* 2, 171-181.

Iwasa, Y. (1998) Proc.Int.Workshop on Risk Evaluation and Management of Chemicals. 1, 42-29.

Jobling, S., D. Sheahan, J. A. Osborne, P. Matthiessen and J. P. Sumpter (1996) *Environ. Toxicol. Chem.* 15, 194-202.

Foley, P. (1994) *Conservation Biology* 8, 124-137.

Gray, M. A. and C. D. Metcalfe (1997) *Environ. Toxicol. Chem.* 16, 1082-1086.

Lande, R. (1993) *Amer. Natur.* 142, 911-927

Lande, R. (1998) *Res. Popul. Ecol.* in press.

Matsuda, H. (1998) Proc.Int.Workshop on Risk Evaluation and Management of Chemicals. 1, 119-126.

Primack, R. B. (1993) *Essentials of Conservation Biology.* Sinauer and Assoc.

Soule, M. E. (1987) *Viable Populations for Conservation* . Cambridge University Press, Cambridge.