Contamination levels, monthly variations, and predictions of neonicotinoid pesticides in surface waters of Gifu Prefecture in Japan

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ABSTRACT

The neonicotinoid pesticides acetamiprid (ACE), clothianidin (CTD), dinotefuran (DIN), imidacloprid (IMI), nitenpyram (NTP), thiacloprid (THI), and thiamethoxam (TMX) are widely used in over 120 countries. These pesticides have been regulated in many jurisdictions, including the European Union (EU), the United States, and the United Kingdom, due to adverse effects on non-target organisms, whereas some of these pesticides are permitted in Japan. In the present study, we have 1) measured levels of these pesticides at 103 locations (n = 672) across Gifu Prefecture, 2) analyzed the monthly trends and regionality using R and ArcGIS, and 3) created a predicted contamination map by an ordinary kriging analysis. The concentration levels of the seven neonicotinoid pesticides in surface waters were determined using liquid chromatography with tandem mass spectrometry (LC/MS/MS) and ranged from < 2.0 to 530 ng/L during the ten-month period. In a total of 672 samples, the top three pesticides detected at high frequency were DIN (76.9%), CTD (48.4%), and IMI (19.6%). The concentration of the neonicotinoid pesticides in environmental waters varied with the time periods of application, physiochemical properties of the pesticides, land use, geological properties of the contamination sources, and other factors. Potential contamination sources were depicted in the predicted contamination maps by using ordinary kriging models, which showed that DIN and CTD are widely present in Gifu Prefecture. Monthly variance of the concentration of IMI differed in the two geological regions, due to differences in the time of application and agricultural products yield. The results of our study contribute to a better understanding of the contamination status of neonicotinoid pesticides by providing reference data (actual pesticide concentrations) as well as predicted contamination maps.

Key words: neonicotinoid pesticide; surface water; Gifu Prefecture; predicted contamination; environmental behavior; dinotefuran

INTRODUCTION

Neonicotinoid pesticides are currently the most widely used class of pesticides in over 120 countries (Simon-Delso et al., 2015). In Japan, an estimated 431 tons of neonicotinoid pesticides were used in 2018 in the production of rice, vegetables, flowers and fruits (National Institute for Environmental Studies, 2018). The seven major neonicotinoid pesticides are acetamiprid (ACE; CAS RN®: 160430-64-8), clothianidin (CTD; CAS RN®: 210880-92-5), dinotefuran (DIN; CAS RN®: 165252-70-0), imidacloprid (IMI; CAS RN®: 138261-41-3), nitenpyram (NTP; CAS RN®: 150824-47-8), thiacloprid (THI; CAS RN®: 111988-49-9), and thiamethoxam (TMX; CAS RN®: 153719-23-4), all of which are hydrophilic in nature (Chemicalize, 2020). These pesticides dissolve in water and enter vegetation through the roots, and, in small quantities, these pesticides are
Neonicotinoid concentrations in surface waters

Effective insecticides (Wood and Goulson, 2017). Nicotinoinds are considered to be less toxic to vertebrates than other commonly used pesticides and are relatively safer for the environment (Nauen et al., 2001; Tomizawa and Casida, 2005) due to their specific mode of action as inhibitors of insect nicotinic acetylcholine receptors (nAChRs) (Palmer et al., 2013). However, the findings of prior studies suggest that these pesticides may have adverse direct or indirect effects on children and non-target organisms in the environment (Hallmann et al., 2014; Blacquière et al., 2012; Millot et al., 2017; Van Dijk et al., 2013; Han et al., 2018; Woodcock et al., 2016; Gupta, 2018).

Due to the adverse effects of neonicotinoid pesticides, the governments of several European countries and the United States (US) have established restrictions. The governments of the US and the United Kingdom restricted the use of all neonicotinoid pesticides in 2017 (US Congress, 2017; Department for Environment, Food and Rural Affairs, 2017). In 2018, the European Union (EU) banned all outdoor uses of the three neonicotinoid pesticides (CTD, IMI, and THI) (European Commission, 2018). In contrast, Japanese regulators, with the aim of promoting widespread application of neonicotinoids in agricultural production, had loosened relevant laws in 2015 (Ministry of Health, Labour and Welfare, 2015) and in 2017 (Ministry of Health, Labour and Welfare, 2017). Therefore, particularly in Japan, evaluating the effects of neonicotinoid pesticides on human bodies, organisms, and the environment is important.

There have been a number of reports in Japan of contamination from these pesticides in surface waters (Yamamoto et al., 2012; Sato et al., 2016; Nishino et al., 2018), effluents (Nishino et al., 2018), tap water (Sato et al., 2016; Kamata et al., 2020), and underground water sources (Hayashi et al., 2017). Due to the hydrophilic nature of nicotinoids, there is the possibility that these pesticides, when applied to agricultural fields, will enter the environment through runoff (Pietrzak et al., 2020). Most of the agricultural fields in Japan are used to grow rice, and 70% of these are “wet fields.” (Ministry of Agriculture, Forestry and Fisheries, 2019). Thus, runoff of hydrophilic pesticides from rice paddy fields is a major concern when examining contamination of the aquatic environment.

In this study we carried out a monthly survey (over 10 months) of the occurrence of seven neonicotinoid pesticides (ACE, CTD, DIN, IMI, NTP, THI, and TMX) in surface waters at 103 locations in seven river systems across Gifu Prefecture. We found that nicotinoids had been widely applied to rice paddy fields, vegetable fields, orchards, and golf courses. We used geostatistical analysis (ArcGIS) with ordinary kriging to obtain predicted contamination distribution maps of CTD, DIN, and IMI across Gifu Prefecture.

MATERIALS AND METHODS

MATERIALS AND CHEMICALS

Analytical standards of each neonicotinoid pesticide mixture (20 mg/L each in acetonitrile [ACN]), as well as deuterium-labeled standards of thiamethoxam-d₄, imidacloprid-d₄, and acetamiprid-d₄, and formic acid of LC/MS grade (99.5%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Standard stock solutions were prepared in ACN at 1,000 μg/L and stored in a freezer at -20°C. Acetone and ACN for pesticide residue grade were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Pure water (≤ 0.5 μS/m) was purchased from ADVANTEC (Tokyo, Japan). Solid phase extraction cartridges (InertSep Slim-J Pharma FF, 230 mg), and graphite carbon-SPE cartridges (InertSep Slim-GC, 400 mg) were purchased from GL Sciences (Tokyo, Japan) and both were conditioned with 5 mL acetone and 10 mL pure water prior to use. L-column 2 (2.1 mm × 150 mm, 3 μm) was purchased from Chemicals Evaluation and Research Institute (Tokyo, Japan).

SAMPLE COLLECTION AND EXTRACTION

Surface water samples were collected at 37 locations of seven major river systems (Ibi-, Jintsuu-, Kiso-, Nagara-, Shou-, Shonai-, and Yahagi-river) and 66 locations in their tributaries (n = 672) in Gifu Prefecture, Japan, from May 2016 to February 2017 (Fig. S1). The samples were collected after the approval of local laws, and the GPS coordinates of the sampling locations are given in Table 1. All water samples were collected at the surfaces of the center streams and stored in polypropylene bottles in a refrigerator at 4°C until sample extraction.

The extraction procedure of the neonicotinoid pesticides in surface water is shown in Fig. S2. The pesticides in the sampled water were collected by passing 250 mL of the water through an InertSep Slim-J Pharma FF cartridge (Pharma FF). After loading the samples, an InertSep Slim-GC (Slim-GC) cartridge was connected to the exit of the sample containing Pharma FF, the pesticides were eluted by passing 5 mL acetone through the Pharma FF connected to the Slim-GC. The eluted solution was collected in a glass tube and concentrated to 0.1 mL or less under a gentle stream of nitrogen gas. The concentrated solution was reconstituted with ACN/water (1/9, v/v) to 1 mL, and 1 μL of this solution was applied to LC/MS/MS measurement.

INSTRUMENTAL CONDITIONS

The pesticides were analyzed by selected reaction monitoring (SRM) with an API5500 electrospray tandem quadrupole mass spectrometer (Sciex Pte., Ltd., Framingham, MA, USA) interfaced with LC800 HPLC systems (GL Sciences Inc., Tokyo, Japan) equipped with L-column 2 and mobile phase of ACN and 0.1 v/v% formic acid in water. The details of the instrumental parameters are presented in Table S1. Analyte peaks were identified with the retention times (± 0.05 min), and the ratio of quantitative to quantitative transition-ion responses (± 20%) as well as predefined sets of SRM transitions. ACN, TMX, and IMI were quantified with deuterium-labeled standards as the surrogate, and the other pesticides were quantified using acetamiprid-d₄ as the clean-up spike. Five-point calibration curves ranging from 0.5 to 10 μg/L were made. The regression coefficients (r²) with equal weighting quadratic regression were ≥ 0.999.

VALIDATION OF THE QUANTITATIVE ANALYSIS

The analytical method was validated throughout the entire analysis. A volume of surface water (250 mL each, n = 3) was spiked with 10 ng of each of the seven pesticides, and 1 ng of each mixture of the three deuterium-labeled standards was used for evaluating the recovery efficiencies. Non-spiked surface waters (procedural blanks) were prepared and analyzed.
The neonicotinoid pesticides were not found in the surface water, thus the surface water was sufficient for quality matrix spike experiments.

**GEOSTATISTICAL ANALYSIS OF POTENTIAL SOURCES OF PESTICIDE CONTAMINATIONS**

Gifu Prefecture, located in central Japan, can be divided into five basic geographical regions: Chuunou, Gifu, Hida, Seinou, and Tounou (Gifu Prefecture, 2019). There are seven major river systems: Ibi, Jintsuu, Kiso, Nagara, Shonai, Shou, and Yahagi. Potential neonicotinoid contamination sources are: rice fields, vegetable crops, fruit and mulberry trees, and tea cultivation. These areas were identified using the GIS land use shape files (specific to Gifu Prefecture) provided by the Ministry of Land, Infrastructure, Transport, and Tourism (Ministry of Land, Infrastructure, Transport and Tourism, 2018; Ministry of Land, Infrastructure, Transport and Tourism, 2018). The other land use type in our analysis is golf courses, which were identified from GPS information of Google Maps (Google LLC, California, USA).

Areas with potential contamination by neonicotinoid pesticides were calculated using ArcGIS (Environmental Systems Research Institute, ver. 10.5) and then overlaid on maps showing the agricultural areas and golf courses. At each sampling point, annual average concentrations of CTD, DIN, and IMI were calculated using R (R Core Team, 2018) and plotted with locations in ArcMap by coordinating with GCS_JGD_2011. Concentrations of CTD, DIN, and IMI in non-sampling areas were predicted by geostatistical analysis with ordinary kriging method. The weighted average of adjacent sampling locations, and the distribution of predicted values of pesticide concentrations were plotted in the maps of Gifu Prefecture. We used a cross-validation procedure to ensure high accuracy and low bias in our maps (Boken et al., 2004; Santra et al., 2008; Buchanan and Triantafilis, 2009). This analysis focused on predicting average annual concentrations of the neonicotinoid pesticides in Gifu Prefecture without considering other environmental factors such as flow speed, precipitation, and monthly trends.

**RESULTS AND DISCUSSION**

**VALIDATION OF THE QUANTITATIVE ANALYSIS**

The average recoveries and their relative standard deviations are 92.8%–99.3% and 0.9%–1.8%, respectively. In Table S2 we present the values for recovery rate and the limit of detection (LODs), which were calculated by dividing the instrumental detection limit (IDLs) with the sample volume. Each of the IDL values were determined as the concentration at which the chromatographic peak signal was five times higher than the baseline noise. For quality assurance, a concentration of the standard mixture was injected after every 15 of the sample measurements. In the quality assurances, the target recovery value was 100% ± 20%.

**LC/MS/MS DETECTION OF NEONICOTINOID PESTICIDES IN SURFACE WATERS**

Annual average concentrations of the neonicotinoid pesticides in surface water at sampling sites (n = 672) are shown in Table 1. DIN was detected at the highest concentrations (22.8 ng/L on average), followed by CTD (12.0 ng/L on average), TMX (8.6 ng/L on average), and IMI (7.5 ng/L on average). ACE, NIT, and THI were rarely detected above the LOD thresholds (5.8, 3.5, 5.0 ng/L on average, respectively). Throughout this 10-month period, we detected DIN and CTD at the highest frequencies (76.9% and 48.4%, respectively).

Monthly concentration profiles of the seven neonicotinoid pesticides in the seven major river systems in Gifu Prefecture are shown in Fig. 1. Each of the Y-axis values was normalized by the ratio of the neonicotinoid concentration divided by annual average concentration of each neonicotinoid from all the samples. Surface water samples of the Shou-river were collected from locations deep in the mountainous area, away from human activities. Consequently, the neonicotinoid pesticides were detected in only three samples from the Shou-river throughout the ten-month period (including trace levels of DIN and CTD).

In all river systems, the concentration ratios of DIN were close to 1.0, meaning there were higher concentrations in August followed by October and November, except for samples from the Shou-river system (Fig. 1). Similarly, the concentration of CTD tended to be higher in samples collected in May in all the river systems except the Shou-river system. In the Yahagi-river system, the concentration ratios of CTD were above 1.0 during most of the study period. Both the Kiso- and Yahagi-river systems exhibited concentration ratios of TMX at higher concentrations from October to December. In the Kiso- and Nagara-river systems, ratio trends of IMI stayed above 1.0 for more than four months and concentrations in this period were higher than the ten-month average. These detection patterns reveal monthly trends in neonicotinoid concentrations in surface water.

**CORRELATION BETWEEN CONCENTRATIONS OF NEONICOTINOID PESTICIDES AND LAND USE IN GIFU PREFECTURE**

**LAND USE AND AREAS OF POTENTIAL CONTAMINATION SOURCES OF THE NEONICOTINOID PESTICIDES**

In Gifu Prefecture (9,562 km²), the area of potential contamination sources is 1,057 km², including rice paddies (817 km²), vegetable farms (133 km²), golf courses (42.9 km²), fruit orchards (33.3 km²), mulberry tree cultivation (25.3 km²), and tea fields (6.1 km²). The corresponding areas were calculated for each of the five regions within Gifu Prefecture: Chuunou, Gifu, Hida, Seinou, and Tounou (Table 2).

Hida is one of the typical mountainous regions in Japan and although it is the largest geological area in Gifu Prefecture (as shown in Table 2), the farmable area is very small (144 km²). Compared to Hida, the other regions, namely Seinou, Gifu, Chuunou, and Tounou, have larger farming areas and golf courses, and, therefore, more frequent use of neonicotinoid pesticides is expected in these regions. In Fig. 2 we show the distribution of the potential sources of contamination of the neonicotinoid pesticides. The distribution pattern of contamination sources in rice paddy fields and vegetables farms is very similar, and both were spread over the entire area of Gifu Prefecture (with the exception of Hida region, as explained above). Golf courses were mainly located in Chuunou and Tounou, fruits orchards and tea fields in Seinou, and mulberry
| River system | Sample ID | Region | Sampling location | GPS | AChE<sup>a</sup> (ng/L) | CTD<sup>b</sup> (ng/L) | DIN<sup>c</sup> (ng/L) | IMI<sup>d</sup> (ng/L) | NTP<sup>e</sup> (ng/L) | TMX<sup>f</sup> (ng/L) | THI<sup>g</sup> (ng/L) |
|-------------|-----------|--------|-------------------|-----|---------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Ibi IB-1    | T S      | Haginaga bridge | 136.57096 35.47277 | 10 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| Ibi IB-2    | M G      | Goto bridge | 136.64259 35.39044 | 3 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| Ibi IB-3    | T S      | Neo bridge | 136.63326 35.43981 | 3 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| Ibi IB-4    | T S      | Minazugawa bridge | 136.61414 35.43113 | 10 | <2 | 4.7 | 45.6 | 3.2 | <2 | <2 | <2 |
| Ibi IB-5    | T S      | Hanada river | 136.62968 35.47891 | 3 | <2 | 4 | 30.0 | 2.3 | <2 | <2 | <2 |
| Ibi IB-6    | T S      | Shimokura bridge | 136.63664 35.51545 | 3 | <2 | 15 | <2 | <2 | <2 | <2 | <2 |
| Ibi IB-7    | M S      | Obagai bridge | 136.65319 35.44433 | 3 | <2 | 3.7 | <2 | <2 | <2 | <2 | <2 |
| Ibi IB-8    | T S      | Hachibe bridge | 136.62032 35.29848 | 4 | <2 | 30.8 | <2 | <2 | <2 | <2 | <2 |
| Ibi IB-9    | T S      | Ar river | 136.58466 35.37445 | 10 | <2 | 5.6 | 22.4 | 2.2 | <2 | <2 | <2 |
| Ibi IB-10   | T S      | Ichinose bridge | 136.48036 35.16586 | 10 | <2 | 6.9 | 10.7 | <2 | <2 | <2 | <2 |
| Ibi IB-11   | T S      | Nakano river | 136.64156 35.33545 | 3 | <2 | 4.3 | 14.7 | <2 | <2 | <2 | <2 |
| Ibi IB-12   | M S      | Fukuoka bridge | 136.64832 35.22713 | 4 | <2 | <2 | 6.7 | <2 | <2 | <2 | <2 |
| Ibi IB-13   | T S      | Fukuoka ohashi bridge | 136.68945 35.29264 | 10 | <2 | 5.5 | 91.5 | 2.7 | <2 | <2 | <2 |
| Ibi IB-14   | M S      | Kaido bridge | 136.62974 35.16033 | 3 | <2 | <2 | 8.3 | <2 | <2 | <2 | <2 |
| Ibi IB-15   | T S      | Manju bridge | 136.65679 35.10088 | 4 | <2 | 3.8 | 239 | 5.3 | <2 | <2 | <2 |
| Ibi IB-16   | M C      | Ise bridge | 136.69096 35.08464 | 3 | <2 | 2 | 14 | <2 | <2 | <2 | <2 |
| Ibi IB-17   | M O      | Most downstream of Biwa river | 136.70315 35.44601 | 2 | <2 | <2 | 10 | <2 | <2 | <2 | <2 |

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<sup>a</sup> AChE: Acetylcholinesterase
<sup>b</sup> CTD: Chlorpyrifos
<sup>c</sup> DIN: Dinitrophenol
<sup>d</sup> IMI: Imidacloprid
<sup>e</sup> NTP: Neonicotinoid pesticides
<sup>f</sup> TMX: Tetramethrin
<sup>g</sup> THI: Thioclothropridic acid

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Table 1: Annual average concentrations of seven neonicotinoid pesticides in surface water of the seven major river systems in Gifu Prefecture
In the present study, DIN was found at high concentrations in all the river systems in August. Since the annual concentrations of DIN (Fig. 3) approximately correspond to the areas of rice fields (Table 2), we infer that a large amount of DIN application to rice fields in August would increase DIN concentrations in the surface water.

In the DIN predicted-distribution map (RMSE = 0.98, ME = −0.07), the locations of rice paddy field and vegetable farms are concordant with higher concentrations of DIN (> 30 ng/L) in the Northern Hida, Southern Seinou, Southern Gifu, and Southern Chūnou (Fig. 4). In most parts of Gifu Prefecture, the concentrations were predicted to be more than 2.98 ng/L. This result is possibly due to the continuous activities of rice and vegetable farms and suggests that these two land use types are probable sources of DIN. The distribution of predicted DIN concentration raises concerns about the adverse health effects from chronic DIN exposure. The no-observed-adverse-effect-levels for pregnant women has been determined to be 784 mg/kg/d by the US Environmental Protection Agency (Sheets et al., 2015), and because some residents of Gifu Prefecture are still using well-water for drinking water, monitoring of concentrations of DIN runoff in surface and underground water sources would help to prevent adverse health effects.

**REGIONAL AND MONTHLY VARIATION IN NEONICOTINOID CONTAMINATION AND MAPPING OF PREDICTED CONTAMINATION**

CTD, DIN, IMI, and TMX were the most frequently detected neonicotinoid pesticides (as shown in Table 1). The annual averages, medians, minimums, and maximums of the four pesticides in the five geological regions are shown in Fig. 3. Using the quantitative values, geostatistical analysis (ArcGIS) with ordinary kriging was carried out to create maps of predicted contamination distribution of the whole Gifu Prefecture for CTD, DIN, and IMI. We note that the prediction of TMX concentration distributions could not be achieved using ordinary kriging analysis due to the low detection rate.

**DINOTEFURAN (DIN)**

The range of annual average concentrations and the detection rates of DIN were <2.0–239 ng/L and 50.0%–93.8% in each sampling location. DIN and CTD have been used for exterminating insect pests of rice (Lanka et al., 2014). Generally, in Japan, DIN is applied to rice fields around August (Tsueda et al., 2002; Suzuki, 2005; Hashimoto, 2005), and in the present study, DIN was found at high concentrations in all the river

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### Table: Predicted Contamination of Neonicotinoids

| River system | Sample ID | M/T | Region | Sampling location | GPS East | GPS North | ACE (ng/L) | CTD (ng/L) | DIN (ng/L) | IMI (ng/L) | NTP (ng/L) | TMX (ng/L) | THI (ng/L) |
|--------------|-----------|-----|--------|-------------------|----------|-----------|-----------|-----------|-----------|------------|------------|------------|------------|
| 72 Nagara NA-9 | T | C | Kawaura river | 35.49864 | 10 | 32.8 | 37.1 | 15.5 | 2 | 2 | 1 | 2 |
| 73 Nagara NA-10 | T | C | Sakura bridge | 35.47144 | 10 | 14.3 | 34.3 | 3.3 | 2 | 2 | 1 | 2 |
| 74 Nagara NA-11 | M | G | Kagoshima bridge | 35.41987 | 3 | 12 | 2 | 2 | 2 | 2 | 2 | 2 |
| 75 Nagara NA-12 | T | G | Take bridge | 35.42997 | 3 | 3 | 3 | 4 | 4 | 2 | 2 | 2 |
| 76 Nagara NA-13 | T | G | Naeda bridge | 35.41015 | 10 | 11 | 10.4 | 2 | 2 | 1 | 2 |
| 77 Nagara NA-14 | T | S | Sai river | 35.38500 | 3 | 9.7 | 2.7 | 2 | 2 | 1 | 2 |
| 78 Nagara NA-15 | T | G | Gokuyu river | 35.31875 | 4 | 14.5 | 11.5 | 2 | 2 | 1 | 2 |
| 79 Nagara NA-16 | M | G | Nanno bridge | 35.27986 | 3 | 11.3 | 2 | 2 | 2 | 2 | 2 | 2 |
| 80 Nagara NA-17 | T | G | Kowbara river | 35.25517 | 10 | 14.1 | 2 | 2 | 1 | 2 | 2 | 2 |
| 81 Nagara NA-18 | M | S | Tokai bridge | 35.22531 | 3 | 11 | 2 | 2 | 1 | 2 | 2 | 2 |
| 82 Nagara NA-19 | M | O | Ise bridge | 35.83289 | 3 | 11.7 | 2 | 2 | 2 | 2 | 2 | 2 |
| 83 Nagara NA-20 | M | O | Most downstream of Nagara river | 35.55672 | 2 | 9 | 2 | 2 | 1 | 2 | 2 | 2 |
| 84 Shou SH-1 | M | H | Makiko | 36.54829 | 4 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| 85 Shou SH-2 | M | H | Miboro basin | 36.51362 | 3 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| 86 Shou SH-3 | M | H | Nairu basin | 36.35954 | 6 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| 87 Shonai SN-1 | M | T | Minamami bridge | 35.36757 | 10 | 10.8 | 20.6 | <2 | <2 | 2 | 2 | <2 | <2 |
| 88 Shonai SN-2 | T | T | Kase bridge | 35.32562 | 4 | <2 | 30.5 | 10 | <2 | <2 | <2 | <2 |
| 89 Shonai SN-3 | T | T | Harako bridge | 35.30967 | 10 | <2 | 17.4 | 15.3 | <2 | <2 | <2 | <2 |
| 90 Shonai SN-4 | T | T | Hida bridge | 35.36190 | 10 | <2 | 5.8 | 2 | <2 | <2 | <2 | <2 |
| 91 Shonai SN-5 | M | T | Sankyo bridge | 35.30302 | 10 | <2 | 13.1 | 22.5 | <2 | <2 | <2 | <2 |
| 92 Shonai SN-6 | T | T | Miyuki bridge | 35.39573 | 10 | 2.2 | 2.4 | <2 | <2 | <2 | <2 | <2 | <2 |
| 93 Shonai SN-7 | T | T | Suzuki bridge | 35.27061 | 10 | <2 | 3.1 | 2.3 | <2 | <2 | <2 | <2 |
| 94 Shonai SN-8 | M | T | Kumanaga bridge | 35.28648 | 3 | <2 | 9.7 | 2.3 | <2 | <2 | <2 | <2 |
| 95 Shonai SN-9 | T | T | Ohbara river | 35.33047 | 3 | <2 | 4.7 | 6 | <2 | <2 | <2 | <2 |
| 96 Shonai SN-10 | M | T | Toki river | 35.31407 | 3 | <2 | 8.3 | 21.7 | <2 | <2 | <2 | <2 |
| 97 Yahagi YA-1 | T | T | Sekiwa bridge | 35.40501 | 10 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| 98 Yahagi YA-2 | M | T | Ohkawa bridge | 35.30870 | 10 | <2 | 1.2 | 2 | <2 | <2 | <2 | <2 |
| 99 Yahagi YA-3 | M | O | Sasado dam | 35.30387 | 3 | <2 | 6 | 19.3 | <2 | <2 | <2 | <2 |
| 100 Yahagi YA-4 | T | T | Akechi river | 35.38475 | 10 | <2 | 12.1 | 14 | <2 | <2 | <2 | <2 |
| 101 Yahagi YA-5 | T | O | Okuna river | 35.39290 | 10 | <2 | 23 | 3.4 | <2 | <2 | <2 | <2 |
| 102 Yahagi YA-6 | M | O | Ayumi bridge | 35.31699 | 2 | <2 | 7.5 | 16.5 | <2 | <2 | <2 | <2 |
| 103 Yahagi YA-7 | M | O | Shiida bridge | 35.31437 | 2 | <2 | 6.5 | 16.5 | <2 | <2 | 2 | 2 | <2 | <2 |

a) M: Main stream  
T: Tributary  

b) C: Chūnou  
G: Gifu  
H: Hida  
S: Seinou  
T: Tōnou  
O: Outside of Gifu-prefecture  

c) n: Sample size  

b) DIN: dinotefuran  
CI: clothianidin  
IMI: imidacloprid  
NTP: nitenpyram  
THI: thiacloprid  

**tree plantations in Chūnou and Seinou.**
Fig. 1 Monthly concentration profiles of the seven neonicotinoid pesticides in the seven major river systems in Gifu Prefecture

Table 2 Areas of potential neonicotinoid contamination sources in the five geological regions of Gifu Prefecture

| Source of Contamination                      | Gifu-prefecture (km²) | Chuunou (km²) | Gifu (km²) | Hida (km²) | Seinou (km²) | Tounou (km²) |
|----------------------------------------------|-----------------------|---------------|------------|------------|--------------|--------------|
| Whole area of Gifu prefecture                | 9,562                 | 2,193         | 798        | 4,033      | 1168         | 1,371        |
| Farming and golf courses                     |                       |               |            |            |              |              |
| Rice fields                                  | 817                   | 185           | 147        | 112        | 234          | 140          |
| Vegetable farms                              | 133                   | 39.6          | 29.6       | 25.7       | 13.1         | 24.5         |
| Golf courses                                 | 42.9                  | 23.5          | 3.8        | 0.9        | 0            | 14.6         |
| Fruits farms                                 | 33.3                  | 2.9           | 12         | 2.9        | 10.3         | 5.2          |
| Mulberry tree fields                         | 25.3                  | 7.7           | 2.8        | 2.7        | 5.2          | 7            |
| Tea fields                                   | 6.1                   | 1.7           | 0.1        | 0.4        | 3.5          | 0.4          |
| Total                                        | 1,057                 | 261           | 195        | 144        | 266          | 192          |
CLOTHIANIDIN (CTD)

The range of annual average concentration and the detection rates of CTD were <2.0–62.2 ng/L and 20.0%–60.0% in each sampling location. Although CTD is applied to rice fields, the regional annual average concentration profile was different from that of DIN (Fig. 3). CTD concentrations were higher in Chuunou and Tounou (maximum values of 62.2 and 30.5 ng/L, respectively) than in the other regions. The CTD concentrations in a tributary of the Yahagi-river system, which is located close to a golf course, showed a different monthly change from the other sampling points. Golf courses are an expected source of contamination due to the fact that most golf courses in Gifu Prefecture are located in Chuunou and Tounou, and these golf courses are
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often near tributaries and rivers. For this reason we would expect higher concentrations of CTD in these water bodies.

From the predicted CTD distribution map (RMSE = 0.99, ME = 0.30), areas of higher concentrations (>30 ng/L) in Southern Chuunou and Tounou were also matched with the locations of golf courses (Fig. 4). However, in the Northern Hida region, this pattern of high concentration of CTD did not correspond to the proximity of golf courses. It is likely that elevated concentration of CTD in surface waters in Northern Hida are due to other contamination sources, in particular rice and vegetable cultivation areas, as indicated by their distribution. CTD is found in most areas in Gifu Prefecture, and golf courses are the likely sources (Bradford et al., 2018). The adverse health effects from chronic exposure to low-dose CTD are still unknown. As an intervention in cases of residential chronic exposure to CTD, the monitoring of CTD concentrations in surface and underground water sources should be considered.

**IMIDACLOPRID (IMI)**

The range of annual average concentrations and the detection rate of IMI were <2.0–11.5 ng/L and 4.0%–50.0%, respectively, in each sampling location. IMI was found at higher frequencies in Gifu and Chuunou, where vegetable farms are larger in size than in other regions (Table 2). IMI was found at concentrations higher than 10 ng/L only in Chuunou and Gifu (sites #72, NA-9 and #78, NA-15 in Table 1), downstream of the vegetable farms.

The map of predicted IMI distribution (RMSE = 0.97, ME = −0.04) was produced by a cross-validation procedure. IMI has been observed in two hot spots in the Southern Gifu, where elevated concentrations could be due to the high yield of agricultural products in Chuunou and Gifu.

**THIAMETHOXAM (TMX)**

The range of the annual average concentrations and the detection rates of TMX were <2.0–15.7 ng/L and 0%–40%, respectively, in each sampling location. TMX was found only in Tounou. Although TMX has been reported to be a precursor of CTD (Nauen et al., 2003), the regional annual average concentration profile was not similar to that of CTD. These results suggest that TMX might be quickly metabolized in environment or might be applied for another crops.

**ESTIMATING THE ENVIRONMENTAL BEHAVIOR OF THE NEONICOTINOID PESTICIDES**

The neonicotinoid pesticides, as well as other organic chemicals, tend to adsorb on black carbon in soil (Motoki et al., 2015). However, factors affecting the adsorption of the pesticide molecules to black carbon in soil are complex and unclear. We used Log $K_{oc}$ (with EPI Suite™, USEPA), with the organic carbon/water partition coefficient, to briefly examine the adsorption/desorption behavior of the neonicotinoid pesticides in an aqueous environment. In Table 3 we list the concen-

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Table 3 Annual detection levels of the neonicotinoid pesticides in underground water and three comparable factors (chemical structure, Log $K_{oc}$, and Log $K_{ow}$) possibly affecting levels

| Name | NTP | THI | ACE | CTD | IMI | TMX | DIN |
|------|-----|-----|-----|-----|-----|-----|-----|
| underground water average $^*$ ** (ng/L, n = 118) | <0.3 | <0.3 | <0.3 | 0.3 | 0.8 | 0.9 | 8.6 |
| Log $K_{oc}$ | 3.2 | 3.1 | 2.7 | 3.0 | 3.0 | 2.4 | 2.1 |
| Log $K_{ow}$ | 0.4 | 2.3 | 2.6 | 0.6 | 0.6 | 0.8 | <0.2 |
| Half-life of photolysis in water (DT$_{50}$ in days) | NA | 10–63 | 34 | <1 | <1 | 2.7–40 | <2 |
| Half-life of hydrolysis in water (DT$_{50}$ in days) | Stable | Stable | Stable | Stable | Stable | Stable |

$^*$ A, B, C: Substructures of increasing $K_{oc}$ value (A: +0.39 B: +0.22 C: +0.18)
$^*$ D: Substructure of decreasing $K_{oc}$ value (D: −0.09)
$^*$ ** average concentrations of summer and winter surveys (2 days)
$^*$ * Morrisey et al. (2015)
NTP: nitenpyram THI: thiacloprid ACE: acetamiprid IMI: imidacloprid
CTD: clothianidin TMX: thiamethoxam DIN: dinotefuran
tation levels of the neonicotinoid pesticides in underground water through summer/winter surveys (Hayashi et al., 2017) with three comparable factors (chemical structure, Log $K_{ow,\pi}$ and Log $K_{ow}$) possibly affecting the levels. Pesticides containing a nitrile-group showed the strongest adsorption onto soil particles (Substructure A in Table 3), followed by nitro-group (Substructure B), and pyridine-ring (Substructure C). NTP, THI, and ACE, which were not found in underground water through summer/winter surveys (Hayashi et al., 2017), have multiple substructures of A–C within their chemical structures. The above results suggest that pesticide molecules substituted with nitrile-group, nitro-group, and pyridine-ring were rarely detected in underground waters due to their high affinity to soil components. It has been suggested that molecules with a nitrile-group in their structures might be adsorbed on black carbons in soil by $\pi$–$\pi$ interaction (Bucheli and Gustafsson, 2003; Sobek et al., 2009). On the other hand, the annual average concentrations of CTD, IMI, TMX, and DIN in underground water sources were found to be in the range of 0.3–8.6 ng/L (Hayashi et al., 2017). This might be due to substructures, making it harder to be adsorbed by soil particles, or hydrophilic substructures decreasing the Log $K_{ow}$ value (Substructure D in Table 3), as well as a low Log $K_{ow}$. These results suggest that the physiochemical properties of the neonicotinoid pesticides, such as chemical structure, Log $K_{ow}$ and Log $K_{ow}$, might be the key parameters affecting the detection frequencies and the concentrations in environmental waters.

**CONCLUSION**

Seven neonicotinoid pesticides were found in surface waters at concentrations ranging from < 2.0 to 530 ng/L through the ten-month period. DIN was detected at the high rate of 76.9% within 672 samples, followed by CTD (48.4%), and IMI (19.6%). The distributions have shown monthly and regional patterns, and these results suggest that detection varies with the type of agricultural products, practices, and types of land use. Pesticides that are highly adsorbed on soil particles have a tendency of not being detected in surface waters. Predicted contamination maps showed that DIN and CTD are found in a wide range of areas, and that the concentrations were especially high near estuaries. IMI contamination showed significant regionality, and the concentrations were predicted to be high only near estuaries.

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**DECLARATION OF COMPETING INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**SUPPLEMENTARY MATERIAL**

Fig. S1, Sampling locations and the river systems in Gifu Prefecture; Fig. S2, Extraction procedure for seven neonicotinoid pesticides in surface water samples; Table S1, Instrumental parameters of the HPLC / MS / MS measurement; Table S2, Recoveries and limits of detection of the seven neonicotinoid pesticides in surface water ($n$ = 3).

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