Studies on the behavior and ecotoxicity of pesticides and their transformation products in a river

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To clarify the properties of pesticide transformation products (TPs) for which the risk to aquatic organisms should be evaluated, I monitored the concentrations of paddy pesticides and their TPs in the Sakura River, Japan, during the rice-growing season in 2007–2010. I also conducted algal growth inhibition tests of herbicides and their TPs using a diatom and a green alga and acute toxicity tests of insecticides and their TPs using a caddisfly and a daphnid. Moreover, on the basis of the results of pesticide monitoring and toxicity tests, I attempted to evaluate the risk of these compounds to the riverine organisms as well as the risk of mixtures of insecticides and their TPs for caddisflies and cladocerans. The TPs were detected in the river water depending on the half-lives of the parent compounds and of the TPs in water and soil. The toxicities of the parent compound and its TPs may be related to their hydrophobicities and chemical structures. Some toxic and persistent TPs that formed rapidly in water and soil posed a risk to the organisms over a long period. The physicochemical properties and chemical structures of a parent compound and its TPs can be key factors in evaluating the pesticide TP risk to aquatic organisms in rivers. © Pesticide Science Society of Japan

Keywords: paddy pesticide, metabolite, degradate, pesticide mixtures, river water, ecological risk assessment.

Introduction

Pesticides applied to arable or nonarable land can be degraded by chemical processes or microorganisms in the environment, and transformation products (TPs) of the pesticides can be transported to waterways and flow into rivers. Public concern about the adverse effects of pesticides and their TPs on water quality and aquatic organisms is increasing. However, few detailed surveys with high-frequency (weekly) monitoring of seasonal changes in the concentrations of pesticides and their TPs in rivers during the growing season1,2) and few experimental studies that have produced data comparing the ecotoxicities of pesticide TPs with those of parent compounds3,4) have been conducted; therefore, the ecotoxicities and behavior of pesticide TPs in the environment are poorly understood.

Europe and the United States have established regulations5,6) for pesticide registration, requiring environment fate and ecotoxicity data to be provided on pesticide TPs as necessary. A separate ecological risk assessment of the parent compound, as well as its TPs, is conducted as necessary, using the environment fate and ecotoxicity data for each compound, based on guidance7,8) from the assessment of pesticide risk to aquatic organisms in Europe and the United States.

In Japan, pesticide registration criteria based on ecological risk assessment are set by Japan’s Ministry of Environment under the Pesticide Regulation Law9). Under the risk assessment scheme, acute toxicity tests are conducted for fish, daphnids, and algae, and then the acute effect concentration (AEC) is determined as the minimum value of the median effective concentration (EC50) or the median lethal concentration (LC50) 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 basin10). Finally, if the PEC is less than the AEC, the short-term aquatic risk is deemed to be insignificant, and the pesticide is considered to meet the criteria. However, under the risk assessment scheme, pesticide TPs are not assessed, and information on the behavior and ecotoxicities of pesticide TPs is insufficient.

Pesticides applied to paddy fields, which account for about 33% of all arable land in Japan (total area of arable land, 4.5 million ha; paddy fields under rice cultivation, 1.5 million ha in
flows through a region with many paddy fields, not only paddy pesticides but also some TPs have been detected during the rice-growing season. However, it is not known how a mixture of paddy pesticides and their TPs affects aquatic organisms in rivers or which TPs pose a risk to aquatic organisms in the presence of a mixture of paddy pesticides and their TPs. Therefore, it is necessary to clarify which pesticide TPs should be evaluated for risk to aquatic organisms in rivers.

The present study (1) monitored the concentrations of paddy pesticides and their TPs in the Sakura River during four rice-growing seasons (2007–2010); (2) conducted toxicity tests of paddy herbicides and their TPs to an attached diatom, *Mayamaea atomus*, and a green alga, *Pseudokirchneriella subcapitata*, and acute toxicity tests of paddy insecticides and their TPs to a riverine caddisfly, *Cheumatopsyche brevilineata*, and a daphnid, *Daphnia magna*; and (3) conducted a separate evaluation of the risk these compounds pose in the Sakura River to these aquatic organisms at acute exposure, as well as an evaluation of the risk that mixtures of insecticides and their TPs pose to caddisflies and cladocerans. Finally, on the basis of these results, I describe key points of the evaluations that should be conducted to assess pesticide TP risks to aquatic organisms in rivers.

1. **Paddy pesticides and their TPs**

Forty-three pesticides widely used for rice-crop protection in Japan and in the rice-growing area around the Sakura River (Fig. 1) in 2007–2010 and their 17 TPs were selected for the monitoring of their concentrations in river water. Among these compounds, 4 herbicides, 6 insecticides, and their 15 TPs were selected for toxicity tests and evaluation of their risk to aquatic or-

![Fig. 1. Map of the Sakura River basin in Japan, showing the river water sampling site.](image)

Table 1. Toxicity data and risk quotients of selected herbicides and their transformation products to *Mayamaea atomus* and *Pseudokirchneriella subcapitata*

| Compounds                        | M. atomus | P. subcapitata |
|----------------------------------|-----------|----------------|
|                                  | 72 hr EC₅₀ (µg/L) | 95% Confidence interval (µg/L) | Risk quotient for diatoms | 72 hr EC₅₀ (µg/L) | 95% Confidence interval (µg/L) | Risk quotient for green algae |
| Bromobutide                      | >3480(3500, 4.5%) | —<0.002 <0.004 <0.004 <0.004 | <0.004 74300 39200–139000 —<0.004 <0.008 <0.009 <0.008 |
| Bromobutide desbromo             | >3480 (3500, 2.7%) | — <0.001 <0.001 <0.001 <0.001 | <0.001 1580 (2,000, 7.2%) — <0.001 <0.001 <0.001 <0.001 |
| Cafenstrol                       | >478 (500, 2.9%) | — <0.001 <0.002 <0.002 <0.002 | 15.5 9.7–25.9 0.028 0.038 0.037 0.053 |
| Cafenstrol descarbamoyl           | >23600 (25000, 14.6%) | — <0.001 <0.001 <0.001 <0.001 | <0.001 4810 (5000, 3.9%) — <0.001 <0.001 <0.001 <0.001 |
| Clomeprop                        | >15.1 (35, 2.1%) | — <0.007 <0.005 <0.003 | <0.007 15.2 (35, 2.1%) — <0.007 <0.005 <0.003 |
| Clomeprop proponic acid          | >30200 (50000, 6.3%) | — <0.001 <0.001 <0.001 | <0.001 74300 39200–139000 — <0.001 <0.001 <0.001 <0.001 |
| Pyrazolynate                     | >39.4 (56, 4.1%) | — <0.001 <0.001 <0.001 <0.002 | <0.001 38.9 (56, 7.8%) | — <0.001 <0.001 <0.001 <0.002 |
| Pyrazolynate destosyl            | 21900(15000–33300) | <0.001 <0.001 <0.001 <0.002 | <0.001 1470(1010–2150) <0.001 <0.001 <0.001 <0.001 |

— Value indicates that the 72 hr median effective concentration (EC₅₀) was more than what is shown because the inhibition was <0.9–14.6% at the nominal concentration in the limited test. Concentration in parentheses indicate the nominal concentration and the percentage of inhibition in the limit test, respectively. — No value could be calculated either because the river water concentration was not measured, or because only a limited acute toxicity test was performed. Transformation product. Provisional values because of low recoveries with the analytical method.

2016, flow easily into rivers via drainage canals. Pesticides and their TPs runoff from paddy fields may pose a risk to aquatic organisms in rivers during the rice-growing season. In fact, in the Sakura River (Ibaraki Prefecture, Japan; Fig. 1), which...
Table 2. Acute toxicity data and risk quotients of selected insecticides and transformation products for Cheumatopsyche brevilineata and Daphnia magna

| Compounds                  | C. brevilineata | D. magna |
|----------------------------|-----------------|----------|
|                            | 48 hr EC50 (µg/L) | 95% Confidence interval (µg/L) | Risk quotient for caddisflies | 48 hr EC50 (µg/L) | 95% Confidence interval (µg/L) | Risk quotient for cladocerans |
| Benfuracarb                | 2.43            | 2.29–2.57 | 0.018<sup>a</sup> | 2007 | 0.010<sup>a</sup> |
| Carbofuran<sup>b</sup>     | 0.374<sup>a</sup> | 0.340–0.388<sup>b</sup> | <0.001<sup>b</sup> | 2008 | 0.004<sup>b</sup> |
| Carbofuran-3-hydroxy      | 14.1            | 13.8–14.3 | 0.181 | 2009 | 0.002<sup>b</sup> |
| Carbofuran phenol         | 472             | 429–513   | <0.001<sup>b</sup> | 2010 | 0.002<sup>b</sup> |
| Diazinon                  | 21300           | 19900–22300 | — | 2007 | 0.004<sup>a</sup> |
| Diazinon oxon<sup>c</sup> | 1.17            | 1.13–1.21 | 0.115 | 2008 | 0.004<sup>a</sup> |
| Fenitrothion              | 4.76            | 4.50–5.00 | 0.813 | 2009 | 0.004<sup>a</sup> |
| Fenitrothion oxon<sup>d</sup> | 3.77          | 3.57–3.96 | 0.009 | 2010 | 0.004<sup>a</sup> |
| Fenthion                  | 1.67            | 1.51–1.81 | 0.015 | 2007 | 0.004<sup>a</sup> |
| Fenthion oxon<sup>c</sup> | 1.84            | 1.57–2.03 | 0.014 | 2008 | 0.004<sup>a</sup> |
| Fenthion sulfone<sup>c</sup> | 3.75           | 3.41–4.11 | 0.043 | 2009 | 0.004<sup>a</sup> |
| Fenthion sulfoxide<sup>c</sup> | 17.1            | 15.2–18.2 | 0.092 | 2010 | 0.004<sup>a</sup> |
| Fipronil                  | 0.133           | 0.112–0.148 | 0.201 | 2007 | 0.004<sup>a</sup> |
| Fipronil carboxamide<sup>c</sup> | 4.95            | 3.23–2.60 | — | 2008 | 0.004<sup>a</sup> |
| Fipronil desulfinyl<sup>c</sup> | 0.177           | 0.155–0.200 | — | 2009 | 0.004<sup>a</sup> |
| Fipronil sulfide<sup>c</sup> | 0.052           | 0.042–0.059 | — | 2010 | 0.004<sup>a</sup> |
| Fipronil sulfone<sup>c</sup> | 0.066           | 0.054–0.078 | — | 2007 | 0.004<sup>a</sup> |

<sup>a</sup> Provisional value because of low recoveries in the analytical method.<sup>b</sup> Transformation product.<sup>c</sup> No value could be calculated either because the river water concentration was not measured, or because only a limited acute toxicity test was performed.<sup>d</sup> Value indicates that the 48 hr median effective concentration (EC50) was more than the value because the immobility was less than 5% at a nominal concentration of 10 µg/L in the limited test.

2. Monitoring in the Sakura River basin

2.1. Geography of the Sakura River basin

The Sakura River basin is located in southern Ibaraki Prefecture, Japan. The Sakura River, which is 53.4 km long and has a basin area of 335 km² (calculated using a geographical information system), flows into Lake Kasumigaura (Fig. 1). Paddy fields are distributed along the river. The paddy-field area (95 km²) corresponds to approximately 30% of the basin and approximately 10% of all paddy fields in Ibaraki Prefecture (990 km²). In the basin, 80% of the paddy-field area is under rice cultivation. The rice-cultivation season is from late April to mid-September. Rice seedlings are transplanted in early May. Heading occurs from late July to early August, and harvest starts in early September, after drainage of the paddy water in late August. Paddy pesticides and their TPs were monitored at the sampling site (Kimi-jima Bridge), which is also located in the paddy-field area and 41 km downstream from the head of the river, once a week from April to August in 2007–2010. In the present paper, I especially discuss the 2009 results, in which most of these compounds were detected in the river water.

2.2. Behavior of herbicides and their TPs in river water

Figure 2<sup>14</sup> shows changes in the concentrations of two herbicides and their transformation products in Sakura River water in 2009.

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<sup>1</sup> Studies on the behavior and ecotoxicity of pesticides and their transformation products in a river. Vol. 43, No. 4, 297–304 (2018)
icides and their TPs in 2009. The herbicides applied just after rice transplanting in the basin reached peak concentrations immediately after rice transplanting (from early May to mid-May). Changes in the concentrations of the parent compound and the corresponding TP differed among herbicides. The maximum concentration of bromobutide desbromo (0.318 µg/L) was much lower than that of bromobutide (13.7 µg/L) (Fig. 2A). I attribute this difference in concentration to the fact that bromobutide can be degraded not only to bromobutide desbromo but also to many other TPs in natural water by sunlight\(^{15}\) and that bromobutide has a long half-life (\(DT_{50} = 11–13\) weeks)\(^{15}\) and in soil (\(DT_{50} = 25–34\) days).\(^{16}\)

In contrast, the maximum concentration of clomeprop propionic acid (0.925 µg/L) was much higher than that of clomeprop (0.073 µg/L) (Fig. 2B). The detected frequency of clomeprop propionic acid was also much higher than that of clomeprop. Clomeprop is rapidly hydrolyzed to clomeprop propionic acid in soil (\(DT_{50} = 3\) days)\(^{17}\); therefore, the rapid degradation of clomeprop may have been the reason for its low maximum concentration in the river water.

2.3. Behavior of insecticides and their TPs in river water

Benfuracarb, carbosulfan, and fipronil are insecticides applied to the nursery box in which rice seedlings are initially raised just before rice transplanting. In 2009, clear peaks of carbofuran, the main TP of benfuracarb and carbosulfan, and of fipronil were observed in early May (Fig. 3A, B).\(^{13}\) The concentrations of carbofuran and fipronil decreased gradually in late May, but they continued to be detected in July and August. Carbofuran is formed very rapidly by the hydrolysis of carbosulfan (\(DT_{50} = 11.4\) hr at pH 7)\(^{18}\) or by the rapid degradation of benfuracarb in soil (\(DT_{50} = 4–28\) hr at pH 7),\(^{18}\) and it has a longer half-life in both water (\(DT_{50} = 121\) days at pH 7)\(^{18}\) and soil (\(DT_{50} = \text{approximately} 30–60\) days)\(^{18}\) than do its parent compounds. Carbofuran-3-hydroxy, which is formed from carbofuran by photodegradation, make up only a very small amount of the TP of carbosulfan and benfuracarb detected in both water and soil.\(^{18}\)

No clear peaks of fipronil TPs were observed; however, along with fipronil, fipronil desulfinyl (formed by photolysis in water), fipronil sulfide (formed by reduction in soil),\(^{19}\) and fipronil sulfone (formed by oxidation in surface soil) were detected at low concentrations (<0.0001–0.0011 µg/L) from May to August in 2009 (Fig. 3B).\(^{13}\) Because the mixture of fipronil and its desulfinyl, sulfide, and sulfone TPs have a long half-life in soil (\(DT_{50} = 188\) days),\(^{20}\) fipronil and its TPs can be expected to be present in paddy fields and river water for several months after transplanting. Fipronil carboxamide, a hydrolysate of fipronil, was not detected, probably because of the long half-life of fipronil in water (\(DT_{50} = 100\) days)\(^{19}\) and because fipronil probably does not hydrolyze at typical environmental pH values.\(^{19}\)

The organophosphorus insecticide fenthion is mainly applied to paddy fields, with the exact timing depending on the pest pressure and the emergence of pest insects over the long period of time from transplanting to a few weeks before harvest. In 2009, no clear peak of fenthion was observed; however, two clear peaks of fenthion sulfoxide, the TP of fenthion detected at the highest concentration in the river water, were observed (Fig. 3C).\(^{13}\) Fenthion degrades easily to fenthion sulfoxide in soil under aerobic conditions,\(^{18}\) and the half-life of fenthion sulfoxide in soil under both aerobic and submerged conditions is longer than that of the parent compound.\(^{21}\) For these reasons, fenthion sulfoxide formed rapidly in the paddy fields, causing its concentration to be consistently higher than that of the parent compound. However, fenthion sulfone and fenthion oxon, oxides of fenthion, were detected rarely and at similar or lower concentrations than the parent compound (fenthion sulfone, <0.02 µg/L; fenthion oxon, <0.001 µg/L). Oxon compounds derived from diazinon and fenitrothion were also detected rarely and at low concentrations.\(^{13}\) Because oxon compounds of organophosphorus insecticides hydrolyze faster than do the parent
compounds, oxon compounds can be expected to be present only rarely and in low concentrations in paddy fields and river water.

In the Sakura River, the appearance of clear peaks of paddy pesticides and their TPs depended on the pesticide application timing. The TP behavior depended on the half-lives of the parent compounds and on the stability of the TPs in water and soil. TPs formed from rapidly degradable pesticides, such as clometho prop propionic acid, carbofuran, and fenthion sulfioxide, were detected at much higher peak concentrations than the parent compounds. In addition, persistent TPs in the environment, such as fipronil desulfinyl, sulfide, and sulfone, continued to be detected in the river water for several months after transplanting.

3. Toxicity of paddy pesticides and their TPs

3.1. Toxicity of herbicides and their TPs to a diatom and a green alga

Algal toxicity tests of the four herbicides and their TPs were conducted according to a growth inhibition test method for *P. subcapitata* with a 96-well flat-bottom microplate based on the test guidelines of Environment Canada using two unicellular freshwater algae, *Mayamaea atomus* (diatom) and *Pseudokirchneriella subcapitata* ATCC22662 (green alga).

The calculated 72 hr EC50 value to *M. atomus* was 21,900 µg/L for pyrazolynate destosyl, and the values to *P. subcapitata* were 15.5 µg/L for cafenstrol, 74,300 µg/L for clomeprop propionic acid, and 1,470 µg/L for pyrazolynate destosyl (Table 1). All of the TPs had 72 hr EC50 values greater than 1,000 µg/L, indicating that the toxicity of the TPs to these two algae was relatively low. The toxicity of the TPs of some herbicides, such as atrazine, metsulfuron methyl, chlorsulfuron, and bensulfuron methyl, to green algae or cyanobacteria was found to be lower than the toxicity of the parent compounds. To my knowledge, no studies have investigated the toxicity of herbicide TPs to diatoms. The toxicity of certain herbicide TPs to the diatom, *M. atomus*, and to the green alga, *P. subcapitata*, can be relatively low.

3.2. Toxicity of insecticides and their TPs to a caddisfly and a daphnid

Acute toxicity tests for a riverine caddisfly, *Cheumatopsyche brevilineata* (Iwata) (Trichoptera: Hydropsychidae) Strain M, and for a freshwater cladocera, *Daphnia magna* (Straus) (Cladocera: Daphniidae), were conducted according to the test method for *C. brevilineata* established by Yokoyama et al. and to test guideline 202 of the Organization for Economic Cooperation and Development, respectively.

Both *C. brevilineata* and *D. magna* have 48 hr EC50 values in the same range or order of magnitude (i.e., less than 10-fold differences) for the majority of the compounds studied, except for carbofuran-3-hydroxy, for which *D. magna* is 15 times more sensitive than *C. brevilineata*, and particularly fipronil and its TPs, for which *D. magna* is 300 times less sensitive than *C. brevilineata* (Table 2). *Chironomus annularius* was reported to be much more sensitive to fipronil than *Daphnia pulex*, since *C. annularius* bioaccumulated fipronil more than did *D. pulex*. Although bioaccumulation analyses were not conducted in the present study, the different sensitivity to fipronil and its TPs between *C. brevilineata* and *D. magna* might be due to the differences in their bioaccumulation among those species, as with that of *C. annularius* and *D. pulex*. The toxicities of benfuracarb and carbosulfan and their common TPs (carbofuran, carbofuran-3-hydroxy, and carbofuran phenol) to *C. brevilineata* and *D. magna* can be ranked roughly as carbosulfan > benfuracarb > carbofuran > carbofuran-3-hydroxy > carbofuran phenol (Table 2). The toxicity of a parent compound and its TPs is considered to depend mainly on the presence of toxicophores, which are necessary chemical moieties for specific toxic mechanisms; the compound’s hydrophobicity (as indicated by the n-octanol/water partitioning coefficient, or Kow); and its dissociation constant (pKa), because these parameters affect a compound’s uptake to the body of an organism and its ability to cross cell membranes. Carbofuran phenol having no toxicophore was much less toxic than the parent compounds and the other TPs. The lower toxicity of carbofuran relative to the parent compounds may reflect its lower hydrophobicity (log Kow values, 4.2, 5.3, and 1.5 for benfuracarb, carbosulfan, and carbofuran, respectively). Although the log Kow value of carbofuran-3-hydroxy has not been reported, the TP of carbofuran is thought to have a similar or higher polarity than carbofuran. Thus, even carbofuran and carbofuran-3-hydroxy having the toxicophore, which are less hydrophobic than the parent compounds, were also less toxic than the parent compounds.

The 48 hr EC50 values between organophosphorus insecticides (diazinon, fenitrothion, and fenthion) and their oxons were within less than 13-fold differences (Table 2). Similar differences in toxicities between other organophosphorus insecticides and their oxon compounds for *D. magna* were reported by Damásio et al. and Guilhermino et al. Therefore, the toxicity of organophosphorous insecticide oxons to aquatic organisms appears to be similar to, or slightly lower than, that of their parent compounds.

The toxicities of fipronil TPs to *C. brevilineata* and *D. magna* were similar to or slightly higher than that of the parent compound, except that of fipronil carboxamide to *C. brevilineata* and those of fipronil carboxamide and fipronil desulfinyl to *D. magna* (Table 2). The EC50 values of fipronil carboxamide and fipronil desulfinyl to *D. magna* could not be calculated because the percentage of the immobility (48 hr) at a nominal concentration of 10 µg/L in their limited tests was less than 5%. The lower toxicity of fipronil carboxamide to *C. brevilineata* compared with the parent compound and the other fipronil TPs is similar to the toxicity data of these compounds for *Chironomus tentans* (chironomid) and for *Aedes aegypti* (mosquito). The toxicities of fipronil desulfinyl, fipronil sulfide, and fipronil sulfone to *A. aegypti* and *C. tentans*, and *Procambarus* sp. (crayfish) were also similar to or higher than that of fipronil. The similar
or higher toxicities of the desulfanyl (log \( K_{OW} \) value, 4.63), sulfide (4.77), and sulfone (3.68) TPs of fipronil compared with that of the parent compound (4.01)\(^{40}\) may thus reflect the similar or larger log \( K_{OW} \) values of these three TPs compared with that of the parent compound.

4. Risk assessment

4.1. Risk quotients of individual compounds for aquatic organisms

The risk of each parent compound and TP was evaluated on the basis of the risk quotient, which is also used in the ecological risk assessment scheme in Japan,\(^9\) defined as the ratio of the PEC to the AEC. In the present study, to evaluate the risk to diatoms, green algae, caddisflies, and cladocerans at acute exposure, the risk quotient was calculated by dividing the maximum concentration measured in the river water as a substitute of the PEC by the AEC of each compound. The concentrations obtained by the limited tests of the compounds were used instead of the EC\(_{50}\) values to calculate the risk quotients of those compounds.

For diatoms and green algae, the risk quotients of each of four herbicides and their TPs were much lower than 1 (Table 1). Even the quotient of clomeprop propionic acid, whose maximum concentration in the river water was higher than that of the parent compound, was much lower than 1 (<0.001). For caddisflies and daphnids, the risk quotients of each of the organophosphorus insecticides, fipronil, and TPs were also lower than 1 except for that of fenitrothion for daphnids in 2007 (the quotient, 1.41)\(^{\text{Table 2}}\). Even the risk quotients of carbofuran (≤0.224) and fenthion sulfoxide (≤0.117), whose maximum concentrations in the river water were higher than those of their parent compounds, and of fipronil and its TPs (≤0.391), which were more highly toxic than the other compounds to \( C. \ brevilineata \), were lower than 1. These quotients suggest that the risk posed by each individual compound to diatoms, green algae, caddisflies, and daphnids in the Sakura River might be almost acceptable.

4.2. Risk quotients for mixtures of the insecticides and their TPs for caddisflies and cladocerans

To examine seasonal changes in the risk of mixtures of insecticides and their TPs at acute exposure in 2009, the risk quotient for the mixture (RQ\(_{\text{mix}}\)) of these compounds was calculated using the equation reported in a previous study,\(^{41}\) except that instead of the predicted noneffective concentration in the equation, I used the AEC:

\[
\text{RQ}_{\text{mix}} = \sum_{i=1}^{n} \frac{C_i}{\text{AEC}_i}
\]

where \( C_i \) is the concentration of the compound, in the river water, \( \text{AEC}_i \) is the AEC of the compound, and \( n \) is the number of compounds. This approach to RQ\(_{\text{mix}}\) assumes concentration addition\(^{42}\); toxicities are additive or nearly so, and there are no synergistic, antagonistic, or other interactions. This assumption may be most applicable when the modes of action of compounds in a mixture are similar; however, there is evidence that even with chemicals having dissimilar modes of action, additive or nearly additive interactions are common.\(^{43,44}\) Moreover, to assess the effect of the input variables (\( C_i \) and \( \text{AEC}_i \)) of each compound on the output variable (RQ\(_{\text{mix}}\)), the sensitivity of output variables to changes in the input variables on May 11, July 15, and July 27, 2009, was investigated for caddisflies and cladocerans: sensitivity ratio \( \frac{(Y' - Y)}{[(X' - X)X]} \), where \( X \) is the baseline value of the input variable, \( X' \) is the value of the input variable obtained by multiplying \( X \) by 1.1, \( Y \) is the baseline value of the output variable obtained using the baseline values of the input variables, and \( Y' \) is the value of the output variable obtained by using the \( X' \) values of the input variables. When the sensitivity ratio is positive or negative, the output variable increases or decreases, respectively, with an increase in input variables.

Figure 4\(^{13}\) shows growing-season changes in the RQ\(_{\text{mix}}\)s of those insecticides and TPs for caddisflies and cladocerans in 2009. Figure 5\(^{13}\) shows the sensitivities of the RQ\(_{\text{mix}}\)s for caddisflies and cladocerans to changes in the input variables on May 11, June 15, and July 27, 2009, when RQ\(_{\text{mix}}\) peaks were observed for caddisflies and cladocerans. The RQ\(_{\text{mix}}\) peak on May 11 (Fig. 4), observed after transplanting, was attributed to the nursery-box application of benfuracarb and carbosulfan before transplanting. Carbofuran made a notable contribution to the peak for both caddisflies and cladocerans (Fig. 5A, B), and fipronil, which is also applied to nursery boxes, and its TPs also contributed to the May 11 peak for caddisflies (Fig. 5A). The highest RQ\(_{\text{mix}}\)s were observed on June 15 (RQ\(_{\text{mix}}\) for caddisflies and cladocerans, 1.178 and 0.986, respectively) (Fig. 4). These peaks
were attributed to the application of the organophosphorus insecticide diazinon, fenitrothion, or fenthion sprayed on the rice crop 1 and 1.5 months after transplanting (Figs. 4 and 5C, D). The RQmix of just the organophosphorus insecticides and their TPs for cladocerans (0.931) was larger than that for caddisflies (0.635) (Fig. 4). On July 27, fenthion and its TPs increased the RQmix (Fig. 4), making notable contributions to the observed peaks for both caddisflies and cladocerans (Fig. 5E, F). Fipronil and its TPs contributed to all three RQmix peaks for caddisflies (Fig. 5A, C, E), and the RQmix of fipronil and its TPs was high (0.163–0.570) from May to August. In contrast, fipronil and its TPs contributed little to the RQmix for cladocerans (RQmix of fipronil and its TPs for cladocerans, <0.005) (Fig. 4). These results suggest that nursery-box applications may affect caddisflies more than cladocerans in May; applications of organophosphorus insecticides sprayed on the growing rice crops in June and July may affect cladocerans more than caddisflies; and fipronil and its TPs may affect caddisflies over periods longer than the duration of the rice-growing season. Taken together, these findings indicate that the risk of mixtures of insecticides and TPs changes seasonally and differs between caddisflies and cladocerans, depending on the application timing of the insecticides and on the persistence and toxicity of the TPs that are easily formed in the environment.

No RQmix of only parent compounds exceeded 1, whereas the RQmix of parent compounds and their TPs exceeded 1 for caddisflies in mid-June 2009 (Fig. 4). The risk of a mixture of diuron, a herbicide, and its TPs to daphnids was found to have increased greatly compared with that of diuron alone, because its TP, 1-(3,4-dichlorophenyl)-3-methyl-urea, was highly toxic. In the Sakura River, mixtures of insecticides and their TPs may have affected caddisflies in mid-June 2009. For cladocerans, the TPs of carbamate and organophosphorus insecticides sporadically increased the RQmix. Therefore, in the environment, the presence of some TPs along with the applied pesticides can result in a higher risk to aquatic organisms than the risk of the parent compounds alone. In addition, the persistence of TPs, which are similarly or more toxic than the parent compounds, may pose a risk beyond the rice-growing season.

On the basis of these results, evaluation of the risk of a pesticide TP to aquatic organisms should be conducted if the TP (1) contains a toxicophore and (2) is formed rapidly from a parent compound in the environment and/or (3) is more hydrophobic than the parent compound. In particular, the persistent TP with the above features should be evaluated carefully because it can pose a risk to aquatic organisms over long periods of time. Therefore, the DT₅₀ values in water and soil, log KOW values, and chemical structures of a parent compound and its TPs can be key factors in deciding whether the risk of a pesticide TP to aquatic organisms should be evaluated.

Concluding remarks

It is not always necessary to evaluate the ecological risk of all pesticide TPs. The present study revealed (1) key aspects of the TPs for which ecological risk assessment will be required and that (2) the risk of mixtures of insecticides and TPs changes seasonally and differs between aquatic organisms, depending on the insecticide application timing and on the persistence and
toxicity of the TPs that are easily formed in the environment. A risk evaluation is recommended for the TP whose acute toxicity to aquatic organisms is similar to or greater than that of the parent compound. However, our current knowledge about the physicochemical properties, environmental fate, and ecotoxicity of TPs is limited. Further study is needed to speculate about the potential toxicity of TPs using a model of quantitative structure–activity relationships. In addition, for a more detailed evaluation of the risk of persistent pesticide TPs to aquatic organisms in rivers, a chronic toxicity study is also necessary.

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