Studies on ecological risk assessment of pesticide using species sensitivity distribution

Takashi NAGAI
Institute for Agro-Environmental Sciences, NARO, 3–1–3 Kannondai, Tsukuba, 305–8604, Japan
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Species sensitivity distribution (SSD) is a key concept of probabilistic analysis for quantifying ecological risk. I developed a method of probabilistic ecological risk assessment in Japan with a case study of the herbicide simetryn. Then, risk comparison among eleven herbicides was conducted using the developed method. However, one of the most important limitations of SSD application is the lack of sufficient toxicity data for SSD analysis. Thus, an ecotoxicity database was developed for the application of SSD to a wide range of pesticides. After that, I proposed that species batteries of the five species should be the standardized dataset for the SSD analysis of insecticides and herbicides. Finally, I have published a technical guidance document for SSD analysis written in Japanese to promote the application of SSDs in Japan. The remarkable point is that the supplemental Excel-based tool makes it easy to analyze SSDs and conduct ecological risk assessments. © Pesticide Science Society of Japan

Keywords: database, algae, arthropod, risk management.

Introduction

Recently, there has been concern about the potential degradation of biodiversity caused by inadequate pesticide use. Governments are encouraging eco-friendly (sustainable) agriculture with reduced pesticide use. However, scientific evidence is still insufficient regarding the relationship between reducing pesticide use and actual environmental conservation outcomes.

To conserve the ecosystem, the objective should not be "reducing the amount of pesticide use", but "reducing the ecological risk". Excessive reduction of pesticide use would increase risks to agricultural production, thus contradicting the purpose of sustainable agriculture. Farmers take various measures to protect biodiversity, such as reducing their use of pesticide, switching to safer pesticides, and preventing pesticide runoff into river water. The efficiency of various risk management options should be quantitatively evaluated. This information would be useful for determining the effective risk management option. Such a risk analysis is proposed as a "solution-focused risk assessment" and is different from a deterministic risk analysis (good or bad) assessment at the pesticide registration examination.

To quantify the ecological risk, probabilistic analysis is useful. This is a method for evaluating risk as an exceedance probability of environmental concentration to the toxicity of aquatic organisms by considering the uncertainty of toxicity and pesticide exposure. For example, Fig. 1 shows the variability of species sensitivity and environmental concentration, and the probability of concentration > sensitivity as the result of round-robin comparisons between five species and six concentrations. Three of 30 combinations indicate potential effects, and therefore the potentially affected fraction is calculated to be 10%. The magnitude of the ecological risk can be compared using the value as the probability.

The present paper describes the concept of species sensitivity distribution (SSD) as a key concept of probabilistic analysis and introduces the application of SSD to ecological risk assessment.

1. Species sensitivity distribution

1.1. Differences in species sensitivity

In Japan, pesticide registration criteria based on ecological risk assessment are set by Japan's Ministry of Environment under the Pesticide Regulation Law. Under the risk assessment scheme, acute toxicity tests are conducted for fish (*Cyprinus carpio*), daphnids (*Daphnia magna*), and algae (*Pseudokirchneriella subcapitata*), and then the acute effect concentration is determined as the minimum value of the 50% effect concentration (EC$_{50}$) or the 50% lethal concentration (LC$_{50}$) divided by an uncertainty factor that considers the species sensitivity difference (default 10, but depends on the data number for fish and crustaceans and 1 for algae). Subsequently, the predicted environmental concentration (PEC), which is the peak concentration in river water at
the time of pesticide application, is calculated using an environmental model based on a standard scenario in a model basin. Finally, if the PEC is less than the acute effect concentration, the short-term aquatic risk is deemed to be insignificant and the pesticide is considered to fulfill the criteria.

There are various organisms in aquatic ecosystems, and pesticide sensitivities are dramatically different among species. In the case of the insecticide imidacloprid, the toxicity of the three species (fish LC$_{50}$ > 105,000 µg/L, daphnid EC$_{50}$ = 85,000 µg/L, and algal EC$_{50}$ > 98,600 µg/L) resulted in the registration criteria of 85,000/10 = 8500 µg/L. On the other hand, acute toxicity data for the insecticide imidacloprid was obtained for 27 genera of freshwater organisms (Fig. 2). If the environmental concentration is just below the registration criteria (8500 µg/L), most arthropod species would be affected. *Daphnia magna*, which is generally sensitive to insecticides and therefore is regarded as the standard species for toxicity testing of arthropods, is not always sensitive, especially to neonicotinoids. Moreover, it is noted that the EC$_{50}$ and LC$_{50}$ values ranged from 0.65 to 85,000 µg/L (>10,000-folds) for arthropods. This drawback of the most sensitive species approach has been argued for more than 30 years. Instead, a species battery approach, which combines species that represent an aquatic community, is essential for properly assessing the effect of pesticides that have a wide variety of species sensitivities.

1.2. Species sensitivity distribution

Species sensitivity to environmental contaminants varies markedly, and this variation can be described by the statistical distribution (often a log-normal distribution) estimated from sample data.

![Fig. 1. Conceptual diagram of probabilistic ecological risk assessment.](image)

![Fig. 2. Genus mean acute values of acute toxicity (EC$_{50}$ and LC$_{50}$) of imidacloprid for each taxonomic group.](image)

![Fig. 3. Conceptual diagram of SSD. The variability of toxicity values (EC$_{50}$ or LC$_{50}$) of six species is fitted to a log-normal distribution. Arrow 1 indicates the derivation of HC$_{50}$ and arrow 2 indicates the calculation of the PAF from the pesticide concentration.](image)
pled toxicity data (EC50 or LC50s) and visualized as a cumulative distribution function (Fig. 3).6) This is termed SSD.

SSD has been used to determine hazardous concentrations so as to ensure the protection of ecosystems and to reveal ecological risks. The fifth percentile of a distribution (called the 5% hazardous concentration, HC5) has been used in the United States, the European Union, and Australia to derive threshold concentrations that protect most species in a community.7–9) Semi-field experiments (microcosm/mesocosm) have provided more realistic ecological effects of pesticides than single-species toxicity experiments in the laboratory. Several studies4,10–13) have shown that the HC5 values based on acute toxicity were protective against adverse ecological effects with single short-term exposure in freshwater microcosm/mesocosm experiments. Their results indicate the validity of HC5 values as predicted no-effect concentrations in aquatic ecosystems. The SSD approach has now been adopted by a tier system in the European pesticide risk assessment scheme.14)

The toxicity data for insecticides were separated into arthropods and other species because arthropods are the most sensitive taxonomic groups to insecticides, and the SSD showed a clear separation of arthropods from other species (Fig. 4).4,10) The toxicity data for herbicides were separated into primary producers and other species because primary producers are the most sensitive taxonomic group to herbicides, and the SSD showed a clear separation of primary producers from others (Fig. 4).11,15) The toxicity data for fungicides were all used for SSD analysis because the sensitivity difference among the taxonomic groups was not clear.12) The HC5 values for imidacloprid were calculated to be 0.43 (90% CI of 0.073–2.4) µg/L; this value is more than 10000 times lower than the registration criteria.4)

2. Development of probabilistic ecological risk assessment

2.1. Probabilistic ecological risk assessment using uncertainty analysis: A case study for simetryn

SSD has also been used for the probabilistic ecological risk assessment of pesticides (Fig. 1), such as diazinon16) and aldicarb17) insecticides and atrazine herbicide.18) The potentially affected fraction (PAF) was used as an index of the magnitude of ecological risk. Specifically, it is defined here that species are affected by a pesticide when the environmental concentration is greater than the EC50 or LC50 values. The PAF represents the effect on species diversity, which is a quantitative index of the biodiversity effect.

I conducted a probabilistic ecological risk assessment of the

![Fig. 4. Typical SSD curves for sensitive and insensitive taxonomic groups of freshwater organisms. Genus mean acute values and cumulative probabilities were plotted as the x-axis and y-axis, respectively. The maximum likelihood estimation (solid line) with a 90% confidence interval (dashed line) of the log-normal distribution is shown.](image)

![Fig. 5. Comparison of PEC distributions and SSD for freshwater algae (left) and the joint probability curve (risk curve) for simetryn (right). The area under the curve (shaded area) is the EPAF.](image)
herbicide simetryn which is used in Japanese paddy fields, as a case study. The EC₅₀ values for 31 algal genera were fitted to a log-normal distribution, and the HC₅ was estimated to be 8.2 µg/L (Fig. 4). The PEC of simetryn was calculated to be 0.71 µg/L using an environmental model and the standard scenario as defined by the Ministry of Environment, Japan. Then, the distribution of the PEC was quantified using Monte Carlo analysis considering the regional variability of environmental model parameters such as the paddy rice cropped area, river flow, pesticide usage ratio, soil density, and soil organic matter content. As a result, the mean of the PEC was 0.77 µg/L, and the 95 percentile was 2.8 µg/L. The joint probability curve was derived by comparing the SSD and the distribution of the PECs, and the probability of exceeding HC₅ was estimated to be 1.5% (Fig. 5).

2.2. Comparison of ecological risks of 11 paddy herbicides

Paddy rice is the most important crop, and paddy fields occupy more than half of the total agricultural land in Japan. Many paddy herbicides are used to prevent weeds after rice is planted. From a few percent to more than 50% of the applied pesticide runs off from paddy fields into open aquatic systems through drainage channels. Therefore, the runoff of paddy herbicides is greater than that of upland field pesticides. Thus, a probabilistic ecological risk assessment of 11 herbicides commonly used in Japanese paddy fields was conducted. The effect assessment was based on the SSD. The acute EC₅₀ values of standard toxicity tests for aquatic primary producers were collected from the available literature and then fitted into log-normal distributions. The PEC was calculated using an environmental model defined by the Ministry of Environment, Japan. The regional variations of the PEC were quantified using Monte Carlo analysis the same as above. A joint probability curve was derived by comparing the SSD and the PEC distribution, and the area under the curve was defined as the expected potentially affected fraction (EPAF) as the quantitative risk index (Fig. 5). EPAF means the national average PAF and is useful for comparing various pesticide risks. The EPAF values for 11 herbicides are shown in Fig. 6, and the highest EPAF is 6.2% for bensulfuron-methyl.

2.3. Application of probabilistic ecological risk assessment

Probabilistic risk assessment would be useful for considering effective risk management option. However, it should not be used for the deterministic (good or bad) evaluation. Moreover, it should not be used only to establish the risk ranking. Risk management should not simply involve prohibiting the use of high-risk pesticides, because risks are always a tradeoff. Therefore, risk tradeoff must be considered whenever proposing alternative actions. Moreover, overemphasis on a specific species is dangerous to ecosystem protection because the effects on other species may be neglected. An action that protects a specific species may have side effects on other species. This is another kind of risk tradeoff. Therefore, the effect on biodiversity covering a wide range of species must be evaluated. The SSD approach is very useful for comparing the ecological risks of pesticides considering risk tradeoffs.

3. Development of an ecotoxicity database and SSD

3.1. Development of an ecotoxicity database

Data requirements for SSD analysis range from n=4 to 10, depending on the organization. A number of reports support the validity of SSD analysis using data from five species. Therefore, the minimum data requirement for SSD analysis in my study was set at five for each most-sensitive taxonomic group, as described above (arthropods for insecticide, primary producers for herbicides, and all species for fungicides). However, one of the most important limitations of SSD application is the lack of sufficient toxicity data for SSD analysis. The development of an ecotoxicity database is an important issue for the application of SSD to a wide range of pesticides.

Unique characteristics of pesticide use in Japan is a large amount use of paddy pesticides. Their ecotoxicity data is highly insufficient because they are not largely used in Western countries. Moreover, Japanese literature about the ecotoxicity of paddy pesticides has not been incorporated into Western ecotoxicity databases. Therefore, I developed an ecotoxicity database for paddy pesticides used mainly in Japan. The reliability of the information is categorized into four classes according to...
the Organization for Economic Cooperation and Development (OECD) Manual for the Assessment of Chemicals: 1 = reliable without restrictions, 2 = reliable with restrictions, 3 = not reliable, and 4 = not assignable. The reliability of data in original papers in scientific journals was evaluated according to the method proposed by Hobbs et al.23 In their method, the quality of data presented in published research papers is assessed by awarding scores based on a series of criteria or questions designed to ascertain the scientific rigor of the testing. Their method is robust and independent of the assessors for evaluating the quality of aquatic toxicity data.23 Collected acute toxicity data (EC50 and LC50) were entered in a database, and only the data evaluated as having reliability of “1” or “2” were used for SSD analysis.

As of May 2017, I had 2563 records (1455 for insecticides, 176 for fungicides, and 932 for herbicides) in my ecotoxicity database. The number of pesticides was 82. From the viewpoint of taxonomic groups (216 of all species), the database includes data of 585 primary producers (algae+vascular plants), 1104 arthropods, 678 vertebrates (fish+amphibian), and 196 others (such as shellfish and oligochaetes).

3.2. Standardized dataset for arthropods
I proposed a species battery of the following five species: the water flea Daphnia, the amphipod Hyalella, the freshwater shrimp Paratya, the chironomid Chironomus, and the caddisfly Cheumatopsyche, as the standardized dataset for insecticide SSD analysis. This is because official test guidelines or standard test protocols are available for the five species.24,25 To validate the SSD analysis using data of the five species, SSDs using all taxonomic groups (216 of all species), the database includes data of 585 primary producers (algae+vascular plants), 1104 arthropods, 678 vertebrates (fish+amphibian), and 196 others (such as shellfish and oligochaetes).

3.3. Standardized dataset for algae
As for the algae, I also proposed a species battery of the following five species as the standardized dataset: cyanobacteria Pseudanabaena galeata, green alga Desmodesmus subspicatus, diatom Achnanthidium minutissimum, diatom Nitzschia palea, and diatom Navicula pelliculosa.21 These species were selected because they (1) are widely distributed and frequently observed in river ecosystems in Japan; (2) include a wide range of taxonomic groups (green algae, diatoms, cyanobacteria), and (3) reflect the actual species composition of Japanese rivers. These riverine periphytic algae, those attached to riverbed gravel, play an important role in the ecological function of the river as primary producers and food for invertebrates and fish. However, limited toxicity data on riverine periphytic algal species are available because of the difficulty in testing toxicity due to cell attachment to the test chamber’s surface. Therefore, I developed an efficient and economical high-throughput algal toxicity assay using the five species of algae.21 The use of a microplate assay, in which periphytic algae are attached to the bottom of a microplate, was combined with the fluorometric measurement of algal growth with high measurement sensitivity. Finally, I published the standard test method for a five species algal bioassay.27

I conducted toxicity assays of twenty herbicides using the developed method. Toxicity characteristics were analyzed, focusing on their relationship to the herbicide mode of action.28 A clear relationship between sensitive species and the herbicide mode of action was observed: green alga was most sensitive to

Table 1. Comparative toxicity (EC50 µg/L) of 16 herbicides to 5 periphytic algae and the relationship with mode of action (MoA).26

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>MoA</th>
<th>Pse</th>
<th>Des</th>
<th>Ach</th>
<th>Nit</th>
<th>Nav</th>
</tr>
</thead>
<tbody>
<tr>
<td>bensulfuron-methyl</td>
<td>B</td>
<td>4.1</td>
<td>150</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
</tr>
<tr>
<td>cyclosulfamuron</td>
<td>B</td>
<td>3.1</td>
<td>11</td>
<td>&gt;9700</td>
<td>&gt;9700</td>
<td>&gt;9700</td>
</tr>
<tr>
<td>pyrimisulfan</td>
<td>B</td>
<td>150</td>
<td>120</td>
<td>&gt;5400</td>
<td>4400</td>
<td>&gt;5400</td>
</tr>
<tr>
<td>simetryn</td>
<td>C1</td>
<td>23</td>
<td>16</td>
<td>47</td>
<td>86</td>
<td>34</td>
</tr>
<tr>
<td>pentoxazone</td>
<td>E</td>
<td>&gt;220</td>
<td>0.084</td>
<td>&gt;220</td>
<td>5.7</td>
<td>59</td>
</tr>
<tr>
<td>pyraclonil</td>
<td>E</td>
<td>&gt;5000</td>
<td>0.96</td>
<td>1300</td>
<td>58</td>
<td>1000</td>
</tr>
<tr>
<td>oxadiargyl</td>
<td>E</td>
<td>&gt;1500</td>
<td>0.21</td>
<td>840</td>
<td>56</td>
<td>380</td>
</tr>
<tr>
<td>tefuryltrione</td>
<td>F2</td>
<td>&gt;100,000</td>
<td>&gt;100,000</td>
<td>10,000</td>
<td>23,000</td>
<td>23,000</td>
</tr>
<tr>
<td>benzofenap</td>
<td>F2</td>
<td>&gt;240</td>
<td>&gt;240</td>
<td>130</td>
<td>66</td>
<td>15</td>
</tr>
<tr>
<td>pretilachlor</td>
<td>K3</td>
<td>&gt;4000</td>
<td>62</td>
<td>5100</td>
<td>15,000</td>
<td>1300</td>
</tr>
<tr>
<td>butachlor</td>
<td>K3</td>
<td>&gt;3600</td>
<td>38</td>
<td>5100</td>
<td>6200</td>
<td>950</td>
</tr>
<tr>
<td>mfenacet</td>
<td>K3</td>
<td>&gt;10,000</td>
<td>620</td>
<td>&gt;3000</td>
<td>&gt;3200</td>
<td>5800</td>
</tr>
<tr>
<td>cafenstrole</td>
<td>K3</td>
<td>&gt;11,000</td>
<td>8.3</td>
<td>8600</td>
<td>5700</td>
<td>9500</td>
</tr>
<tr>
<td>fentrazamide</td>
<td>K3</td>
<td>&gt;4100</td>
<td>50</td>
<td>11,000</td>
<td>3600</td>
<td>55,000</td>
</tr>
<tr>
<td>benfuresate</td>
<td>N</td>
<td>&gt;23,000</td>
<td>22,000</td>
<td>10,000</td>
<td>76,000</td>
<td>95,000</td>
</tr>
<tr>
<td>esprocarb</td>
<td>N</td>
<td>1600</td>
<td>350</td>
<td>&gt;3200</td>
<td>4100</td>
<td>1800</td>
</tr>
</tbody>
</table>

Pse: Pseudanabaena galeata; Des: Desmodesmus subspicatus; Ach: Achnanthidium minutissimum; Nit: Nitzschia palea; Nav: Navicula pelliculosa.

Inhibitor of acetolactate synthase; C1: inhibitor of photosynthesis by photosystem II; E: inhibitor of protoporphyrinogen oxidase; F2: inhibitor of 4-hydroxyphenyl-pyruvate-dioxygenase; K3: inhibitor of very long-chain fatty acid synthesis; N: inhibitor of lipid synthesis.
inhibitors of protoporphyrinogen oxidase and very long-chain fatty acid synthesis; diatoms were most sensitive to inhibitors of 4-hydroxyphenyl-pyruvate-dioxygenase; cyanobacterium was most sensitive to inhibitors of acetalactate synthase (Table 1). Moreover, the differences in species sensitivities were markedly large (1000 or more times) for some herbicides. These results clearly showed that a single algal species cannot represent the sensitivity of an algal assemblage. Therefore, multispecies algal toxicity data are essential for substances with specific modes of action.

3.4. Expansion of the number of pesticide for SSD analysis
As described above, I constructed the framework for building data, resulting in SSD analyses for 68 pesticides that cover a wide range of pesticides commonly used in Japan. Nevertheless, this number is much smaller than the number of all registered pesticides. Therefore, I developed a method of SSD estimation using single-species toxicity data and information regarding the pesticide mode of action. This method was based on two assumptions: (1) the slopes of the SSD of pesticides with the same mode of action are the same, and (2) the relative sensitivities of standard algae in the SSD of pesticides with the same mode of action are the same. The outcome of the SSD estimation method was validated by comparing the estimated SSDs using the proposed method with the generated SSDs, using toxicity data that is independent of the method development. These SSDs were very consistent, and considering information regarding the mode of action improved the accuracy of estimating the SSD markedly. As a result, the application of the SSD for ecological risk assessment is possible for most pesticides using this estimation method.

4. Application of SSD

4.1. Publication of a technical guidance document on SSD
The application of SSDs to pesticides in Japan has been very limited, probably because of the lack of a technical guidance document written in the Japanese language. Therefore, I have published a technical guidance document for SSD analysis. The document includes basic information, historical context, application worldwide, a case study, and its application to ecological risk assessment. The document (PDF) and analytical tools (Microsoft Excel sheet) can be downloaded from the website of the National Institute for Agro-Environmental Sciences (http://www.niaes.affrc.go.jp/techdoc/ssd/). The remarkable point is that supplemental tool makes it easy to analyze SSD and conduct ecological risk assessment (Fig. 7). Moreover, there is a function for calculating EPAF (Fig. 5) by entering information regarding exposure distribution.

4.2. Validation of the registration criteria
To validate the pesticide registration criteria, the values of these 68 pesticides were compared with their HC₅ values, which cor-

Fig. 7. Microsoft Excel worksheet for calculating the PAF (in Japanese only). SSD parameters for 68 pesticides are already input, and the PAF can be calculated by selecting the pesticide name and inputting the environmental concentration (µg/L). Moreover, the evaluation of PAF values (four classes) is also shown as a reference.

Fig. 8. Comparison between the HC₅ and registration criteria for 26 insecticides. The solid lines show 1:1, and dotted lines show 1:10 and 10:1. Mode of action (MoA) indicates the classification by the Insecticide Resistance Action Committee.
respond to the predicted no-effect concentration for aquatic ecosystems.26) The differences between them were small (less than tenfold) for 50 of the 68 pesticides. This suggests that the current registration criteria are an appropriate index of the threshold level for toxicity to aquatic ecosystems for such pesticides. However, there were greater than tenfold differences for nine insecticides and nine herbicides (Fig. 8). These pesticides tended to have specific modes of action: GABA-gated chloride channel blockers, nicotinic acetylcholine receptor competitive modulators, and nicotinic acetylcholine receptor allosteric modulators for insecticides and inhibitors of acetolactate synthase, protoporphyrinogen oxidase, and 4-hydroxyphenyl-pyruvatedioxygenase for herbicides. In particular, the differences were markedly large (511–16,820-fold) for neonicotinoid insecticides. After considering these analyses, the additional data requirement of acute toxicity to Chironomus was added for insecticides with above three mode of actions to develop the registration criteria.31)

The ecological effect level (described as the PAF) under the registration criteria was calculated using each SSD and the registration criteria.26) The PAF values ranged from <0.1 to 98.3%, with a median of 5.1%. Thus, half of the registration criteria corresponded to an effect level of <5% and the other half to an effect level of >5%. This result indicates that the ecological effect levels under the registration criteria are not consistent among pesticides.

**Concluding remarks**

SSD is a powerful tool for ecological risk assessment, as described above. This will provide helpful information for implementing appropriate risk management measures. Further issues are expanding objective taxonomic groups and validating ecological risk assessment using a field ecological survey. SSD analyses were biased toward specific taxonomic groups (fish, arthropods, and algae) at this time. Aquatic fungi, vascular plants, and soil organisms have not been tested much before, however they should be subjected to more ecotoxicological research for more appropriate SSD analysis. Furthermore, the ecological relevance of ecological risk assessment using SSD, for example what can happen to a realistic ecosystem when the PAF is calculated to be 10%, has not been well studied. Recently, field-based SSD has been proposed to evaluate the relationship between the PAF value and the actual ecological response in a multi-stress environment.32) The investigation of such a relationship is important for further study.

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