Ecotoxicological study procedure on aquatic organisms

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[**Abstract**] Individual, population, community and ecosystem are 4 successive levels in aquatic ecotoxicological study, and in this order the experimental complexity increases. Toxicity evaluation methods adopted at each level were reviewed in this paper. It is no doubt that the extrapolated results are more accurate at higher experimental level. However, if a contaminant proves to be safe even at individual level, then it is of no need to do any more work. Summarized from the studies all these years, recoverability and recovery rate of the toxic effect are more important in risk evaluation as the sublethal effect becomes more popular. Also, the interaction among different contaminants on toxicity as well as recovery needs to be observed. As a last step, all the experimental results should be carefully detected in field situation for the difference between experimental conditions and the actual outdoors.

[**Key words**] aquatic ecotoxicology; aquatic organisms; ecotoxicity; risk evaluation

[**CLC number**] Q948.8 [**Document code**] A [**Article ID**]1000-2782(2001)06-070-06

Ever since ecotoxicology was founded, a lot of studies have been conducted on aquatic ecosystem[1] because almost all contaminants including wastewater from factories and pesticides sprayed in agricultural fields may enter ecosystem through various ways. To predict or assess the impact of contaminants on aquatic ecosystem, usually the studies are conducted at 4 levels, i.e. individual, population, multiple species and ecosystem. This paper is attempted to summarize the ecotoxicological study procedure on aquatic organisms.

1 Individual level

This is the first step of aquatic toxicological research work and usually it is conducted in the laboratory. Just as other toxicity research work, this step is aimed to set up the relationship between contaminant concentration and response. Based on this step, the concentration response model can be made and NOEC (no observant effect concentration) or LOEC (lowest observant effect concentration) may be extrapolated. If the PEC (predicted effluent concentration) is lower than NOEC or LOEC, it is acceptable. Otherwise work should be done to decrease PEC. In other words, NOEC or LOEC is very important for safety threshold determination.

Statistically, if the concentration of contaminant is below NOEC or LOEC, it will does no harm. However, at present, contaminants enter aquatic ecosystem is somewhat unavoidable. So it is more reasonable to ensure there is no significant effect rather than no effect. What's more, NOEC or LOEC depends much upon the concentrations that have been chosen. Comparatively, ECx is more reproducible and may be extrapolated from concentration response model. Because of this, Erikson et al[2] suggested to preset up an insignificant standard such as 10 percent or 20 percent first, then if the effect at PEC is statistically higher than that, it is unacceptable, otherwise acceptable. Also, EPA[3] has used EC_{25} as wastewater effluent standard.

In the traditional concentration response model, the factors involved are only probability and concentration. It is well known that many other factors such as water temperature, water pH, and

[收稿日期] 2000-12-04
[基金项目] 福建省教育厅科技项目(JA 01038)
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healthy situation of tested organisms may affect test results. But they are just regulated by a standard procedure, which has made test results of limited use. Another thing is the information from experiment is not fully used. For example, in outside aquatic ecosystem, fish can escape from contaminated area, so the response of fish at different treated time is worth mentioning. And this can be easily recorded during toxicity test. Unfortunately, all these information has been ignored.

Newman et al[4] proposed an alternative method named time-to-event toxicity test to analyze data. By this method, the effect of external factors such as water temperature, water physiochemical characters, healthy situation or ages of tested organisms on test results may be found, and the safe concentration threshold or time threshold also can be calculated out. But it needs no more extra labor work or cost. All these are just done by making full use of the data obtained from the traditional toxicity tests. The safety threshold is a little like the former NOEC, but it is of higher statistical value.

All these above methods do experiments directly on tested individuals, thus to do an experiment is always time consuming and may kill many individuals. As we know, nowadays, to evaluate the risk of a chemical or to set up a safety threshold for a chemical, effect on more than one species should be known. So if all work is conducted in these ways, it will take a long time and do too much harm. To find a more rapid, more accurate and more sensitive way becomes urgent. That is why microbiotest is used quite often not only in aquatic toxicology but also in other areas of ecotoxicology. Microbiotest is to determine chemical toxicity by means of biomarkers. A biomarker is a biological response to an environmental chemical which gives a measure of exposure, and sometimes also of toxic effect[5]. The biomarkers represent changes of some physiological or biochemical parameters including those of enzyme activities, cellular or subcellular structure. But the biomarkers at molecular level are mostly used. Its theoretical basis is that chemical toxicity always appears at molecular level first and the greatest similarity among different species exists at this level. Because chemicals have effect on both target sites and nontarget sites, biomarkers may be classified as exposure biomarkers and toxicity biomarkers. Exposure biomarkers represent changes of nontarget sites including MFO, GST and esterase. Because the relationship between toxicity and these biomarkers is not clear and they are not stable enough, they are not so useful. Toxicity biomarkers work better for they represent contaminant toxicity directly. AchE is an often-used biomarker of organophosphorus pesticides. To fish, if AchE is 20% inhibited, it is indicative of the existance of organophosphorus pesticides. If AchE is 50% inhibited it is life threatening and 70%- 85% inhibited the fish will die[6~10]. DNA adduct is the biomarker of mutagenic agents and carcinogenic agents. By using ^{32}P post-labeling technique, one adduct in 10^{10} normal nucleotides can be detected[11].

2 Population Level

Many studies confirm that acute mortality estimation is not very predictive of population growth[12]. But from a purely ecological point of view, a population decline is unacceptable[13]. So just as the National Research Council[14] has recommended that chemicals should be studied at the population, community and ecosystem level. According to their own study results, Nicholson[15] and Solobodkin et al[16] reported that losses even as great as 25% might have no long-term impact on a population while losses of 50% may result in only a slight change. On the contrary, Hallam et al[17] concluded that sublethal effects would result in extinction of a species. Despite their conclusion was different, their work has both proved that effect on individual level can not accurately represent that on population level. A population persistence ability is relative to survival chances of individuals (s), and population recruitment which is in turn dependent upon the time needed for an individual to grow to reproductive stage (t) and individual capacity to reproduce (number of offspring one individual re-

produces, n). S, t, and n make up of the components of a population size. To know the effect of a chemical on population, effect on these factors should be studied[18~20].

3 Community Level

Since 1980s, researchers have realized that it is insufficient to evaluate the toxicity of a chemical to aquatic ecosystem just by experimentation on fish. And this is the beginning of multispecies toxicity study. The involved species represent a food chain in an aquatic ecosystem, usually primary producers (algae), primary consumers (daphnids) and predators (fishes)[20].

After multiple species toxicity test, next work is to estimate a safety threshold for an ecosystem. Two ways may be adopted. One is to use safety factor and another is to extrapolate from simulated models.

The safety factor is between 10-1000, much greater than 2-3 fold differences in calculating endpoints. The factor is decided by the based information. Some factors are listed in Table 1[3, 21].

Table 1 Assessment Factors Applied To Derive Environmental Concern Level

Available information	Assessment factor
Lowest acute L(E)C_{50} or QSAR estimate for acute toxicity	1 000
Lowest acute L(E)C_{50} or QSAR estimate for minimal/algae/crustaceans/fish	100
Lowest NOEC value or QSAR estimate for chronic toxicity	10
Lowest NOEC value or QSAR estimate for chronic toxicity for minimal/algae/crustaceans/fish	10
LOAEL to NOAEL	10
Daphnia test result for regional water quality criteria	21.9

Note: LOAEL, Lowest Observant Adverse Effect Level; NOAEL, No Observant Adverse Effect Level.

Besides, safety thresholds also can be extrapolated from certain models, and this has become a more often used method. Isnard[22] based on his researches proposed an equation to extrapolate the safety threshold for aquatic ecosystem. The equation is

$$\log(\text{NOEC microcosm}) = [1.07 \times \log(\text{NOEC monosp})] - 0.25,$$

in which the NOEC monosp. is the lowest NOEC for single species. Results extrapolated from this are near to those from equation $Y = X$.

Factor method or Isnard equation is aimed to protect all the organism species in the aquatic environment. However, since effect of chemicals on aquatic system is somewhat unavoidable, it is not so possible or so necessary to protect all the organism species in the aquatic system. Many researchers agree that 95 percent is enough[23~25]. The equation Kooijman[23] proposed is

$$\log HC_5 = X_m - K_k S_m$$

in this equation, HC_5 is safety threshold for 95 percent species; X_m is the average log NOEC of different species, K_k is a constant varying with sample size and S_m is the standard error of X_m.

The equation of Aldenburg et al[25] has a same form with that of Kooijman except replacing K_k with K_{AL}, which also varies with sample size.

Some K_k and K_{AL} are listed in table 2.

Table 2 A list of K_k and K_{AL}

Sample size	K_k	K_{AL}	Sample size	K_k	K_{AL}
2	3.33	27.70	11	2.29	2.96
3	3.04	8.14	12	2.26	2.87
4	2.88	5.49	13	2.25	2.80
5	2.74	4.47	14	2.24	2.74
6	2.62	3.93	15	2.23	2.68
7	2.52	3.59	20	2.18	2.49
8	2.43	3.37	30	2.06	2.28
9	2.37	3.19	∞	1.62	1.62
10	2.32	3.06			

In table 2, at a same sample size, K_{AI} is always greater than K_k especially at smaller sizes, which indicates that at same sample size, safety threshold extrapolated from Aldenburg equation will be lower than that from Kooijman equation.

4 Ecosystem Level

To find the effect of chemicals on aquatic ecosystem is the final purpose of aquatic ecotoxicology. This is conducted by simulated ecosystems named microcosm or mesocosm system. Microcosm system and mesocosm system are both artificial systems, the greatest difference between them is microcosm system is studied in indoor conditions but mesocosm in outdoors. Microcosm is used more. Compared with the work at lower levels, studies on microcosm system can provide some more and unique information[26], including (1) indirect trophic-level effects e. g. increased abundance of species via increased food supply through fewer competitors or less predatoriness; (2) compensatory shifts within a trophic level; (3) responses to chemicals within the context of seasonal patterns that modify water chemistry and birth and death rates of populations; (4) effects of chemical transformations by some organisms on other organisms and (5) persistence of parent and transformation products.

The advantages of this artificial ecosystem method lie in its strong statistical power, high speed of analysis, high reproducibility among laboratories and modest expenses (compared with field studies).

Usually, a standard aquatic microcosm (SAM) system contains 10 algae and 5 invertebrate species such as Daphnia, ostracods, and amphipods. Fish is not added to avoid algae overgrazing.

5 Discussion

From individual to simulated ecosystem, toxicity test results become increasingly accurate. But the effect on field system should be always kept on close watch especially for those chemicals uneasily degradable or repetitively entering certain areas, because the procedure is conducted in completely indoor conditions which greatly differs from the variable outdoor conditions. Moreover, since the experiments are all designed by human beings, whether they are able to accurately predict the effect outdoors is not always so sure. For example, although in community or microcosm research, it is demanded that the involved species should represent different trophic levels, their representatives are not always stable. For sensitivity differences exist among different species to same or different chemicals even among those belonging to same trophic level and no general laws can be summarized. Another example is, based on the same data, the thresholds extrapolated with different methods will be different, then it is necessary to decide which one is most practical. Generally the factor method is strictest, but it is always unnecessary to choose the lowest one. The only way to determine is to detect in field situation.

As it has been pointed out that our task is not to protect all the organism species. In one area, many organism species always coexist, among which the interaction mechanism is not known for sure. So it is dangerous to draw a conclusion simply from the population sizes of studied species. To protect an ecosystem, our aims include, (1) maintaining biodiversity ensuring that there are no species extinctions (although it should be recognized that local extinctions are a natural albeit usually slow process, associated with species ranges and environmental change); (2) maintaining a certain degree of functionality: e. g. production of a certain biomass of invertebrate food species for fisheries; (3) protecting a certain type of habitat from degradation (wetland, areas of outstanding natural beauty, sites of special scientific interest); (4) protecting rare, threatened or endangered species[27].

It has been mentioned that test results are more accurate in larger experiment scale. But that does not mean that we should conduct experiments at all these levels each time. For those chemicals that can be easily degraded or only enter aquatic

system occasionally and the tested organisms have a long life history, the acute toxicity on individual level result may represent the toxicity on population level well. If the chemicals have been proved to be very safe at individual level, it is no need to do any more work.

As time goes on, lethal effect on aquatic organisms will become rarer and rarer. Most effect is sublethal, and then the recovery study is more important. For example, carbamate and organophosphorus pesticides are all AchE inhibitors. At sublethal concentration, carbamate pesticides inhibited AchE may recover very soon but organophosphorus pesticides inhibited recover very slowly and sometimes even impossible. DNA adduct is also an important biomarker, but it can also recover despite the recovery rates may be slow[13]. Because many chemicals other than mutagenic agents and carcinogenic agents can cause DNA adduct, too. Considering this, risk evaluation will become more accurate. Many factors affect recovery rate. At individual level, contaminant source, contaminant concentration, exposure time and water temperature all can affect recovery rate. At higher levels, the recovery ability is different in different sites and different seasons. It is higher in open areas than in enclosed ones because external populations may immigrate in. It also recovers faster when population size is larger and the reproducibility is higher.

Another thing is that different contaminants may enter aquatic ecosystem simultaneously or alternatively. Interaction among them is important, too. No one doubts that ecotoxicity of contaminants might be changed because of interaction. However, studies on this area are still limited. Besides, our work has shown that recovery rate may also be changed by interaction[28~29]. In one word, studies on interaction should be conducted more.

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水生生物的生态毒理学研究程序

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[摘　要] 个体、种群、群落、生态系统是水生态毒理学研究的 4 个层次, 实验系统的复杂性也按此顺序增加。本研究总结了这 4 个层次的毒性评价方法。无疑, 高层次的研究结果准确性更高, 但若在较低层次即便是在个体水平上能证实污染物的安全性, 就没有必要再做更多工作。根据这些年的研究, 由于污染物的亚致死影响越来越普遍, 故有毒影响的可恢复性及恢复速度在危险性评价中就显得更为重要。另外一点是必须关注污染物间因相互作用对毒性效应及其恢复的影响。最后由于实验室条件与田间实际情况存在差异, 故所有实验结果都必须经实际检验。

[关键词] 水生态毒理学; 水生生物; 生态毒性; 危险性评价
[中图分类号] Q 948. 8　　[文献标识码] A　　[文章编号] 1000-2782(2001)06-070-06