Technical Report

Ecological effect assessment by species sensitivity distribution for 38 pesticides with various modes of action

Takashi Nagai

Institute for Agro-Environmental Sciences, NARO, 3–1–3 Kannondai, Tsukuba, Ibaraki 305–8604, Japan

(Received June 21, 2021; Accepted October 7, 2021)

Species sensitivity distributions (SSDs) of 38 pesticides with various modes of action were analyzed as a higher-tier ecological effect assessment based on collected acute toxicity data. Then the 5% hazardous concentrations (HC₅) based on each SSD were calculated as the predicted no-effect concentrations for aquatic ecosystems. The differences between HC₅ and registration criteria were small (within ten-fold) for 35 of the 38 pesticides. However, there were more than ten-fold differences for a fungicide and two herbicides. These results suggest that the current effect assessment scheme could underestimate the effect of such pesticides. This could be caused by differences in sensitivity of specific properties of the mode of action.

Keywords: SSD, aquatic organisms, hazardous concentration, registration criteria.

Introduction

In Japan, pesticide registration criteria concerning toxicity to aquatic organisms are set by Japan’s Ministry of Environment under the Agricultural Chemicals Regulation Law. To determine the criteria, acute toxicity tests are conducted for fish (basically, Cyprinus carpio), daphnids (Daphnia magna), and algae (Raphidocelis subcapitata), and then the minimum value of the 50% effect concentration (EC₅₀) or 50% lethal concentration (LC₅₀) is 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). The Agricultural Chemicals Regulation Law was revised in 2018, and the method of assessing pesticide registration criteria was also revised. Toxicity tests using aquatic plants such as Lemma sp. in addition to algae will be introduced in the setting of revised criteria for herbicides. The uncertainty factor applied to the algal EC₅₀ was changed from 1 to 10 by default, which is then reduced depending on the number of algal species tested. However, registration criteria for the new method have not yet been developed, and the present paper considered the existing criteria.

Species sensitivity to environmental contaminants varies markedly, and this variation can be described by the statistical distribution (often a log-normal distribution) estimated from sampled toxicity data (EC₅₀s or LC₅₀s) and visualized as a cumulative distribution function (called species sensitivity distribution, SSD; Fig. 1). The SSD has been used to determine hazardous concentrations for the protection of ecosystems and to reveal ecological risks. The 5th percentile of a distribution (called the 5% hazardous concentration, HC₅) has been used in the US, Europe, and Australia for deriving threshold concentrations that protect most species in a community (Fig. 1, arrow 1). The HC₅ values as threshold concentrations were validated by comparing them with the result of semi-field experiments (microcosm/mesocosm), which have provided more realistic ecological effects of pesticides. The SSD has also been used for quantitative ecological risk assessment of pesticides, such as diazinon and aldicarb insecticides, atrazine herbicide, and pesticide mixtures. Our previous studies also conducted probabilistic ecological risk assessment of several paddy insecticides and herbicides. The potentially affected fraction (PAF) is an index of the magnitude of ecological risk (Fig. 1, arrow 2). The PAF represents the effect on species diversity, which is a quantitative index of the biodiversity effect.

Nagai previously analyzed the SSDs of 68 pesticides com-
commonly used in Japanese paddy fields based on collected acute toxicity data. The study showed that variation in species sensitivity greatly depends on the chemical mode of action (MoA). In addition, robust evidence of the relationship between sensitive species and herbicide MoA was found from the analysis of toxicity data for 120 herbicides. However, pesticides with only some MoAs have sufficient toxicity data to assess differences in species sensitivity. Thus, there are many pesticides with MoAs whose sensitivity differences have not yet been analyzed. The main objective of the present study is to analyze SSDs with various MoAs using newly collected toxicity data. For that purpose, the acute SSDs of 38 pesticides were analyzed, and then HC5 values and registration criteria were compared to check the validity of the registration criteria.

**Materials and methods**

1. **Pesticides**

The SSDs for 38 pesticides (15 insecticides, 7 fungicides, and 16 herbicides) were analyzed (Tables 1–3). The MoAs (Tables 1–3) were derived from the Insecticide Resistance Action Committee, the Fungicide Resistance Action Committee, and the Herbicide Resistance Action Committee. The pesticide registration criteria concerning toxicity to aquatic organisms were derived from Japan’s Ministry of Environment (Tables 1–3). When registration criteria had not yet been developed, the equivalent values for comparison purposes were calculated based on toxicity values for the standard test species.

2. **Data collection and evaluation**

Information on the acute (defined as a test duration of 1–7 days and endpoint of growth rate for primary producers and immobility/mortality for animals) effect of pesticides was collected from open literature. The literature includes the Pesticide Handbook, the Pesticide Manual, various risk assessment reports by national and international agencies, the ECOTOX database of the US Environmental Protection Agency (EPA), and other publicly accessible documents written in Japanese. Data collection from the ECOTOX database was limited when the original paper in scientific journals was available. The reliability of the information was categorized into four

---

**Table 1.** Properties of the 15 insecticides studied, including mode of action (MoA), registration criteria, SSD parameters (ln Mean, ln SD, and data number n), HC5 (with 90% confidence intervals), and HC50 (with 90% confidence intervals).

| Insecticides | MoA | Registration criteria (µg/L) | ln Mean | ln SD | n | HC5 (µg/L) | HC50 (µg/L) |
|--------------|-----|-----------------------------|---------|------|---|------------|-------------|
| Methomyl    | 1A  | 1.5                         | 4.47    | 2.08 | 18 | 2.7 (0.61–7.7) | 88 (39–200) |
| Acephate    | 1B  | 5500                        | 11.10   | 1.52 | 11 | 5000 (920–14000) | 66000 (29000–150000) |
| Trichlorfon | 1B  | 0.11                        | 3.96    | 1.96 | 17 | 2.0 (0.35–6) | 53 (22–130) |
| Malathion   | 1B  | 0.3                         | 2.92    | 2.65 | 31 | 0.23 (0.052–0.68) | 19 (8.2–42) |
| Methidathion| 1B  | 0.11                        | 4.03    | 1.96 | 9  | 2.0 (0.15–8.1) | 56 (17–190) |
| Flupyradifurone | 4D | 6.1                         | 6.88    | 3.39 | 5  | 2.3 (0.00064–61) | 970 (38–25000) |
| Fenbutatin-oxide | 12B | 0.2                        | 10.53   | 3.13 | 6  | 160 (0.35–2400) | 37000 (2800–490000) |
| BPPS        | 12C | 1.3                         | 8.50    | 2.95 | 8  | 31 (0.4–290) | 4900 (680–35000) |
| Chlorfenapyr| 13  | 0.7                         | 1.96    | 1.15 | 8  | 0.99 (0.18–2.4) | 7.1 (3.3–15) |
| Bensulat    | 14  | 20                          | 6.05    | 2.81 | 7  | 3.2 (0.03–32) | 420 (53–3300) |
| Amitraz     | 19  | 26                          | 7.90    | 1.83 | 8  | 120 (8–470) | 2700 (800–9200) |
| Tolfenpyrad | 21  | 0.099                       | 1.47    | 1.85 | 5  | 0.16 (0.0019–0.96) | 4.3 (0.75–25) |
| Indoxacarb  | 22  | 60                          | 4.94    | 1.56 | 6  | 9.1 (0.43–36) | 140 (39–500) |
| Spiromesifen| 23  | 9.2                         | 5.22    | 0.71 | 5  | 52 (9.4–100) | 180 (94–360) |
| Cyenopyrafen| 25  | 0.29                        | 3.93    | 1.77 | 5  | 2.2 (0.03–12) | 51 (9.4–270) |

---

(a) IRAC20; 1A, 1B: acetylcholinesterase inhibitors; 4D: nicotinic acetylcholine receptor competitive modulators; 12B, 12C: inhibitors of mitochondrial ATP synthase; 13: uncouplers of oxidative phosphorylation via disruption of the proton gradient; 14: nicotinic acetylcholine receptor channel blockers; 19: octopamine receptor agonists; 21: mitochondrial complex I electron transport inhibitors; 22: voltage-dependent sodium channel blockers; 23: inhibitors of acetyl CoA carboxylase; 25: mitochondrial complex II electron transport inhibitors.
classes according to the Organization for Economic Cooperation and Development (OECD) Manual for the Assessment of Chemicals26): 1 = reliable without restrictions, 2 = reliable with restrictions, 3 = not reliable, and 4 = not assignable. The detailed method of classification was described in a previous paper.19) Collected acute toxicity data (EC 50 and LC 50) were entered into a database.

3. SSD analysis

The SSD analysis was conducted using the collected datasets on toxicity according to the method described by Nagai.19) Only data evaluated belonging to as reliability classes 1 or 2 were used for SSD analysis. The data for insecticides were separated into arthropods and other species, because arthropods are the most sensitive to insecticides among the taxonomic groups, and the SSD showed a clear separation of arthropods from other species.9) The data for herbicides were separated into primary producers and other species, because primary producers are the most sensitive to herbicides among the taxonomic groups and the SSD showed a clear separation of primary producers from others.10) The data for fungicides were separated into aquatic fungi (including fungus-like organisms) and other species, because aquatic fungi are generally the most sensitive to fungicides among the taxonomic groups.27) It should be noted that all species data were used for fungicide SSD analysis in a previous study,19) because the sensitivity difference among the taxonomic groups is small.

### Table 2. Properties of the seven fungicides in the same form as in Table 1.

| Fungicides       | MoA | Registration criteria (µg/L) | Fungi          | HC5 (µg/L) | HC50 (µg/L) |
|------------------|-----|------------------------------|----------------|------------|------------|
| Hydroxyisoxazole | A3  | 2800                         | 8.57 3.51 5     | 10 (0.0021–300) | 5300 (190–150000) |
| Orysatrobin      | C3  | 120                          | 7.61 0.71 5     | 570 (100–1100) | 2000 (1000–4000)  |
| Kasugamycin      | D3  | 6600                         | 9.25 1.11 5     | 1400 (99–4200) | 10000 (3600–30000) |
| Isothiolane      | F2  | 920                          | 9.65 1.15 5     | 2000 (120–6100) | 16000 (5200–46000) |
| Ipconazole       | G1  | 150                          | 6.29 1.80 5     | 22 (0.28–120) | 540 (97–3000)    |
| Tricyclazole     | I1  | 2100                         | 10.19 0.71 5    | 7500 (1300–15000) | 27000 (14000–53000) |
| Ferimzone        | U   | 620                          | 8.55 0.76 5     | 1300 (220–2800) | 5200 (2500–11000) |

a) FRAC22); A3: DNA/RNA synthesis (proposed); C3: complex III, cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene); D3: protein synthesis (ribosome, initiation step); F2: phospholipid biosynthesis, methyltransferase; G1: C14-demethylase in sterol biosynthesis (erg11/cyp51); I1: cytochrome c oxidase in melanin biosynthesis; U: unknown mode of action. b) Registration criteria have not yet been developed, therefore a calculated value based on the LC50 and EC50 for fish, crustacean, and algae (using default uncertainty factors) is shown.

### Table 3. Properties of the 16 herbicides in the same form as in Table 1.

| Herbicides       | MoA | Registration criteria (µg/L) | Primary producers | HC5 (µg/L) | HC50 (µg/L) |
|------------------|-----|------------------------------|-------------------|------------|------------|
| Cyhalofop-butyl  | 1   | 33                           | 7.07 0.93 7       | 240 (50–500) | 1200 (600–2300) |
| Sethoxydim       | 1   | 7200                         | 10.38 0.88 10     | 7200 (2500–13000) | 32000 (19000–53000) |
| Flucetosulfuron  | 2   | 7900                         | 10.53 0.80 6      | 9200 (1900–19000) | 37000 (19000–72000) |
| Trifluralin      | 3   | 24                           | 3.65 1.19 9       | 5.1 (1.1–12) | 38 (18–80)    |
| Pendimethalin    | 3   | 14                           | 5.62 2.13 11      | 7.5 (0.7–30) | 280 (87–880)  |
| 2,4-D            | 4   | 9800                         | 10.28 2.67 15     | 330 (31–1500) | 29000 (8600–97000) |
| MCPA             | 4   | 6100                         | 10.11 1.61 13     | 1600 (330–4300) | 25000 (11000–54000) |
| Glyphosate       | 9   | 6200                         | 10.74 0.82 13     | 12000 (5200–19000) | 46000 (31000–69000) |
| Glufosinate      | 10  | 10000                        | 10.45 2.81 11     | 290 (13–1800) | 35000 (7400–160000) |
| Chlorotriphenol  | 14  | —                           | 4.57 2.19 7       | 2.2 (0.056–13) | 96 (19–480)    |
| Asulam           | 18  | 9000                         | 9.85 1.64 11      | 1200 (190–3400) | 19000 (7700–46000) |
| Diquat           | 22  | 13                           | 3.95 1.98 10      | 1.8 (0.16–6.9) | 52 (16–160)    |
| Chlorpropham     | 23  | 370                          | 7.96 1.49 15      | 230 (63–540) | 2900 (1500–5600) |
| Benzylophene     | 27  | 34                           | 6.38 0.10 6       | 500 (410–540) | 590 (550–640)  |
| Dichloflonil     | 29  | 150                          | 7.44 2.29 12      | 36 (3.2–150) | 1700 (520–5600) |
| Oxaziclonemone   | 0   | 830                          | 7.94 1.01 6       | 480 (66–1200) | 2800 (1200–6500) |

a) FRAC22); 1: inhibition of acetyl CoA carboxylase; 2: inhibition of acetolactate synthase; 3: microtubule assembly inhibition; 4: auxin mimics; 9: inhibition of EPSP synthase; 10: inhibition of glutamine synthetase; 14: inhibition of protoporphyrinogen oxidase; 18: inhibition of dihydrodiperoxide synthase; 22: photosystem-I-electron diversion; 23: inhibition of microtubule organization; 27: inhibition of 4-hydroxyphenyl-pyruvate-dioxigenase; 29: inhibition of cellulose synthesis; 0: unknown. b) Registration expired.
groups was not clear at that time. The minimum data requirement for SSD analysis in the present study was set to five genera for each most-sensitive taxonomic group. All data were reduced to genus-level data according to the US EPA guideline. The genus-level dataset on the most-sensitive taxonomic group was fitted to a log-normal distribution using the maximum likelihood method. The maximum likelihood parameters of the distribution, logarithmic mean (referred as ln Mean) and logarithmic standard deviation (referred as ln SD), were obtained by fitting. The values of HC₅ and the 50th percentile of SSD (HC₅₀) were calculated (Fig. 1, arrow 1) as follows:

\[
\ln HC_p = (\ln \text{Mean}) - K_p (\ln \text{SD})
\]

where the \(K_p\) values are derived from a normal distribution table, for example, \(K_5 = -1.65\) for HC₅ and \(K_{50} = 0\) for HC₅₀. The 90% confidence intervals (from the 5th to the 95th percentiles) associated with HC₅ and HC₅₀ were calculated by the method of Aldenberg and Jaworska. In their method, \(K_p\) values can vary based on the noncentral \(t\)-distribution depending on the data number (\(n\)). For example, the 5th and 95th percentiles of HC₅ can be calculated using \(K_5\) values of \(-4.20\) and \(-0.82\), respectively, when \(n = 5\).

The values of HC₅ were compared with the registration criteria (Tables 1–3). In addition, the ecological effect level (described as the PAF) for the pesticide concentration equivalent to the registration criteria was calculated using each SSD and the registration criteria (Fig. 1, arrow 2).

**Results**

The typical SSD curves for the insecticide malathion, the fungicide ferimzone, and the herbicide glufosinate are shown in Fig. 2. The SSDs showed a clear separation of sensitive (arthropods for malathion, aquatic fungi for ferimzone, and primary producers for glufosinate) and insensitive taxonomic groups. The results of SSD analysis of 38 pesticides are summarized in Tables 1–3. The sensitivity of each genus of freshwater aquatic organisms is listed in Supplemental Table S1, and each SSD curve is shown in Supplemental Fig. S1. The following numbers of genera used for SSD analysis indicate the reliability of SSD: 5–31 for insecticides, 5 for fungicides, and 6–15 for herbicides. The HC₅ values indicate the predicted no-effect concentration, which were 0.16–5,000 \(\mu\)g/L for insecticides, 10–7,500 \(\mu\)g/L for fungicides, and 1.8–12,000 \(\mu\)g/L for herbicides. The HC₅₀ values indicate the geometric mean of toxicities: 4.3–66,000 \(\mu\)g/L for insecticides, 540–27,000 \(\mu\)g/L for fungicides, and 38–46,000 \(\mu\)g/L for herbicides. The values of ln SD indicate the slopes of the SSD: 0.71–3.39 for insecticides, 0.71–3.51 for fungicides, and 0.10–2.81 for herbicides.

The HC₅ values, which correspond to the predicted no-effect concentration for aquatic ecosystems, and pesticide registration criteria were compared (Fig. 3). In particular, the cases where the registration criteria were more than tenfold higher than the HC₅ were focused, because such cases indicate substantial underestimation of toxicity in the registration criteria. The differences between them were small (tenfold or less) for 35 of the 38 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, the differences for the fungicide hydroxysinoxazole and two herbicides glufosinate and 2,4-D were more than tenfold. An aquatic fungus *Rhizophyllum*, a cyanobacterium *Pseudanabaena*, and an aquatic vascular plant *Myriophyllum* were the most sensitive to hydroxysinoxazole, glufosinate, and 2,4-D, respectively (Supplemental Table S1). The differences in species sensitivity (described as ln SD in Table 1–3) were large (more than 2.5) for these three pesticides. Ecological effect assessment of pesticides that have large ln SD values should be considered cautiously.

The ecological effect level (described as the PAF) under the
registration criteria was calculated using each SSD and the registration criteria. The PAF values ranged from <0.1% to 42.8%, with a median of 4.8%, average of 10.9%, and standard deviation of 13.1%. Thus, half of the registration criteria corresponded to an effect level of <5% and the other half to an effect level of >5%, which is consistent with the previous analysis of 68 pesticides. This result indicates that the ecological effect levels under the registration criteria were not consistent among the pesticides.

**Discussion**

The SSD approach can be applied to both developing registration criteria (Fig. 1, arrow 1) and the quantification of ecological risk (Fig. 1, arrow 2). First, the contribution of SSD analysis to the development of registration criteria (Fig. 1, arrow 1) is discussed. A previous study showed that the relationships between registration criteria and \( HC_5 \) were specific to the MoA, and the differences for 18 of the 68 pesticides were more than tenfold. In particular, the registration criteria at that time were not appropriate for insecticides with specific MoAs (GABA-gated chloride channel blockers, nicotinic acetylcholine receptor competitive modulators, and nicotinic acetylcholine receptor allosteric modulators). This was attributed to the fact that aquatic insects are much more sensitive than \( D. magna \) to these insecticides. Then, the additional data requirement of acute toxicity to aquatic insect *Chironomus* was introduced to assess the effect of these insecticides in Japan.

An aquatic fungus *Rhizophydium* was the most sensitive to hydroxyisoxazole in the present study. Although pesticide regulations have yet to be based on ecological risk assessment using fungal toxicity data, this has been suggested as a challenge for the future. The guidance document for risk assessment of plant protection products for aquatic organisms in the European Union suggests that further research into potential effects on fungi is needed and that the selection of relevant species for which standardized ecotoxicity tests may be developed should be identified as a research need.

For herbicides which are less toxic to algae and duckweed *Lemna* such as 2,4-D, testing an additional aquatic vascular plant *Myriophyllum* is required for ecological risk assessment in the European Union. However, differences in species sensitivity among vascular plants have not yet been sufficiently investigated due to the lack of a method for testing a wide range of aquatic plant species. Therefore, we have recently developed a novel bioassay method for simultaneously determining the difference in species sensitivity of five species of vascular plants. The five species of vascular plants were shown to be more sensitive than algae and duckweed to herbicide 2,4-D. This economical and efficient bioassay would be useful for improving the development of registration criteria.

A cyanobacterium *Pseudanabaena* was the most sensitive to glufosinate in the present study. As part of the revised criteria in 2018, algal species (a green alga *Desmodesmus*, diatom *Navicula*, and cyanobacteria *Synechococcus* and *Anabaena*) can be optionally subjected to testing in addition to a standard algal species *Raphidocelis*. However, the cyanobacterium *Synechococcus* was not sensitive to glufosinate (Supplemental Table S1), and *Pseudanabaena* is generally more sensitive than *Synechococcus*. Therefore, *Pseudanabaena* is a potential cyanobacteria test species.

Next, quantification of the ecological risk of pesticides using SSD as the PAF (Fig. 1, arrow 2) is discussed. Monitoring the actual environmental concentration of pesticides and risk assessment as a post-registration study is important for risk management. Quantification of the ecological risk is a useful tool for risk management in addition to comparison between environmental concentration and registration criteria. A technical guidance document for SSD analysis with a Microsoft Excel worksheet for calculating the PAF (in Japanese only) was recently published. The SSD parameters for 68 pesticides are already provided in this worksheet, and the PAF can be calculated by selecting the pesticide name and inputting the environmental concentration.
concentration (µg/L). Moreover, a cumulative ecological risk assessment tool (NIAES-CERAP) considering the mixture toxicity of multiple pesticides has also been published. These risk assessment tools make it easy to conduct quantitative ecological risk assessment.

One of the most important limitations of SSD application is the lack of sufficient toxicity data for SSD analysis. However, this study determined the SSD parameters of 38 pesticides covering pesticides with a wide range of MoAs. These SSD parameters will be available for ecological risk assessment tools in the future. Therefore, the future application of SSDs for ecological risk assessment and the management of pesticides is expected to increase.

Acknowledgements

This research was partly supported by the sponsored research “Technological development of aquatic ecological risk assessment of pesticides” by the Ministry of the Environment, Japan.

Electronic supplementary materials

The online version of this article contains supplementary materials (Supplemental Table S1 and Fig. S1), which are available at http://www.jstage.jst.go.jp/browse/jpestics/.

References

1) MOE: “Assessment report of the pesticide registration criteria concerning toxicity to aquatic organisms,” Japan’s Ministry of Environment, Tokyo (in Japanese). http://www.env.go.jp/water/sui-kaitei/kijun.html (Accessed April, 2021).
2) MOE: “Setting of Pesticides Registration Criteria Regarding the Effect on Living Environmental Animals and Plants (Initial Report),” Central Environment Council, Japan’s Ministry of Environment, Tokyo, 2019 (in Japanese).
3) L. Posthuma, G. W. Suter and T. P. Traas (eds.): “Species Sensitivity Distributions in Ecotoxicology (Environmental and Ecological Risk Assessment),” Lewis Publisher, Boca Raton, USA, 2001.
4) U.S. EPA: “Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses,” U. S. Environmental Protection Agency, 1985.
5) RIVM: “Guidance document on deriving environmental risk limits,” National Institute of Public Health and the Environment, 2001.
6) EC: “Technical guidance for deriving Environmental Quality Standards,” European Commission, 2011.
7) ANZECC: “Australian and New Zealand guidelines for fresh and marine water quality,” Australian and New Zealand Environment and Conservation Council, 2000.
8) T. Nagai and A. Yokoyama: Comparison of ecological risks of pesticides for nursery-box application using species sensitivity distribution. J. Pestic. Sci. 37, 233–239 (2012).
9) L. Maltby, N. Blake, T. C. M. Brock and P. J. van den Brink: Insecticide species sensitivity distributions: The importance of test species selection and relevance to aquatic ecosystems. Environ. Toxicol. Chem. 24, 379–388 (2005).
10) P. J. van den Brink, N. Blake, T. C. M. Brock and L. Maltby: Predictive value of species sensitivity distributions for effects of herbicides in freshwater ecosystems. Hum. Ecol. Risk Assess. 12, 645–674 (2006).
11) L. Maltby, T. C. M. Brock and P. J. van den Brink: Fungicide risk assessment for aquatic ecosystems: Importance of interspecific variation, toxic mode of action and exposure regime. Environ. Sci. Technol. 43, 7556–7563 (2009).
12) R. P. A. van Wijngaarden, L. Maltby and T. C. M. Brock: Acute tier-1 and tier-2 effect assessment approaches in the EFSA Aquatic Guidance Document: Are they sufficiently protective for insecticides? Pest Manag. Sci. 71, 1059–1067 (2015).
13) J. M. Giddings, L. W. Hall Jr. and K. R. Solomon: Ecological risks of diazinon from agricultural use in the Sacramento-San Joaquin River basins, California. Risk Anal. 20, 545–572 (2000).
14) D. R. J. Moore, R. P. Thompson, S. I. Rodnay, D. Fischer, T. Ramana-Rayanan and T. Hall: Refined aquatic risk assessment for aldicarb in the United States. Integr. Environ. Assess. Manage. 6, 102–118 (2010).
15) K. R. Solomon, D. B. Baker, R. P. Richards, K. R. Dixon, S. J. Klaine, T. W. La Point, R. J. Kendall, C. P. Weisskopf, J. M. Giddings, J. P. Giesty, L. W. Hall Jr. and W. M. Williams: Ecological risk assessment of atrazine in north American surface waters. Environ. Toxicol. Chem. 15, 31–76 (1996).
16) D. de Zwart: Ecological effects of pesticide use in the Netherlands: Modeled and observed effects in the field ditch. Integr. Environ. Assess. Manag. 1, 123–134 (2005).
17) T. Nagai, K. Inao and T. Horio: Probabilistic ecological risk assessment of paddy herbicide in Japanese river waters using uncertainty analysis: A case study for simetryn. J. Pestic. Sci. 33, 393–402 (2008), (in Japanese).
18) T. Nagai, K. Inao, A. Yokoyama, T. Iwafune and T. Horio: Probabilistic ecological risk assessment of eleven paddy herbicides. Jpn. J. Risk Assess. Manag. 20, 279–291 (2010), (in Japanese).
19) T. Nagai: Ecological effect assessment by species sensitivity distribution for 68 pesticides used in Japanese paddy fields. J. Pestic. Sci. 41, 6–14 (2016).
20) T. Nagai: Relationship between herbicide mode of action and difference in species sensitivity of aquatic primary producers. Jpn. J. Environ. Toxicol. 19, 83–91 (2016), (in Japanese).
21) IRAC: “Modes of Action,” Insecticide Resistance Action Committee. http://www.irac-online.org/modes-of-action/ (Accessed June, 2021).
22) FRAC: “FRAC Code List 2021: Fungicides sorted by mode of action,” Fungicide Resistance Action Committee. https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2021-final.pdf?sfvrsn=7ftec499a_2 (Accessed June, 2021).
23) HRAC: “Global Herbicide Classification Lookup,” Herbicide Resistance Action Committee. https://hracglobal.com/tools/classification-lookup (Accessed June, 2021).
24) Japan Plant Protection Association (ed.): “Noyaku-handbook 2016,” Japan Plant Protection Association, Tokyo, 2016 (in Japanese).
25) C. Tomlin (ed.): “Pesticide Manual 17th edition,” BCPC Publications, 2015.
26) OECD: “Manual for investigation of HPV chemicals,” Organization for Economic Cooperation and Development, Paris.
27) T. Nagai: Sensitivity differences among five species of aquatic fungi and fungus-like organisms for seven fungicides with various modes of action. J. Pestic. Sci. 45, 223–229 (2020).
28) OECD: “Guidance document for aquatic effects assessment,” Organization for Economic Cooperation and Development, Paris, 1995.
29) W. Naito, Y. Gamo and K. Yoshida: Screening-level risk assessment of di(2-ethylhexyl) phthalate for aquatic organisms using monitoring data in Japan. Environ. Monit. Assess. 115, 451–471 (2006).
30) T. Aldenberg and J. S. Jaworska: Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicol. Environ. Saf. 46, 1–18 (2000).
31) MOE: “Handling of the difference in species sensitivity concerning the development of pesticide registration criteria,” Japan’s Ministry of Environment, 2016 (in Japanese).

32) EFSA Panel on Plant Protection Products and their Residues: Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. 

33) K. Ueda and T. Nagai: Development of the seed germination and seedling growth test method for determining the difference in species sensitivity of 5 vascular plant species simultaneously. Jpn. J. Environ. Toxicol. 21, 21–32 (2018), (in Japanese).

34) T. Nagai: Sensitivity differences among seven algal species to 12 herbicides with various modes of action. J. Pestic. Sci. 44, 225–232 (2019).

35) NIAES: “Technical guidance document on species sensitivity distribution analysis for pesticide ecological risk assessment,” National Institute for Agro-Environmental Sciences, 2016 (in Japanese). https://www.naro.affrc.go.jp/archive/ and https://www.naro.affrc.go.jp/publicity_report/publication/laboratory/niaes/manual/079666.html (Accessed April, 2021).

36) NIAES: “An assessment tool of cumulative ecological risk of multiple pesticides NIAES-CERAP,” Institute for Agro-Environmental Sciences, NARO, 2018 (in Japanese). https://www.naro.go.jp/publicity_report/publication/laboratory/niaes/manual/079666.html (Accessed April, 2021).