Chapter 12
Further Perspectives

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12.1 Analysis and Assessment of the Microcosm N-System for OECD Standardization

Positioning of the various docimasy of the bioassay and the fundamental utility of the microcosm for ecosystem impact assessments are shown in Figs. 12.1 and 12.2. In addition to various factor analysis at the ecosystem level using the microcosm N-system, the combination of the general interaction analysis through the use of a simulated environmental microcosm (mesocosm) and the underlying interactions analysis through both the two-species cultures and the single-species cultures, are important for the advancement of ecosystem impact assessment. It is the microcosm test that becomes essential for evaluating ecosystem impacts, including those on the human body, based on the study of marmots. Additionally, although the docimasy varies in various bioassays, the microcosm test remains at the core.

A procedure for assessing the impacts of chemical substances by culturing, data collection, and branching-type analyses of variance using the microcosm N-system docimasy is shown in Fig. 12.3. Specific examples of the evaluation in this docimasy are shown below.

1. Environmental assessment of 2,3,4,6-tetrachloro phenol (TCP), an insecticide, sterilizer, wood preservative, and formicide. The most susceptible creature, water

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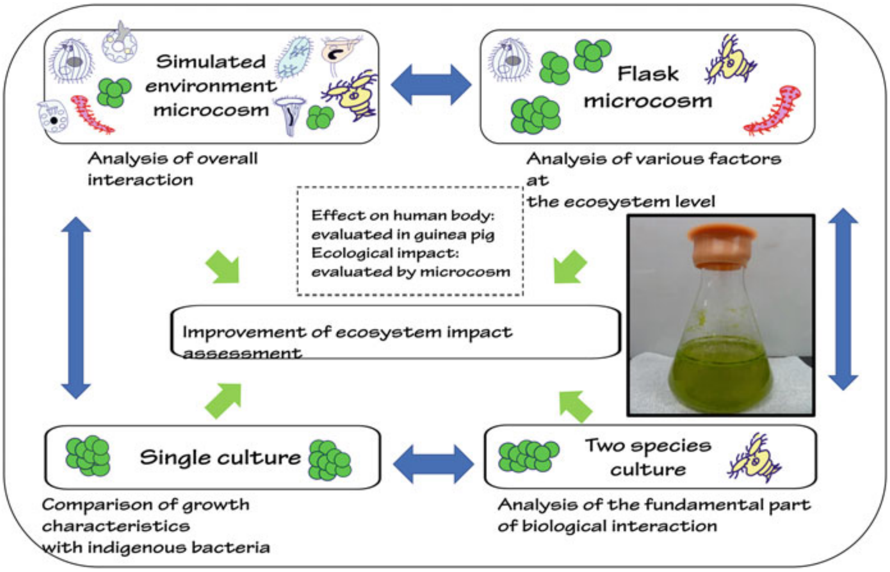

Fig. 12.1 Basic principle for optimizing of environmental impact risk assessment

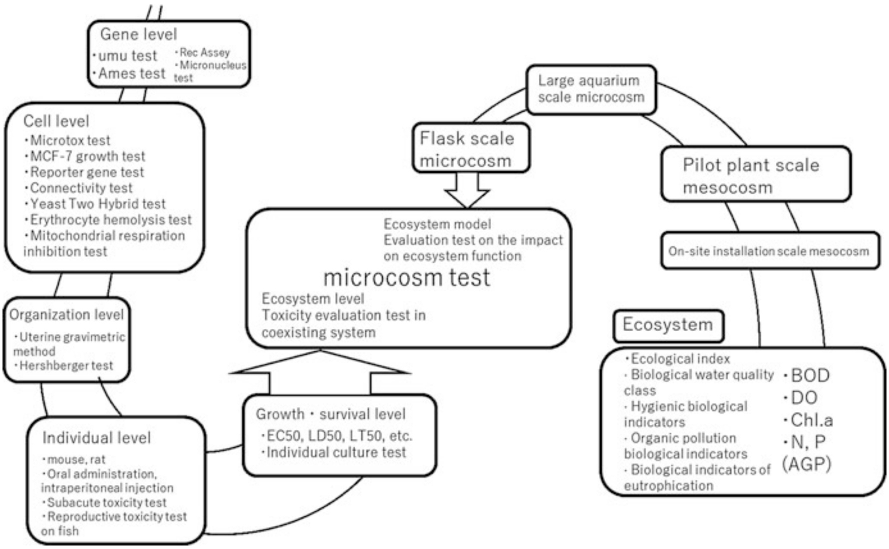

Fig. 12.2 Placement of bioassay test and microcosm test

fleas; ECOSAR class, phenol. The amount of production and respiration decreased even with the addition of 1 mg/L (Fig. 12.4). The amount at which the influence on production became 0 was 10 mg/L, and it was strongly indicated

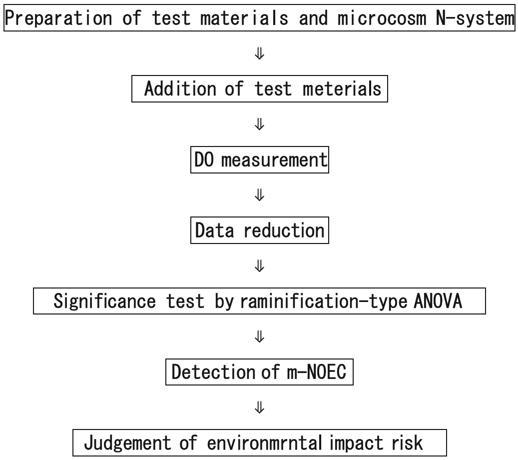

Fig. 12.3 Flowchart of microcosm test for environmental impact risk assessment

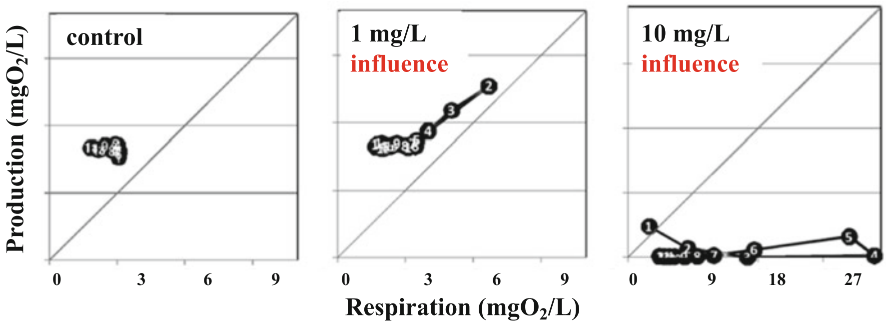

Fig. 12.4 Time course of P/R ratio in TCP added microcosm

that TCP had a greater influence on production than on consumption. As a result of having let match document investigations, a similar tendency was shown in the mesocosm examination, and there was a close association found in the final examination.

2. Environmental assessment of carbendazim, a fungicide. The most susceptible creature, water fleas; ECOSAR classes, imidazoles and carbamate esters. In both systems, it was at approximately one that the P/R ratio stably fluctuated, but, after recovery following attenuation in the 10 mg/L addition system, the amplitude increased with the addition of 20 mg/L (Fig. 12.5). The m-NOEC of the Benrate hydration agent was estimated as ~20 mg/L. Because a Benrate hydration agent is the main product of carbendazim and is composed primarily of ~50% benomyl, and the benomyl is converted into carbendazim with a 2-hour half-life in water, the m-NOEC of the carbendazim is thought to be ~10 mg/L.
3. Environmental assessment of chlorpyrifos, an insecticide. The most susceptible creature, water fleas; ECOSAR classes, esters and monothiophosphates. There

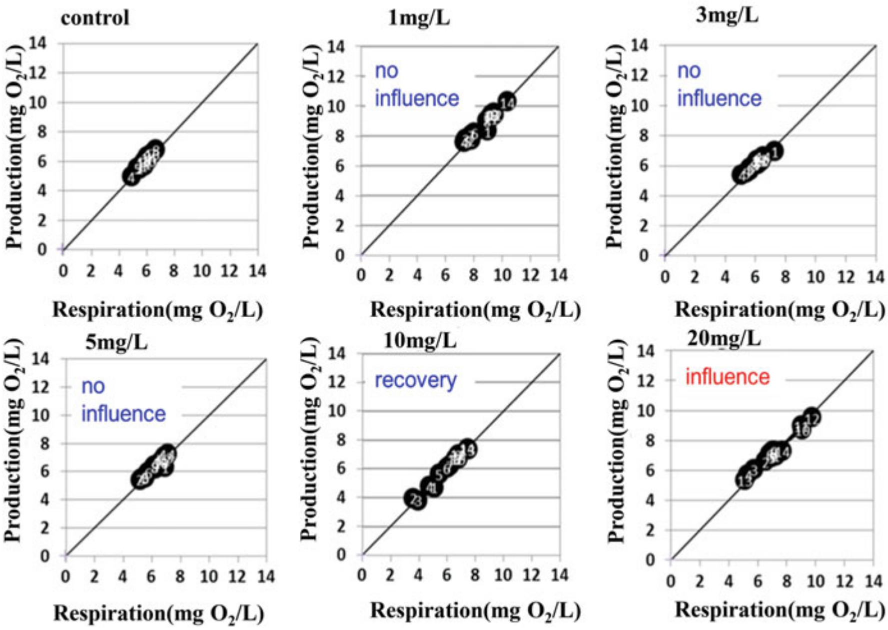

Fig. 12.5 Time course of P/R ratio in carbendazim added microcosm

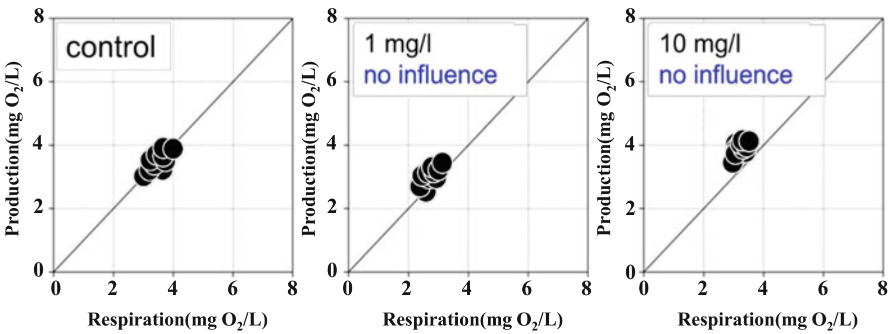

Fig. 12.6 Time course of P/R ratio in chlorpyrifos added microcosm

was no influence on the production (P) or respiration (R) in either concentration zone (Fig. 12.6). However, chlorpyrifos has notable hydrolysis and soil adsorption characteristics, and intermittent administration is performed in outdoor dispersion experiments (i.e., in natural ecosystem experiments/mesocosms). It is thought that the difference between the experimental conditions is related to the presence or absence of its influence.

4. Environmental assessment of alachlor, a weed killer. The most susceptible creature, algae; ECOSAR class, haloacetamides. There was no statistically

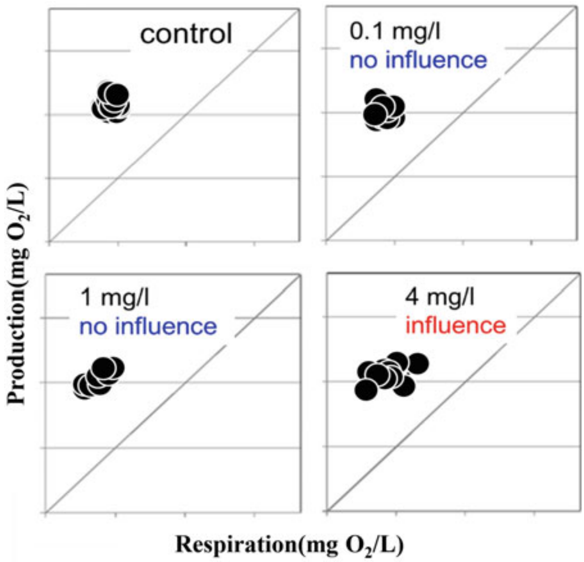

Fig. 12.7 Time course of P/R ratio in alachlor added microcosm

significant difference at concentrations of 0.1 mg/L or 1 mg/L, though significant differences were detected at 4 mg/L (Fig. 12.7). The m-NOEC of the alachlor was 1–4 mg/L.

5. Environmental assessment of linuron, a weed killer. The most susceptible creature, algae; ECOSAR class, substituted urea. It was observed that a nearly stable state was reached at a concentration of 0.1 mg/L, but the amount of production and respiration decreased in all of the systems with more than 0.5 mg/L (Fig. 12.8). Thus, the m-NOEC of linuron was estimated as 0.1 mg/L in the impact assessment based on the P/R ratio.

The results of the impact assessment of P and R data by a ramification-type ANOVA using MS Excel revealed that there was no influence at addition concentrations of 1 mg/L of TCP, 20 mg/L of carbendazim, 4 mg/L of alachlor, or 0.5 mg/L of linuron. There was also no influence with either addition concentration of chlorpyrifos.

Chlorpyrifos, which deviated from the confidence interval from the correlation between the NOEC of the microcosm N-system test and the NOAEC of the outdoor ecosystem test (mesocosm test), exhibited substantial soil adsorption. Additionally, paraquat, which is a weed killer, also deviated from the correlation of this test and the outdoor experiment but can protect nature ecosystems because it is above the value divided by the safety factor (200). Because chlorpyrifos is less toxic in the mesocosm experiment without soil, and the microcosm N-system test also does not have soil, it was estimated to be less toxic in the microcosm test as well. Therefore, it is necessary to consider the influence of the degradability of the test material and the soil.

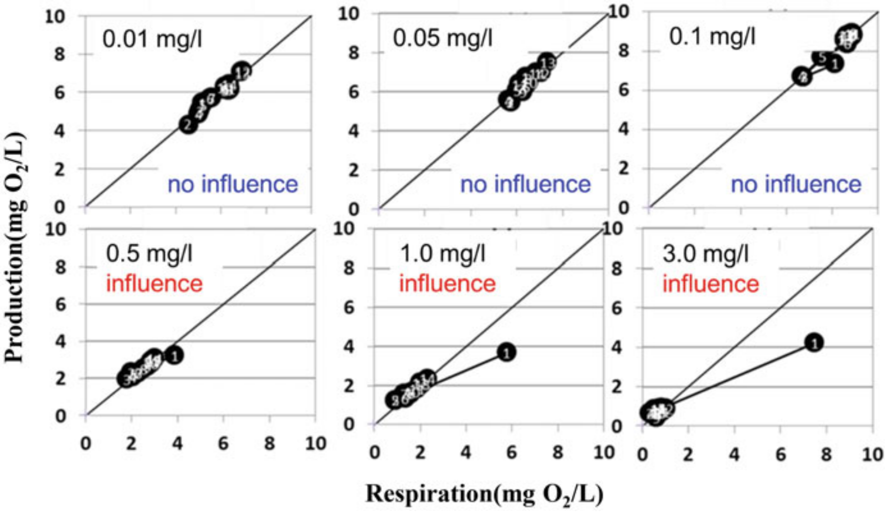

Fig. 12.8 Time course of P/R ratio in linuron added microcosm

Based on the results of these evaluation procedures, widespread chemical substances were grouped based on the characteristics of each chemical substance. The accumulation and inspection of practical ecosystem impact data can support and render possible the OECD international standardization of the microcosm test. When considering the past flow of OECD standardization, the following are needed: (1) confirmed reproducibility by blind testing of the same materials by three institutions, (2) inspection of the versatility using the ring test performed by five domestic institutions, (3) construction of a practical infrastructure by compiling the m-NOEC database, (4) making the general examination methods accessible by completion of the test manual, and (5) inspection of the practicality of the microcosm N-system by developing a NOEC prediction method.

For steps 1–4, the reinforcement, careful investigation, and efficiency of microcosm experiment should be improved, and the microcosm test manual should be accuracy based on the characteristics and addition time of the chemical substances to microcosm, that is, at a steady state or at culture starts. With these procedures, the generalization and practical realization of the microcosm test is possible. For step 5, the database of m-NOEC of the microcosm test and the NOECeco of the mesocosm test will be expanded, the applicable range of the microcosm will be established, and the unevenness of each chemical substance from the correlation formula, the difference in the sensitivity of each ecosystem, and the rationale behind the assessment factor have been already established. Furthermore, the limits of the microcosm test suggested in this study may be clarified based on the evaluable characteristics of chemical substances. It has already been clarified whether this technique can be used to extrapolate to natural ecosystems. In other words, the utilization of this microcosm test and strengthening of the manual are made possible by the expansion of the database, which is the basis for the analyses shown in this manual and which is necessary for expanding the applicable range and optimization of the test method. A close inspection of the assessment factor reinforced in this manual has already been completed.

In the future, the microcosm test will be established as a practical ecosystem impact assessment technique, replacing the mesocosm test by further grouping chemical substances after having thoroughly investigated their characteristics. Because it is anticipated that the correlation between natural ecosystems and microcosms is high, it is thought that the estimated NOEC is more realistic than that derived from evaluations using single-species. It currently remains difficult to omit the test methods for algae, crustaceans, and fish represented by the WET test in the ecosystem impact assessments of chemical substances. However, by using a parallel ecosystem model that includes a food chain and energy flow, it becomes possible to accumulate knowledge, such as that related to the degradability and residual properties of chemical substances, and the recovery and collapse of ecosystem functions. In other words, it may be said that the superiority of the microcosm is enhanced by quantifying and evaluating ecosystem impacts. The WET test is a docimasy for evaluating toxicity that includes combined influences by testing water containing multiple chemical substances, but it is a single-species test that does not allow for the evaluation of individual chemical substances. Although it uses species in different niches in the food chain for an assay, it must be noted that the WET test has a problem when testing under conditions in which there are interactions among material circulations, energy flow, and biological interaction, which are the foundations of natural ecosystems. The microcosm, which is a system of multiple, coexisting species, evaluates the risk of chemical substances at an ecosystem level, and the safety factor is obtained from the results of research on the correlation between the conventional mesocosm test and microcosm test before the PNOEC can be calculated from the accumulation of further data. The merit to the regulations of the chemical industry is remarkable.

The need for evaluating the introduction of chemical substances in model ecosystems has been noted by the OECD and other chemical substance management entities, and regulatory laws and model systems are being developed. However, an international standard for ecosystem impact assessments using mesocosms and microcosms has yet to be undertaken. The methods described in this book allow for the influence of chemical substances on ecosystems to be evaluated in the microcosm, and the effects on the human body, by using marmots and mice, were demonstrated using the microcosm N-system. It is possible to determine that there is no risk to the ecosystem by the docimasy, analysis, and evaluation of the NOEC described in this book. Because a standardized microcosm test could be constructed, this approach should be utilized globally.

12.2 Summary and Further Perspectives

Although ecological risk assessment has been conducted on the risk posed by toxic chemicals to an individual species, the microcosm method can assess the state of recovery and change of an ecosystem by fragmentation, which is not shown in single-species tests. Further, the microcosm test can be used to conduct assessments equivalent to those of conventional mesocosms, measuring changes in the abundance of organisms. The scientific importance of being able to continuously measure changes in the function of an ecosystem is great. This study suggests that even when a microcosm experiences structural effects, the functional stability of the ecosystem can be maintained due to the redundancies at the population or community level. This shows that when a functional effect is emphasized in an ecosystem impact assessment, proper assessment cannot be conducted by measuring structural parameters (species composition, abundance, etc.), which are generally measured, and that it is necessary to measure functional parameters directly.

The microcosm test method was shown as effective for assessing the impacts of biological interactions, which might be overlooked in single-species tests. In particular, there were protozoans and metazoans in the microcosm, which have not conventionally been used, and it was indicated that the existence of these taxa was important for evaluating the influence on the microbial ecosystem. Additionally, it was clarified that the P/R ratio assessment method using the model ecosystem hydrosphere (microcosm) could acquire the basic information needed to determine the influence of various chemical substances on the ecosystem and that it was a useful technique for protecting natural ecosystems using the appropriate management of chemical substances. As for this assessment, an experimental method and data analysis techniques are made available in the manual. Moreover, this ecosystem impact assessment technique has become frequently used in Japan, and it offers the possibility for OECD standardization. It may be said that the significance of this approach for international environmental policy is extremely high. Additionally, the importance of the patterns and environment impact assessment was adopted in the final report of a task group of the Association of National Irradiation Ecology, titled "Ecosystem approach to environmental protection," and this report may possibly have a substantial influence on the International Commission on Radiological Protection (ICRP) or the Organization for Economic Cooperation and Development Nuclear Energy Agency (OECD/NEA). It is expected that the microcosm test method will contribute to an environmental radioprotection policy in Japan through the advice of these international organizations, and it is thought that the ripple effect could be tremendous. Additionally, it is at an early stage of development by the lead laboratory and the domestic ring test in Japan, and it is the establishment and unification of the test method (protocol), inspection (ring test) of the plasticity between facilities, construction of the database, and the clarification of the application range that promote its prospects as an OECD standardized test method with future development of the microcosm test. At this stage, it is necessary to improve the database, inspect correlative analyses with natural ecosystems (mesocosms), and evaluate the relationship with known toxic data. Having passed through these tasks, it will be established as a public, fixed method and could have wide utility after validation by other institutions and peer review (international examination). Furthermore, by following the WET test method and utilizing the results provided by this project to their maximum, it is expected this technique will provide new environmental policy information from Japan for development as an international, standardized OECD test.

In the optimized microcosm, producers, predators, and decomposers are properly structured, and they can be used for highly accurate environmental impact assessments. It can be said that the optimized microcosm test system is not the current OECD test method for individual organisms, such as *Daphnia* or algae, utilized for the WET test, but a test system that approximates natural ecosystems as closely as possible. Therefore, more realistic results at the ecosystem level are expected to be obtained by using the microcosm test method. In order to determine the standard of treated water quality in wastewater treatment, it is important to consider how the trace substances in the treated water affect the aquatic ecosystem at the discharge destination. Also, in promoting environmental impact assessment, it is necessary to evaluate various chemical substances that may affect aquatic ecosystems. According to the OECD test method, representative species are selected one by one from each trophic level of the ecosystem (e.g., *Selenastrum* (producer), *Ceriodaphnia* (primary predator), *Danio rerio* (higher predator), luminescent bacteria (decomposers), etc.), toxicity assessments are then performed, and then the ecosystem impact is calculated based on the toxicity data for the most sensitive species. However, in natural ecosystems, the biological activity of test organisms is different from that of single-species tests because there are biological interactions, material circulation, and energy flows in natural ecosystems. Therefore, toxicity assessments under different biological active conditions are insufficient, particularly as impact assessments of ecosystem function. For this reason, it has been noted that the OECD should conduct various biological tests. It is very important to develop an evaluation tool from aquatic ecosystem models (Takamatsu et al. 1995). To evaluate the toxicity of chemical substances to ecosystems with complex biological interactions, microcosms of model ecosystems composed of producers (algae), predators (microanimals), and decomposers (bacteria) are useful tools. Whole aquatic ecosystems are composed of food webs (i.e., biological interactions, including high-order predators, such as fish), based on microbial loops that are mainly composed of microbes. To evaluate the effect of chemical loads on complex ecosystems, it is important to consider multiple parameters, including the production (P)/respiration (R) ratio. Establishing risk management methods for ecosystems based on environmental changes and the analyzed relationships between the microbial organisms and higher predators that constitute aquatic ecosystems becomes very important (Fig. 10.1). For ecosystem impact assessments, it is effective to comprehensively utilize the kinetic analysis of microbial communities using the microcosm and other multi-species mixed model ecosystems (stable model ecosystems).

This research was conducted to establish a standard test method characterized by both high plasticity and low cost using the microcosm that has been stably subcultured for more than 40 years. The basic manual that affected the testing operations was already built (Ministry of the Environment, Environment study synthesis promotion costs: FY2009–FY2011), and it was frequently used to perform tests between facilities. The OECD examination guidelines progressed through the ring tests, and the establishment of an OECD standard docimasy was planned. The outcomes obtained from this investigation are listed below.

1. Screening test of chemical agents using the microcosm

   The experimental test was advanced by screening 198 listed chemical substances into three groups, those that acted on animals, plants and algae, or bacteria, according to differences in their mechanisms. As a result, the foundational information of an OECD test was provided because the microcosm test did not depend on the classification of chemical substances as mentioned previously, the sensitivity was higher than in a single-species test, and it was strongly correlated with the mesocosm test.
2. Environmental impact assessment of chemical agents using the microcosm

   For establishment as a practical ecosystem impact statement technique for the mesocosm test method, the examination of widespread chemical substances was promoted. A positive ion surfactant, TMAC (1000 mg/L); an environmental hormone, nonylphenol (100 mg/L); a sterilizer, mancozeb (300 mg/L), the weed killers, alachlor (1000 mg/L), linuron (0.1 mg/L), and fomesafen (30 mg/L); and an insecticide, chlorpyrifos (500 μg/L), which were provided for microcosm examination (m-NOEC), were matched with published data, and most of the 26 substances examined exhibited similar behaviors to those in the field experiment (mesocosm test). It became clear that there was a strong association between the microcosm and the mesocosm.
3. Analysis of the relationship between the microcosm test and the mesocosm test

   As described in Chap. 8, the validity of the toxicity evaluation by the microcosm test was considered, and it was shown that 23 of 26 substances adhered to the confidence interval of the regression line and the strength of the correlations among the microcosm, experimental ecosystem (mesocosm), and the natural ecosystem was demonstrated by the means of the m-NOEC and the NOEC of the natural ecosystem. The lower limit of the confidence interval is also a straight line obtained by dividing the mean of the natural ecosystem NOEC by the uncertainty coefficient (coefficient of assessment = 200) calculated in consideration of the differences in ecosystem sensitivity. Only the lower limit line is required to determine the PNOEC of a natural ecosystem, and, if the point on the correlation plot is greater than this (i.e., above the lower limit line), then it is measured in the microcosm (PNOEC) obtained by dividing the NOEC by the coefficient of assessment (200). With a value that is lower than that of the natural ecosystem (mesocosm test) PNOEC, it can protect the natural ecosystem. Most chemicals measured in this investigation fit into this range (i.e., above the lower limit line). On the other hand, while substances exceeding the upper limit can predict the PNOEC, the value is less than in natural ecosystem NOECs. Only 1 substance out of 26 fell above the upper limit line, revealing that precision is high in the microcosm test, regardless of the chemical substance in question.

4. Comparison and interface of the microcosm and mesocosm

   As described in Chap. 8, the two substances that deviated from the confidence interval of the correlation between the mean NOEC of the natural ecosystem and the m-NOEC are characterized by high soil adsorption. Based on the results of this research, it is necessary for most environmental impact assessments of chemical substances to show the limits of the microcosm and plan for OECD international standardization. The outcomes of this investigation are shown on the next page as a poster presented on August 28, 2014 (study briefing session on LRI). The need for developing evaluation techniques for the introduction of chemical substances into model ecosystems has been noted by chemical substance management organizations, including the OECD, and regulatory laws and model systems are being established. However, international standardization of the ecosystem impact statement technique using the mesocosm and microcosm has not yet been completed. The standardization of the microcosm test method for environmental impact assessment should be promoted strongly and as soon as possible.