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Suspect and non-target screening of pesticides and pharmaceuticals transformation products in wastewater using QTOF-MS

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ABSTRACT

Pesticides and pharmaceuticals are widely used in modern life and are discharged into wastewater after usage. However, a large number of transformation products (TPs) are formed through abiotic (hydrolysis/photolysis, etc.) and biotic (aerobic/anaerobic degradation by micro-organisms) wastewater treatment processes, and the structure and potential risk of TPs are still unclear. In this study, a suspect and non-target screening was performed to monitor these chemicals with HPLC-QTOF-MS. We identified 60 parent compounds by suspect screening in three Chinese wastewater treatment plants with the commercial database of pesticides and pharmaceuticals, and they were confirmed by authentic standards. Then, suspect and non-target screening strategies based on the predicted diagnostic fragment ions were used to screen TPs of the 60 parent compounds. We tentatively identified 50 TPs and confirmed thirteen of them with authentic standards. Among 13 quantified TPs, about 40% of them showed higher concentration than their parent compounds in effluent. Especially, cloquintocet, as a TP of cloquintocet-mexyl, had a concentration ratio TP/parent = 14,809 in effluent. Twenty-five TPs had higher predicted toxicity than the corresponding parent compounds by calculating their LC_{50} values towards aquatic organisms using toxicity prediction software. Twenty identified TPs were firstly reported in this study. These results indicate the importance of TP analysis in environmental monitoring in wastewater.

1. Introduction

Pharmaceuticals and pesticides are two types of widely used hazardous chemicals and have been frequently detected in freshwater or coastal waters from ng L^{-1} to μg L^{-1} globally (Agarwal et al., 2015, Ccanccapa et al., 2016, Moreno-González et al., 2016). In 2017, 3.5 million tons of pharmaceuticals and 2.9 million tons of pesticides were produced in China. Pharmaceuticals and pesticides would enter municipal or industrial wastewater treatment plants (WWTPs) after their residential usage or from industrial production (Köck-Schulmeyer et al., 2013, Munz et al., 2017). But conventional WWTPs, designed for the removal of nutrients, only eliminate pharmaceuticals and pesticides to a limit extent (Munz et al., 2017). After treatment process of WWTPs, pharmaceuticals and pesticides are released into receiving water system including rivers, lakes and then coastal water system (Schollée et al., 2018). Coastal waters are vulnerable areas because of their intermediate position between open seas and human activities, and micropollutants in coastal waters are poorly studied (Sánchez-Avila et al., 2012). WWTPs are recognized as an important source of pharmaceuticals, pesticides and their metabolites in coastal water and freshwater system (Gaw et al., 2014, Köck-Schulmeyer et al., 2013). In aquatic environment, pharmaceuticals could cause adverse ecological effect, including reduced feeding rates, impacts on survival, changes in immune response, etc, to aquatic organisms at μg L^{-1} or ng L^{-1} (de Jesus Gaffney et al., 2015, Gaw et al., 2014). And pesticides are one of the most basic contributor to chemical risk in freshwater system (Malaj et al., 2014).

The increasing number of chemicals makes it practically impossible for target analyses dependent on individual standards to cover all potentially occurring chemicals (Moschet et al., 2013). Suspect and non-target screening methods are therefore developed to reveal the full spectrum of occurring chemicals. Despite the lack of authentic standards, suspect screening can be performed with suspect chemical lists, and non-target screening can be performed with no prior information using high-resolution mass spectrometry (HR-MS) (Krauss et al., 2010, Schymanski et al., 2014b, Schymanski et al., 2015). Suspect and non-

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target screening can narrow the scope of target compounds and minimize the related quantification tasks.

Many pesticides and pharmaceuticals are likely to be degraded or metabolized after usage. In WWTPs, biodegradation, including aerobic and anaerobic degradation, is key mechanism for the removal of micropollutants. For example, carbamazepine and atenolol can be efficiently removed in anaerobic process through hydrolysis of amide and urea groups. The biodegradation rate and efficiency vary from compound to compound considering their solubility, functional group and structure. (Tiwari et al., 2017). A study on 86 groundwater samples and 154 surface water samples in the United States revealed that transformation products (TPs) were detected as often as or more frequently than parent compounds, and the toxicity of TPs cannot be neglected (Boxall et al., 2004). In some cases, the concentrations and toxicity of TPs can be higher than those of their parent compounds, warranting environmental risk assessment of TPs (Escher and Fenner, 2011). In European Union Regulation 1907/2006, identification of the major TPs is required to register a substance (Bletsou et al., 2015). However, for the lack of authentic standards and structural information on TPs, TP analysis is still challenging (Hernández et al., 2011).

Many suspect TPs have been identified using suspect screening method, with suspect TP lists built by the TP prediction system and related literatures (Gago-Ferrero et al., 2015, Kern et al., 2009, Moschet et al., 2016). Due to the unavailability of standard MS/MS spectra for many TPs, the fragmentation–degradation relationship was proposed to interpret TPs' MS/MS spectra (Hernández et al., 2011, Kern et al., 2009). This relationship takes advantage of potentially equivalent fragmentation patterns between TPs and their parent compounds; identical or corresponding fragment ions can be found between TPs and parent compounds. However, TPs formed in the industrial synthesis can hardly be predicted by current predicted methods under the specific industrial manufacture conditions (Gómez-Ramos et al., 2011). Due to the limitation of suspect list, suspect screening is unable to identify unknown TPs that may be formed by unknown pathways. To this end, a non-target TP screening strategy with no prior suspect list is needed.

Based on the MS/MS spectra similarity between parent and TPs, software-assisted library searching with no suspect list has become an option (Gao et al., 2015, Gómez-Ramos et al., 2011). In this method, all peaks containing same fragment with parent compounds were extracted and analyzed as potential TPs. The diagnostic fragment ion–based extension strategy (DFIBES) was originally proposed for identifying homologous medicine families based on their common diagnostic fragment ion (DFI) from common structure (Zheng et al., 2009). In this strategy, the DFI is first identified using homologous chemical MS/MS spectra, and extracted ion chromatography is then used to find potentially new homologous chemicals. The DFIBES has also been used in combination with extracted ion chromatography in the screening of medicinal metabolites with the identified DFI (Liang et al., 2010, Wang et al., 2009). The selection of one or several DFIs rather than all fragment ions can avoid many false positive results in complicated matrix non-target analyses. To our knowledge, this DFIBES has not been used in identification of TPs in environment samples.

In our present study, we analyzed wastewater samples from three WWTPs in a Chinese coastal province, Jiangsu, using LC-ESI-QTOF-MS to screen for TPs of pesticides and pharmaceuticals. We selected parent compounds by suspect screening on 1,283 database chemicals, including pesticides and pharmaceuticals in ESI(+) and ESI(-) mode. For the confirmed parent compounds, dual suspect and non-target screening was performed to identify their known and unknown TPs. In suspect TP screening, the lists of suspect TPs were collected from online TP prediction systems and related literatures. In particular, the fragmentation–degradation relationship was used to enhance the confidence of TP identification. In non-target TP screening, we used a modified DFIBES strategy in which DFIs were predicted based on the MS/MS spectra of parent compounds and their predicted TPs. The confirmed parent compounds and TPs were further quantified with authentic standards using LC-MS/MS. The environmental risks of the quantified parent compounds and TPs in the three WWTPs were evaluated. Through all the above tasks, we aimed to establish a framework for a full-spectrum identification of TPs, including knowns and unknowns, to advance our understanding of the fate and impact of pesticides and pharmaceuticals in wastewater.

2. Materials and methods

2.1. Chemicals

Fifty authentic standards, including 29 pesticides and 21 pharmaceuticals, were purchased for validation of parent compound screening method after spiked into wastewater as artificial suspects. They covered chemicals in both ESI(+) and ESI(-) mode and a wide range of physicochemical properties (e.g., log Kow = -2.82–6.64) as is presented in Table S1. Seventy-three authentic standards were purchased for validation of screening results and quantification, including 46 pesticides, 14 pharmaceuticals and 13 TPs. Detailed information can be seen in Table S2.

2.2. Sample preparation

Wastewater samples were collected from influent and effluent of three different WWTPs in a Chinese coastal province, Jiangsu, between March and June 2015. The three WWTPs, denoted as P1, P2, and P3, treat industrial wastewater, a mix of industrial and municipal wastewater, and municipal wastewater, respectively. All three WWTPs apply a pretreatment for solid removal, an activated sludge biological treatment, and a final clarification treatment. The hydraulic retention time of all three WWTPs is about 24 h. At P1, influent and effluent samples at three different time points in a day were collected considering the irregular influent of wastewater in P1. At P2 and P3, we collected one influent and one effluent sample in each WWTP. For each sampling location, 1 L wastewater sample was collected in a polypropylene bottle. Samples were stored under dark conditions at 4 °C and extracted within 48 h.

After filtration with glass fiber filters (1 μm, Whatman, UK) and nylon membrane filters (0.45 μm, Whatman, UK), 1 L wastewater underwent solid-phase extraction with an Oasis HLB column (6 cc, 500 mg, Waters, USA) (Gros et al., 2006). The HLB columns were dried and then eluted with 12 mL methanol. The eluent was evaporated to dryness under nitrogen and reconstituted to 1 mL with methanol. Extracts were filtered using a polytetrafluoroethylene syringe filter (0.45 μm) and stored at −20 °C. A procedure blank was used in each batch of analysis using Milli-Q water treated identically to wastewater.

2.3. Qualitative analysis using LC-ESI-QTOF-MS

An LC system (Agilent 1260, Agilent, USA) was interfaced to a quadrupole time-of-flight mass spectrometer (Triple TOF 5600, AB SCIEX, USA) for analysis in both ESI(+) and ESI(-) modes. The reversed-phase liquid chromatographic separation was performed on a C18 column (Zorbax Eclipse XDB-C18, Agilent, 2.1 mm × 150 mm, 3.5 μm). Detailed information on LC and Q-TOF parameters is presented in Section SI-2. Mass accuracy (< 5 ppm) were checked and corrected every five samples with calibration solution. Fifty authentic standards were spiked into 1 L industrial effluent wastewater 10 ng/L, 50 ng/L, 100 ng/L, 500 ng/L, 1000 ng/L, and treated as samples to 1 mL to evaluate screening method.

2.4. Quantitative analysis using LC-ESI-MS/MS

For higher sensitivity and wider linear range, an LC system (Agilent 1260, Agilent, USA) was interfaced to a triple quadrupole spectrometer (API 4000, AB SCIEX, USA) for confirmation and quantification of

chemicals (Grimalt et al., 2010, Holčapek et al., 2012, Liu et al., 2017). The reversed-phase liquid chromatographic separation was performed on a C18 column (Xbridge BEH C18, USA, 2.1 mm × 50 mm, 2.5 μm, Waters, USA). Detailed instrument information is presented in Section SI-2. The LC-MS/MS method of 73 identified chemicals were optimized using authentic standards, and optimized value of parent ion, daughter ion, collision energy and declustering potential were given in Table S2. To understand the recovery rate of 73 standards after sample pretreatment, 73 standards were spiked into 1 L Milli-Q water at 50 ng/L and 100 ng/L with five replicates. These samples were concentrated to 1 mL and injected to LC-MS/MS for quantification. For the lack of internal standards for most TPs, matrix effect was evaluated by spiking 73 external standards in wastewater samples.

2.5. Selection of parent compounds

In this section, PeakView 1.2 software (AB SCIEX, USA) was used to investigate the presence of parent chemicals. PeakView software included an XIC manager application in which parameters, such as characteristic adducts (e.g., $[M + H]^+$, $[M + NH_4]^+$, and $[M-H]^-$), MS error, isotopic difference, and purity score were set, and calculated results were displayed automatically (Martínez Bueno et al., 2012). The workflow of parent compound suspect screening is shown in Fig. 1. For the suspect chemicals, a list of 1,283 chemicals, comprising of 418 pesticides, 865 pharmaceuticals, was used. This list was sourced from a commercial AB SCIEX database with HR-MS/MS spectra of them. As shown in Fig. 1, screening criteria included (1) procedure blank background subtraction, (2) peak intensity of $>$ 1,000 cps, (3) mass error of $<$ 5 ppm, (4) isotopic difference of $\leq$ 20%, and (5) MS/MS spectra match which requires a purity score of $\geq$ 60 and matched fragments of $\geq$ 2 (Bueno et al., 2012, Gago-Ferrero et al., 2015). The purity score was calculated by PeakView to assess the similarity between standard and experimental MS/MS spectra. Matched fragments were considered to exclude chemicals with only parent ions in their MS/MS spectra. Authentic standards were then purchased to confirm the screening results through retention time and MS/MS spectrum.

2.6. TP screening procedure

For the selected parent compounds by suspect screening, their potential TPs were screened by both suspect screening and non-target screening. In suspect screening, TP suspects were extracted from EAWAG-BBD Pathway Prediction System (http://eawag-bbd.ethz.ch/predict/) and related literatures (Gao et al., 2009). As is presented in Fig. 1, screening was performed based on intensity, mass error, and isotopic pattern using PeakView. After elucidation, MS/MS spectra were interpreted via a literature spectrum search, and two matched fragment ions were required. In the absence of standard MS/MS spectra of TPs, the fragmentation–degradation relationship was used to improve each TP's confidence level based on the MS/MS spectrum of its parent compound (Creek et al., 2014, Schymanski et al., 2014a). Based on the fragmentation–degradation relationship, literature spectrum search, and fragment interpretation, we excluded the unlikely TPs and assigned a confidence level for plausible TPs according to Schymanski et al (Schymanski et al., 2014b): level 5 for exact mass of interest; level 4 for unequivocal molecular formula; level 3 for tentative candidates; level 2a for probable structure by library spectrum match; level 2b for probable structure by diagnostic evidence; and level 1 for correspondence to confirmed structure by authentic standards. Finally, authentic standards were purchased to confirm high-confidence candidates.

In non-target screening of TPs, the MS/MS spectra of target parent compounds were analyzed by PeakView to acquire all explained fragments with an error of $<$ 2 mDa and relative intensity of $>$ 10%. Subsequently, the frequency of fragment structures among predicted TPs (from the EAWAG-BBD Pathway Prediction System) were listed to obtain fragments with the highest frequency and larger structures as the DFI (Qi et al., 2012, Zheng et al., 2009). For the given DFI (error $<$ 2 mDa, ion abundance $>$ 10%), peaks were extracted in MS-DIAL 2.02 (http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/index.html) using MS/MS fragment searcher pane. Extracted peaks were imported into PeakView to calculate their formulas considering MS error ($<$ 5 ppm), MS/MS error ($<$ 2 mDa), isotopic difference ($<$ 20%), and number of explained fragments. TP structures were deduced based on MS/MS spectrum interpretation, especially the

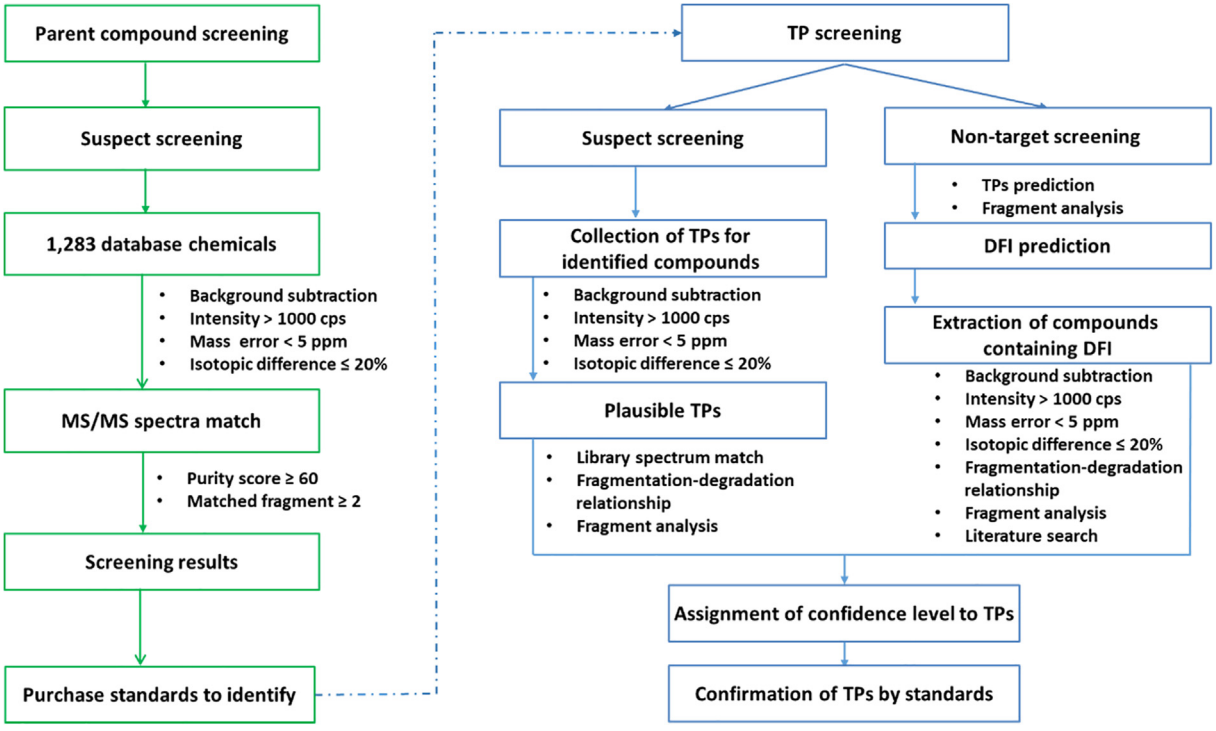


Fig. 1. Workflow of parent compound and transformation product (TP) screening. DFI: diagnostic fragment ion;

Table 1
Information on 57 identified transformation products (TPs).

	Formula	Parent compound	Workflow[1]	Confidence level	Toxicity ratio	Concentration ratio-P1[2]	Concentration ratio-P2	Concentration ratio-P3	Previous study[3]	Structure
TP-1	$C_{11}H_{10}Cl_2N_2O$	Imazalil	S	1	4.75E-01	1.69E+00	N/A	1.62E+00	yes	Fig. S4
TP-2	$C_5H_7ClN_2S$	Thiamethoxam	N	1	1.78E+02	6.95E-01	N/A	N/A	yes	Fig. S5
TP-3	$C_6H_9N_3O_2$	Nicosulfuron	S + N	1	4.63E-01	4.06E-01	N/A	N/A	yes	Fig. S6
TP-4	$C_5H_3Cl_2FN_2O$	Fluroxypyr	S	1	1.38E+01	1.55E+00	N/A	N/A	yes	Fig. S7
TP-5	$C_8H_{15}N_5O$	Atrazine	S	1	5.23E-01	7.62E-02	3.14E+00	3.58E+02	yes	Fig. S8
TP-6	$C_7H_6N_2$	Carbendazim	S	1	1.20E+00	6.35E-03	5.44E-03	2.06E-02	yes	Fig. S9
TP-7	$C_{11}H_8ClNO_3$	Cloquintocet-mexyl	S + N	1	2.96E-04	1.48E+04	N/A	N/A	yes	Fig. S10
TP-8	$C_6H_8ClN_5O_2S$	Thiamethoxam	S + N	1	9.45E-01	4.80E-01	N/A	N/A	yes	Fig. S11
TP-9	$C_{33}H_{62}N_2O_{12}$	Roxithromycin	N	1	8.64E-01	N/A	6.04E-01	7.13E-01	yes	Fig. S12
TP-10	$C_{40}H_{74}N_2O_{15}$	Roxithromycin	S + N	1	3.26E-01	N/A	4.60E-02	7.76E-02	yes	Fig. S13
TP-11	$C_9H_{11}ClN_4$	Imidacloprid	S + N	1	1.08E+01	1.11E-01	1.35E+00	4.73E-01	yes	Fig. S14
TP-12	$C_9H_{10}ClN_3O$	Imidacloprid	S + N	1	2.65E+00	1.25E-02	8.34E-01	1.10E-01	yes	Fig. S15
TP-13	$C_9H_{17}N_5O$	Prometryn	S + N	1	3.21E-01	7.77E-02	3.84E+00	2.21E+00	yes	Fig. S16
TP-14	$C_6H_9ClN_4S$	Thiamethoxam	N	2a	1.42E+02	4.80E+00	N/A	N/A	yes	Fig. S17
TP-15	$C_{21}H_{15}N_3O_5$	Azoxystrobin	S + N	2a	8.48E-02	2.35E-01	1.34E-01	N/A	yes	Fig. S18
TP-16	$C_{12}H_{13}NO_3S$	Carboxin	S	2a	4.24E-01	5.44E+00	N/A	N/A	yes	Fig. S19
TP-17	$C_5H_6ClN_5O_2S$	Thiamethoxam	S + N	2a	5.22E-01	2.86E-01	N/A	N/A	yes	Fig. S20
TP-18	$C_{14}H_{19}NO_4$	Metalaxyl	S + N	2a	1.27E-01	7.51E-04	N/A	N/A	yes	Fig. S21
TP-19	$C_9H_{11}ClN_2O$	Acetamiprid	S + N	2a	8.30E-01	N/A	1.07E+00	N/A	yes	Fig. S22
TP-20	$C_7H_{13}N_5S$	Prometryn	S + N	2a	2.70E-01	1.85E-02	N/A	N/A	yes	Fig. S23
TP-21	$C_8H_{10}ClN_3O_2S$	Thiamethoxam	S + N	2a	3.63E+01	9.29E-01	N/A	N/A	yes	Fig. S24
TP-22	$C_9H_{13}ClN_4OS$	Thiamethoxam	N	2b	1.11E+02	2.70E+01	N/A	N/A	no	Fig. S25
TP-23	$C_5H_5ClN_2S$	Thiamethoxam	N	2b	2.26E+01	7.68E-02	N/A	N/A	no	Fig. S26
TP-24	$C_9H_{12}O_5S_2$	Isoprothiolane	S	2b	3.40E-03	N/A	6.58E-03	N/A	no	Fig. S27
TP-25	$C_6H_9ClN_2S$	Thiamethoxam	N	2b	1.64E+02	5.26E-01	N/A	N/A	no	Fig. S28
TP-26	$C_7H_{10}ClN_3OS$	Thiamethoxam	N	2b	1.32E+02	1.28E+00	N/A	N/A	no	Fig. S29
TP-27	$C_9H_{14}ClN_3OS$	Thiamethoxam	N	2b	1.17E+02	6.28E-03	N/A	N/A	no	Fig. S30
TP-28	$C_{10}H_{19}N_5OS$	Prometryn	N	2b	1.89E-01	7.34E-02	N/A	N/A	no	Fig. S31
TP-29	$C_7H_8ClN_3O_2S$	Thiamethoxam	S + N	2b	3.15E+01	3.15E-02	N/A	N/A	yes	Fig. S32
TP-30	$C_8H_7Cl_2FN_2O_3$	Fluroxypyr	S + N	2b	1.19E+01	5.43E-02	N/A	N/A	yes	Fig. S33
TP-31	$C_6H_8N_3OSCl$	Thiamethoxam	N	2b	1.41E+02	1.12E+01	N/A	N/A	no	Fig. S34
TP-32	$C_{11}H_{15}NO$	Metazachlor	N	2b	7.05E-03	2.83E+00	N/A	N/A	yes	Fig. S35
TP-33	$C_8H_{12}ClN_5OS$	Thiamethoxam	S + N	2b	1.11E+02	1.12E+00	N/A	N/A	yes	Fig. S36
TP-34	$C_8H_{11}ClN_4OS$	Thiamethoxam	S + N	2b	1.18E+02	1.99E+00	N/A	N/A	yes	Fig. S37
TP-35	$C_6H_9ClN_2OS$	Thiamethoxam	N	3	1.51E+02	1.31E-02	N/A	N/A	no	Fig. S38
TP-36	$C_6H_7ClN_2O_2S$	Thiamethoxam	N	3	2.63E-02	1.38E-01	N/A	N/A	no	Fig. S39
TP-37	$C_9H_{13}ClN_4O_2S$	Thiamethoxam	N	3	1.05E+02	4.53E-01	N/A	N/A	no	Fig. S40
TP-38	$C_8H_{11}ClN_6S$	Thiamethoxam	N	3	1.12E+02	1.31E-01	N/A	N/A	no	Fig. S41
TP-39	$C_{13}H_{18}N_2OS$	Levamisole	N	3	6.74E-01	1.45E+00	N/A	N/A	no	Fig. S42
TP-40	$C_7H_4Cl_2O_3$	2,4-D	N	3	1.03E+01	5.40E+02	N/A	N/A	yes	Fig. S43
TP-41	$C_7H_{17}NO_2$	Lidocaine	N	3	2.84E-02	N/A	6.86E-01	N/A	yes	Fig. S44
TP-42	$C_7H_{11}ClN_4S$	Thiamethoxam	N	3	1.33E+02	5.66E-01	N/A	N/A	no	Fig. S45
TP-43	$C_7H_9ClN_2OS$	Thiamethoxam	N	3	1.42E+02	3.38E-01	N/A	N/A	no	Fig. S46
TP-44	$C_{13}H_{14}ClN_3O_3$	Cyproconazole	N	3	3.32E-02	1.63E-01	N/A	N/A	no	Fig. S47
TP-45	$C_{10}H_{23}NO_2$	Lidocaine	N	3	1.56E-01	N/A	2.78E+01	N/A	yes	Fig. S48
TP-46	$C_{10}H_{21}NO_2$	Lidocaine	N	3	5.02E-01	N/A	2.55E+02	2.95E+01	no	Fig. S49
TP-47	$C_9H_{10}NOF$	Flutriafol	N	3	7.79E-01	2.10E-02	N/A	N/A	yes	Fig. S50
TP-48	$C_{15}H_{18}ClN_3O_2$	Cyproconazole	S + N	3	5.19E-01	2.70E-02	N/A	N/A	no	Fig. S51
TP-49	$C_6H_7ClN_2OS$	Thiamethoxam	N	3	2.08E+00	2.59E-01	N/A	N/A	no	Fig. S52
TP-50	$C_9H_{14}ClN_5S$	Thiamethoxam	N	3	1.12E+02	1.17E+00	N/A	N/A	no	Fig. S53
TP-51	$C_{13}H_{13}N_3O_5$	Nicosulfuron	N	4	N/A	3.14E+00	N/A	N/A	N/A	Fig. S54
TP-52	$C_{14}H_{14}Cl_2N_2O$	Propiconazole	N	4	N/A	1.57E+01	1.88E-01	N/A	N/A	Fig. S55
TP-53	$C_{14}H_{14}Cl_2N_2O_3$	Propiconazole	N	4	N/A	1.77E+00	N/A	N/A	N/A	Fig. S56
TP-54	$C_8H_9N_6SCl$	Thiamethoxam	N	4	N/A	4.33E-01	N/A	N/A	N/A	Fig. S57
TP-55	$C_5H_6N_3OSCl$	Thiamethoxam	N	4	N/A	7.65E-01	N/A	N/A	N/A	Fig. S58
TP-56	$C_{10}H_{13}N_4O_2SCl$	Thiamethoxam	N	4	N/A	1.60E+00	N/A	N/A	N/A	Fig. S59
TP-57	$C_{18}H_{31}N_6O_2SCl$	Thiamethoxam	N	4	N/A	6.28E-03	N/A	N/A	N/A	Fig. S60

N/A for not applicable, structures and MS/MS spectra of TPs were given in Fig. S4–Fig. S60.

[1] S for identified by suspect screening workflow, N for non-target screening workflow, S + N for identified by both workflows.

[2] Concentration ratio is calculated by dividing concentration of TP by parent compound concentration in effluent, intensity on LC-QTOFMS is used in calculating while concentration is not available. P1, P2, P3 represent three WWTPs.

[3] Previous study is found in http://scifinder.cas.org.

fragmentation–degradation relationship. Literature was searched for MS/MS spectrum confirmation to obtain a higher confidence level. False positive results, including structures not likely to be TPs and in-source fragmentation behavior of parent compounds, were removed. Identified TPs were each given a confidence level, and standards were purchased for high-confidence TPs. The TP screening workflow is summarized in Fig. 1.

2.7. Toxicity and risk assessment

For confirmed compounds, the risk quotient (RQ) was calculated by dividing measured concentration by predicted no-effect concentration (PNEC) and then multiplying by a dilution factor of 0.1 (considering dilution from wastewater to river) (Xu et al., 2007). PNEC was calculated by dividing LC_{50}, EC_{50}, or NOEC of three organism groups (fish, algae, and daphnia) by a safety factor of 1,000 for acute toxicity and 10

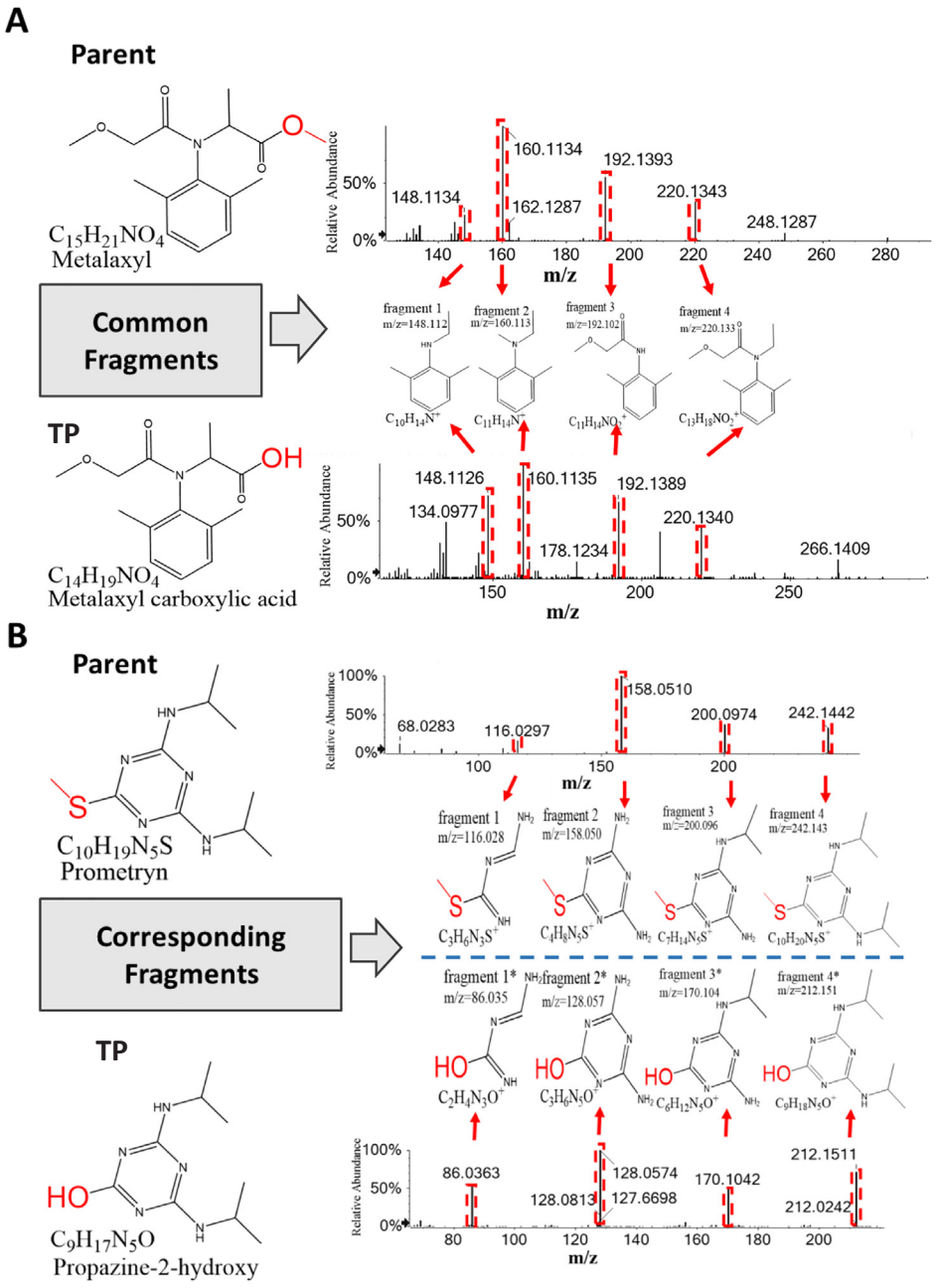


Fig. 2. Tandem mass spectroscopy spectra and fragment analysis for metalaxyl, prometryn, and their transformation products (TPs).

for chronic toxicity (Vryzas et al., 2009). The experimental toxicity data for confirmed chemicals were obtained from the EPA ECOTOX database (http://cfpub.epa.gov/ecotox) and Pesticide Properties DataBase (PPDB) (http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm). We used the geometric mean of collected toxicity data in calculating PNEC for each species, and the most conservative PNEC among three species were used in calculating RQ (Sala et al., 2012). RQ was calculated for the effluent wastewater of all three WWTPs. In the absence of toxicity data for identified TPs, their RQ was calculated using predicted toxicity value by the Ecological Structure Activity Relationships (ECOSAR) Predictive Model (version 1.11) developed by the United States Environmental Protection Agency (Cash and Nabholz, 2001). The most conservative predicted LC_{50} values generated by ECOSAR were used (Burden et al., 2016). Toxicity was calculated according to the structure of TPs in ECOSAR, the most probable structure was used for level 3 TPs, for which there may be several structures. Toxicity ratios of TP to parent compound were calculated by dividing the calculated parent compound LC_{50} by the calculated TP LC_{50}.

3. Results and discussion

3.1. Evaluation of LC-QTOF-MS and LC-MS/MS methods

To evaluate the screening method, 50 mixed standards were spiked into WWTP P1 effluent, treated and injected to LC-QTOF-MS at five different concentrations, and analyzed following the workflow of parent compound screening. As described in Fig. S1, 66% of 50 authentic standards were identified at 10 ng/L, 84% at 50 ng/L, 86% at 100 ng/L, 94% at 500 ng/L, and 94% at 1,000 ng/L.

Through LC-MS/MS analysis of spiked samples at 50 ng/L and 100 ng/L, over 80% of the 73 standards had acceptable recovery rates of between 60% and 120% in two concentrations ($n = 5$, RSD < 20%). Six chemicals, including cimetidine, carboxin, ketoconazole, thiabendazole, 4-amino-3,5-dichloro-6-fluoro-2-pyridone and 1-

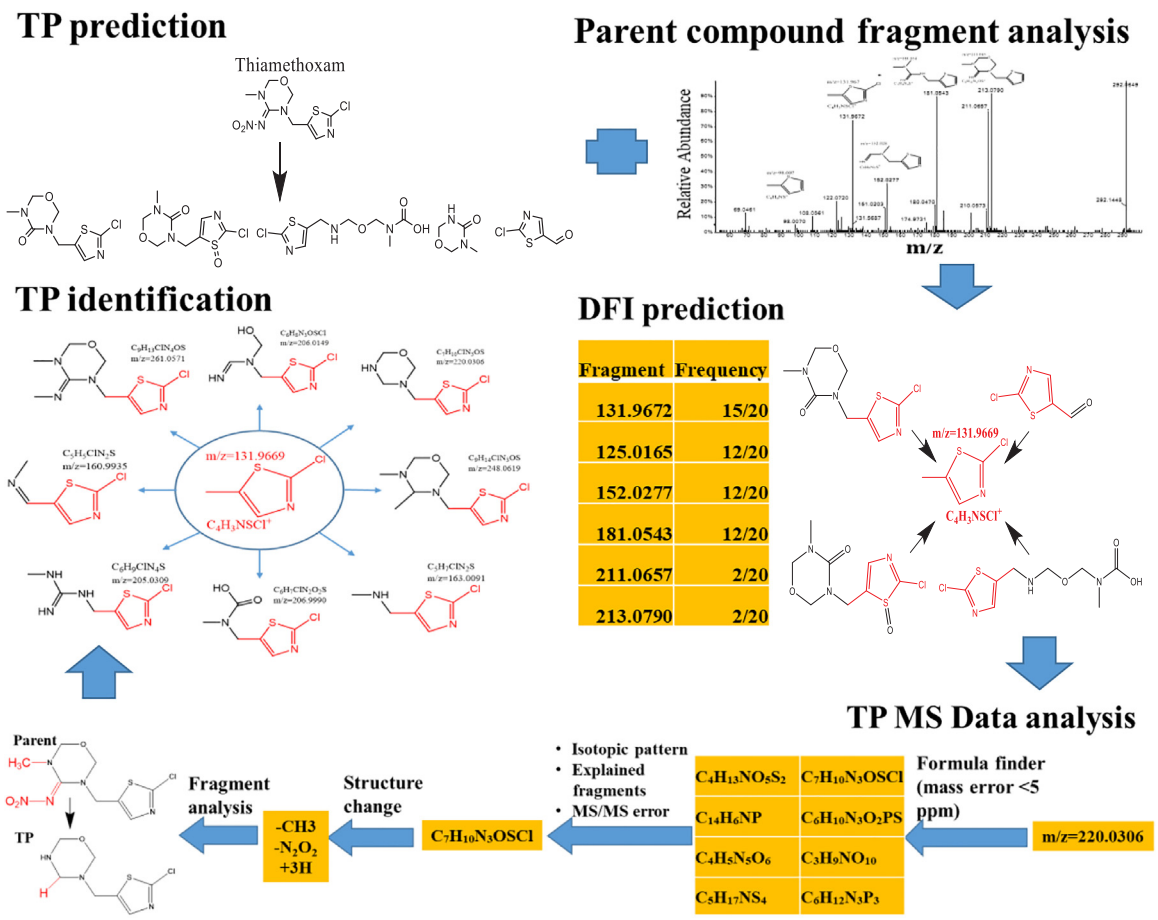


Fig. 3. Workflow for the identification of thiamethoxam transformation products (TPs) by non-target screening. DFI: diagnostic fragment ion; MS/MS: tandem mass spectroscopy.

(2-chlorothiazol-5-yl)-N-methylmethanamine showed recovery rates lower than 40%. The low recovery rate could be attributed to their water solubility and high polarity considering the Kow value, because their high water solubility limits their retention on HLB cartridges (Gómez et al., 2006). Matrix effect was calculated by spiked standards concentration in matrix divided by standards concentration in methanol while the original standards concentration in matrix was subtracted (Bueno et al., 2012). About 80% standards had matrix effect from 60% to 110% in all three WWTP wastewater samples. The recovery rate and matrix effect value for each compound is given in Table S2.

3.2. Suspect screening of TPs

We identified 62 chemicals by parent compound suspect screening, and 60 chemicals including 46 pesticides and 14 pharmaceuticals were confirmed with authentic standards with two false positive results. Sixty confirmed chemicals were quantified in influent and effluent of three WWTPs by authentic standards using LC-MS/MS, concentration of sixty chemicals can be acquired from Table S4. Of the 60 chemicals, 54, 39, and 35 chemicals were detected in P1, P2, and P3, respectively. The highest concentration of the confirmed chemicals ranged from 2.0 ng/L to 260,000 ng/L for P1, 1.6 ng/L to 60,000 ng/L for P2, and 0.7 ng/L to 480 ng/L for P3.

For 60 confirmed chemicals, 334 suspect TPs were collected, yielding 67 hits after elucidation by mass error and isotopic pattern. After interpretation of MS/MS spectra, 24 high-confidence TPs (level 3 or higher confidence) were identified (Table 1). In MS/MS spectra interpretation, literature spectra search was an easy way to assign TPs to level 2a, especially for TPs collected from literatures. For example, the MS/MS spectra of clothianidin, imidacloprid-urea and imidacloprid-guanidine matched with literature spectra and were confirmed with authentic standards (Kamel, 2010). However, for other TPs with no standard MS/MS spectra, fragment analyses were performed for both TPs and their parents to explore potential fragmentation–degradation relationships. For example, metalaxyl carboxylic acid was screened as a transformation product of metalaxyl with methoxy group transformed into hydroxyl group (Fig. 2A). After analysis the MS/MS spectra of metalaxyl and its TP metalaxyl carboxylic acid, four common fragments m/z = 148.1134, 160.1134, 192.1393, 220.1343 were found in both of their spectra. Considering the structures of four fragments, transformation from methoxy group to hydroxyl group influenced none of the four fragments. Thus, metalaxyl carboxylic acid was identified as a transformation product of metalaxyl at level 2b. However, propazine-2-hydroxy, a transformation product of prometryn, showed another pattern (Fig. 2B). For parent compound prometryn, four characteristic fragments m/z = 116.0297, 158.0510, 200.0974, 242.1442 were found while for TP propazine-2-hydroxy m/z = 86.0363, 128.0574, 170.1042, 212.1511 were found. By fragment analysis, transformation from methylthio group to hydroxyl group would lose m/z = 29.9928, which corresponded with fragment change of four characteristic fragments. Thus, propazine-2-hydroxy was assigned to level 2b as a transformation product of prometryn. In both cases, the mass fragmentation behavior remained unchanged after transformation. Corresponding fragments between parent compounds and TPs were used as vital evidence for the assignment of confidence levels to TPs.

In suspect TP screening, 24 TPs were identified, of which 11 TPs were confirmed by authentic standards at level 1, 7 at level 2a, 5 at

level 2b, and one at level 3. Of the 24 identified TPs, 14 TPs were acquired from the EAWAG-BBD Pathway Prediction System, 17 from literatures, and seven from both sources (Huntscha et al., 2012, Reemtsma et al., 2013, Yamamuro et al., 2014). This TP prediction system provides an efficient tool for collecting suspect TPs, but relevant transformation pathways still need to be updated to improve its predictability.

3.3. Non-target screening of TPs

To identify unknown TPs beyond the known suspects, we applied the non-target TP screening strategy to the 60 confirmed parent compounds. In non-target screening, the prediction of DFI is the first step. For each target parent compound, the predicted DFI was select based on the fragment ions in MS/MS spectra of parent compound and the fragment frequency in the structure of predicted TPs. As is presented in Fig. 3, after fragment analysis and TP prediction for thiamethoxam, the most common fragment among the TPs was selected as the predicted DFI, namely $C_4H_3NSCl^+$ with m/z = 131.9672. Based on the selected DFI, all peaks generating m/z = 131.9672 were extracted and analyzed (mass error < 2 mDa, ion abundance > 10%). Taking one identified TP of thiamethoxam as an example (Fig. 3), peak m/z = 220.0306 was extracted because the DFI with m/z = 131.9672 was in its MS/MS spectrum. The formula finder under mass error of 5 ppm returned eight proposed formulas for peak m/z = 220.0306. Further analysis based on isotopic patterns, explained fragments, and MS/MS error excluded seven of these formulas, leaving only one, namely $C_7H_{10}N_3OSCl$. Compared with the parent formula, $C_8H_{10}N_5O_3SCl$, the TP $C_7H_{10}N_3OSCl$ resulted from the loss of a N-nitro group and a methyl group. Based on the structure of the parent compound and the MS/MS spectra of both parent compound and TP (Fig. S29), the structure of this TP was deduced with confidence level 2b and no previous study has been found on this TP.

For TP screening of 60 confirmed parent compounds by both suspect and non-target screening, 57 TPs were identified: 13 were identified at level 1, eight at level 2a, 13 at level 2b, 16 at level 3, and seven at level 4 (Table 1). No false positive results were found in the process of confirming 13 TPs to levels 1 by authentic standards (nine from level 2a, four from level 2b). Among the 50 identified TPs (level 3 or above), 36 had lower Kow values than their parent compounds, whereas 14 had higher Kow values, indicating that compounds were more likely to be degraded into hydrophilic compounds. Twenty TPs have no previous study by searching on http://scifinder.cas.org, including 14 TPs of thiamethoxam, two TPs of cyproconazole, one of isoprothiolane, one of prometryn, one of levamisole, and one of lidocaine (Table 2). Among the twenty novel TPs, only two TPs, TP-24 and TP-48 were identified by suspect screening workflow, which means that TP-24 and TP-48 could be predicted by EAWAG-BBD Pathway Prediction System but not found in any literature. Other eighteen TPs were identified by non-target screening workflow. TPs of thiamethoxam were only detected in P1, which receives wastewater from a factory that produces thiamethoxam. Thus, some identified TPs could also be formed in the synthesis of thiamethoxam. These TPs are formed in specific synthesis conditions and are usually hard to be discovered by laboratory experiments (Gómez-Ramos et al., 2011). Comparing the TPs identified by suspect and non-target screening, we found that the two screening strategies generated different results, although some TPs were identified by both strategies (Fig. 4). Suspect screening is useful for screening for common TPs or those with known pathways, regardless of whether a suitable DFI exists. Non-target screening is more powerful for identifying TPs with unknown pathways with a robust DFI as a premise.

3.4. Quantification of TPs

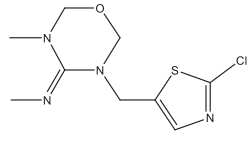
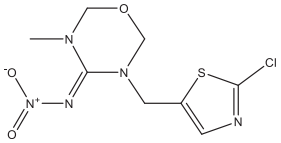
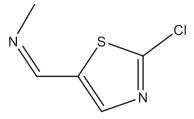
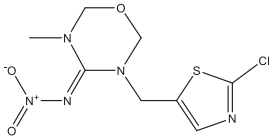
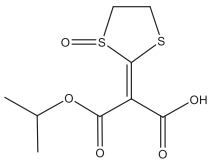
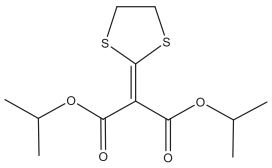
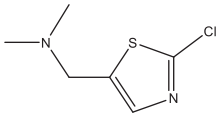
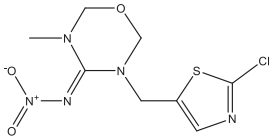
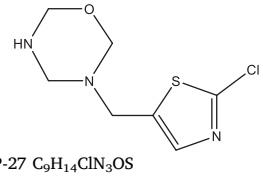
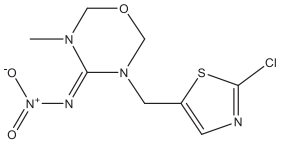
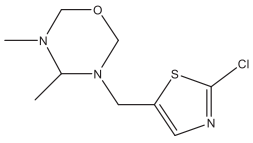
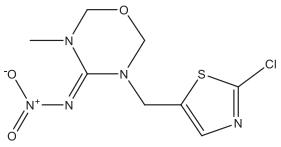
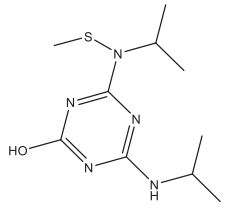
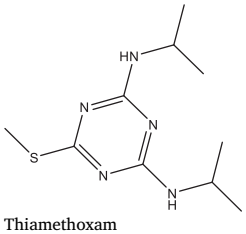
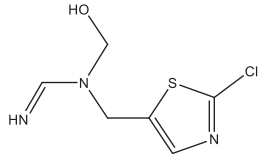
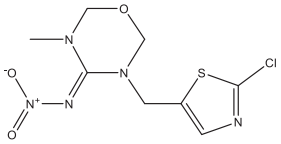
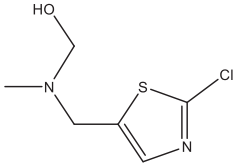
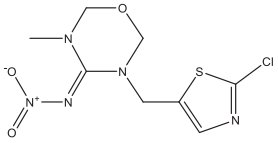
For 50 tentatively identified TPs, 13 of them were further confirmed with authentic standards and the concentration of them in three WWTPs were analyzed using LC-MS/MS. Results were listed in Table S5. We detected eleven, eight and ten TPs in P1, P2 and P3. The concentration of TPs ranged from 8.6 ng/L to 85,000 ng/L in P1, 0.1 ng/L to 143 ng/L in P2, 0.9 ng/L to 390 ng/L in P3. By comparing the concentration of 13 quantified TPs with their parents in effluent wastewater of three WWTPs (Fig. 5A), 41% of TPs had higher concentrations than their parents in average. For all 50 tentatively identified TPs, 39% of TPs had higher intensity than their parents (Fig. 5B). Especially, cloquintocet had a much higher concentration than its parent compound by quantification, cloquintocet-mexyl (with a concentration ratio of TP/P > 1000 in both influent and effluent from P1), indicating that cloquintocet was a major form of cloquintocet-mexyl in wastewater.

3.5. Predicted toxicity and risk of TPs

For the lack of experimental toxicity data for almost all identified TPs, ECOSAR is used to predict toxicity for TPs. ECOSAR has been validated to predict toxicity of many kinds of chemicals, including pesticides, pharmaceuticals and pesticide metabolites (Burden et al., 2016, Madden et al., 2009, Reuschenbach et al., 2008). We also used 60 identified parent compounds as a dataset to evaluate its performance. After comparing ECOSAR predicted and experimental toxicity in fish, daphnid and algae, over 70% of 60 identified chemicals were within 0.1 ~ 10 fold (Fig. S3). The toxicity of 50 identified TPs and toxicity of their parents was predicted using ECOSAR (Fig. 6). The toxicity ratio was calculated by dividing the calculated parent compound LC_{50} by the calculated TP LC_{50}. As is seen in Fig. 6, 50 TPs were identified from 21 parent compounds. Twenty-five TPs with toxicity ratio > 1 were from six parent compounds, thiamethoxam, fluroxypyr, imidacloprid, 2,4-dichlorophenoxyacetic acid (2,4-D), and carbendazim. For 22 TPs of thiamethoxam, 19 had higher predicted toxicity than their parent compounds. The predicted LC_{50} (35.8 μg/L) and experimental LC_{50} (38.3 μg/L) of thiamethoxam is rather close, and pesticide metabolites toxicity prediction using ECOSAR has been proved to be in good performance (Burden et al., 2016). Among 22 TPs of thiamethoxam, only eight TPs have been found in previous studies, with 14 TPs have no previous study. Thiamethoxam is a neonicotinoids developed to replace organophosphate and carbamate insecticides. The effect of neonicotinoids on bees has been widely debated in recent years and the European Union had imposed a 2-year moratorium on the use of neonicotinoids to protect bees in 2013 (Long and Krupke, 2016; Woodcock et al., 2016, 2017). Besides, some metabolites of neonicotinoids have already been found to be as or more toxic than the parent compound (Cimino et al., 2016; Ford and Casida, 2006). Therefore, these discovered TPs of thiamethoxam should be concerned in the further study on neonicotinoids. 1-(2-Chlorothiazol-5-yl)-N-methylmethanamine (TP-2) with the highest toxicity ratio = 178 has been confirmed with standards and quantified as a TP of thiamethoxam (Table 1). TP-2 had a few previous studies but was only mentioned as an intermediate in synthesis (Yang et al., 2014), while no study is found on its occurrence in environment or toxicity. Some TPs of thiamethoxam identified in our study have also been identified as TPs or metabolites before (Ford and Casida, 2006, Kim et al., 2012). Other than thiamethoxam, 2,4-dichlorophenyl hydrogen carbonate (TP-40) was identified as an TP of 2,4-D (Fig. S43), and it had higher predicted toxicity (toxicity ratio = 10) and higher intensity (intensity ratio = 540) than 2,4-D (Table 2). Still, no literature on its occurrence in environment or its toxicity is found.

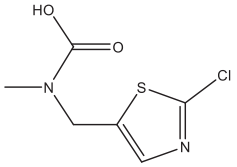
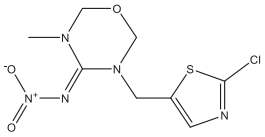
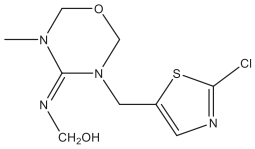
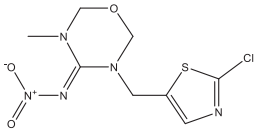
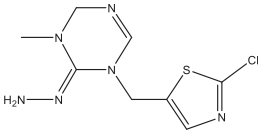
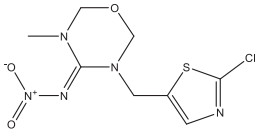
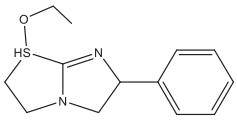
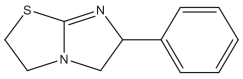
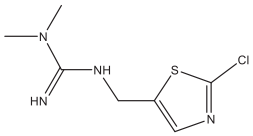
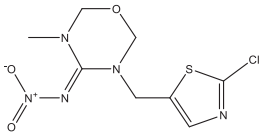
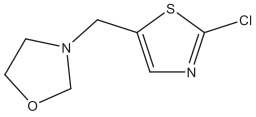
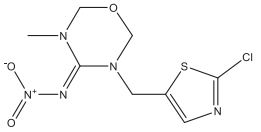
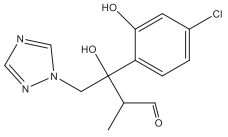
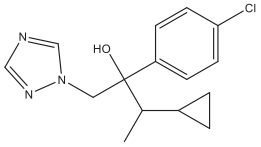
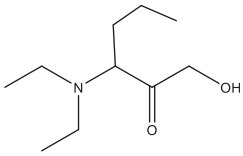
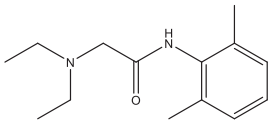
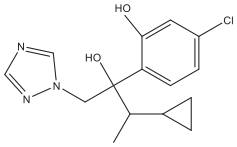
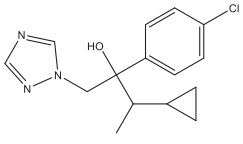
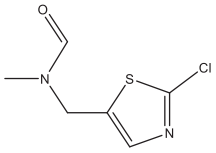
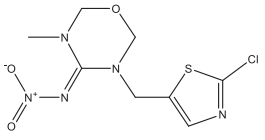
RQs of 13 quantified TPs were also calculated using predicted toxicity and given in Table S5. It's interesting to note that cloquintocet had much higher concentration than parent cloquintocet-mexyl (concentration ratio TP/P = 14809), but the predicted toxicity of cloquintocet is much lower than its parent cloquintocet-mexyl (toxicity ratio TP/P = 0.0003). Thus, the risk of cloquintocet is as low as its parent. However, TPs with high toxicity ratio should be noticed. Two TPs, TP-2 and TP-4, had RQ > 1 from all 13 quantified TPs, while their

Table 2
Identified novel transformation products with no previous study.

Formula and Structure	Parent	Identification workflow	Retention time (min)	Mass error (ppm)	Confidence level	Corresponding fragments with parent
TP-22 $C_9H_{13}ClN_4OS$ 	Thiamethoxam 	Non-target screening	15.16	−0.1	2b	4 fragments
TP-23 $C_5H_5ClN_2S$ 	Thiamethoxam 	Non-target screening	5.22	0.9	2b	2 fragments
TP-24 $C_9H_{12}O_5S_2$ 	Isoprothiolane 	Suspect screening	20.12	2.2	2b	2 fragments
TP-25 $C_6H_9ClN_2S$ 	Thiamethoxam 	Non-target screening	2.13	−0.4	2b	1 fragment
TP-26 $C_7H_{10}ClN_3OS$ 	Thiamethoxam 	Non-target screening	14.27	−1.3	2b	3 fragments
TP-27 $C_9H_{14}ClN_3OS$ 	Thiamethoxam 	Non-target screening	18.53	0.9	2b	3 fragments
TP-28 $C_{10}H_{19}N_5OS$ 	Prometryn 	Non-target screening	20.74	−0.2	2b	4 fragments
TP-31 $C_6H_8ClN_3OS$ 	Thiamethoxam 	Non-target screening	10.09	−0.4	2b	4 fragments
TP-35 $C_6H_9ClN_2OS$ 	Thiamethoxam 	Non-target screening	2.06	−0.4	3	1 fragment

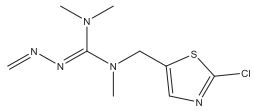
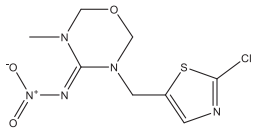
(*continued on next page*)

Table 2 (*continued*)

Formula and Structure	Parent	Identification workflow	Retention time (min)	Mass error (ppm)	Confidence level	Corresponding fragments with parent
TP-36 $C_6H_7ClN_2O_2S$ 	Thiamethoxam 	Non-target screening	15.25	−0.9	3	2 fragments
TP-37 $C_9H_{13}ClN_4O_2S$ 	Thiamethoxam 	Non-target screening	13.86	0.0	3	2 fragments
TP-38 $C_8H_{11}ClN_6S$ 	Thiamethoxam 	Non-target screening	10.94	−0.7	3	1 fragments
TP-39 $C_{13}H_{18}N_2OS$ 	Levamisole 	Non-target screening	16.37	2.9	3	4 fragments
TP-42 $C_7H_{11}ClN_4S$ 	Thiamethoxam 	Non-target screening	7.00	0.6	3	2 fragments
TP-43 $C_7H_9ClN_2OS$ 	Thiamethoxam 	Non-target screening	14.65	−1.4	3	1 fragment
TP-44 $C_{13}H_{14}ClN_3O_3$ 	Cyproconazole 	Non-target screening	17.85	−0.7	3	1 fragment
TP-46 $C_{10}H_{21}NO_2$ 	Lidocaine 	Non-target screening	7.10	0.9	3	2 fragments
TP-48 $C_{15}H_{18}ClN_3O_2$ 	Cyproconazole 	Suspect and non-target screening	19.66	1.0	3	2 fragments
TP-49 $C_6H_7ClN_2OS$ 	Thiamethoxam 	Non-target screening	13.00	−1.1	3	1 fragment

(*continued on next page*)

Table 2 (*continued*)

Formula and Structure	Parent	Identification workflow	Retention time (min)	Mass error (ppm)	Confidence level	Corresponding fragments with parent
TP-50 $C_9H_{14}ClN_5S$ 	Thiamethoxam 	Non-target screening	8.03	−0.8	3	2 fragments

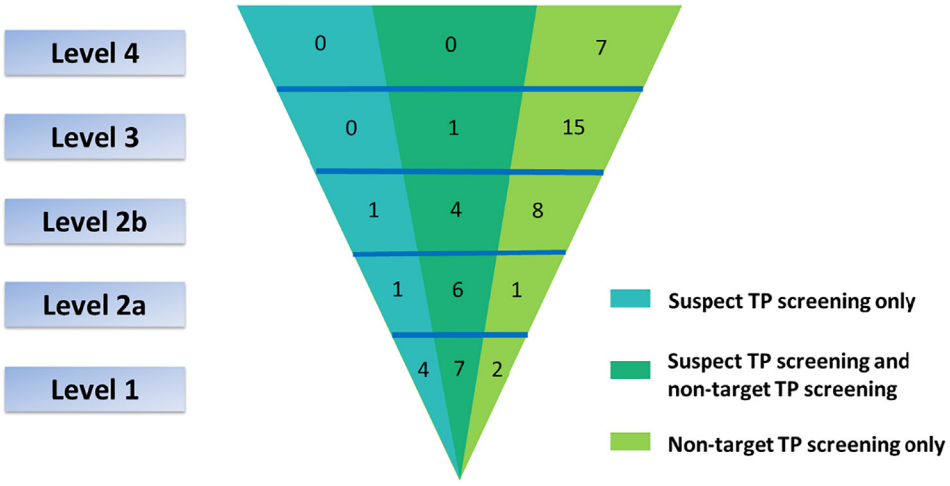


Fig. 4. Number distribution of identified transformation products (TPs) under different confidence levels from suspect and non-target TP screening.

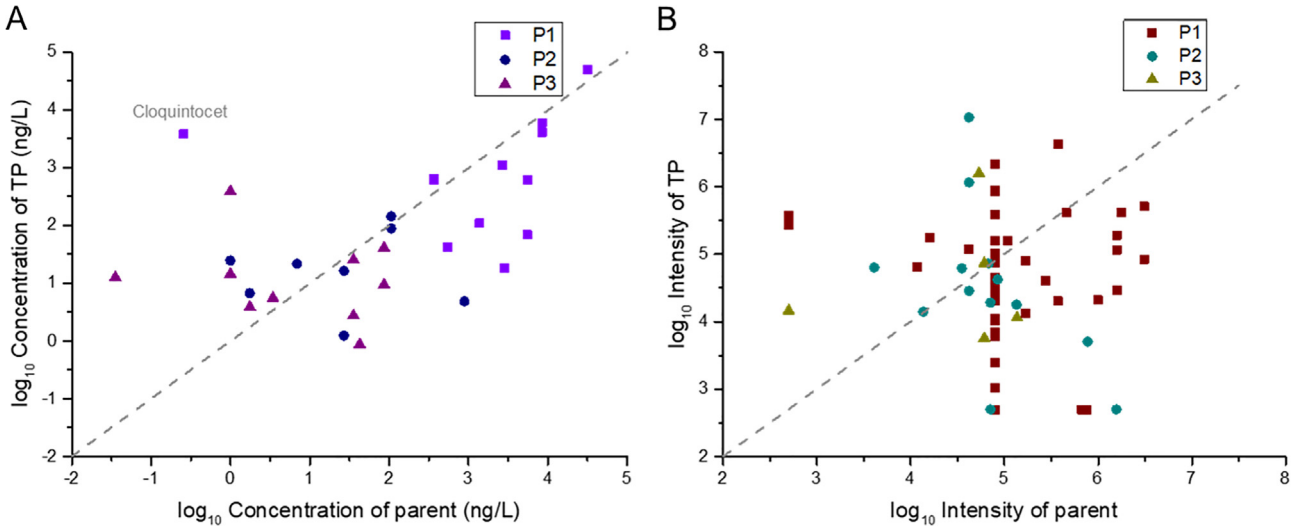


Fig. 5. (A) Concentrations of 13 confirmed TPs and their parents in effluent of three wastewater treatment plants; (B) Intensity of 50 tentatively identified transformation products and their parents in effluent of three wastewater treatment plants.

parent compounds, thiamethoxam and fluroxypyr, both had RQ < 1 (Table S4, Table S5). 4-Amino-3,5-dichloro-6-fluoro-2-pyridone (TP-4) has been found as a TP of fluroxypyr (Hu et al., 2014, Lehmann et al., 1993), but no successive study on its occurrence, toxicity and risk to environment has been done. Based on quantification and its toxicity, we have for the first time reported the concentration of these two TP in wastewater, and they posed potential risk to downstream river water (RQ > 1). These cases confirmed the necessity to include TPs in the risk assessment of wastewater.

4. Conclusion

We identified 60 parent compounds, including 46 pesticides and 14 pharmaceuticals, by suspect screening in three WWTPs. From 60 parents, 57 TPs were identified by suspect and non-target screening, 13 at level 1, eight at level 2a, 13 at level 2b, 16 at level 3, seven at level 4. Twenty identified TPs have not been reported in previous study. About 40% of TPs had higher concentrations than their parents in effluent for all 13 confirmed TPs. Especially, TP cloquintocet had much higher concentration than its parent cloquintocet-mexyl, with a concentration ratio TP/parent = 14809 in effluent. More toxic TPs have been found for six parent compound, thiamethoxam, fluroxypyr, imidacloprid, 2,4-

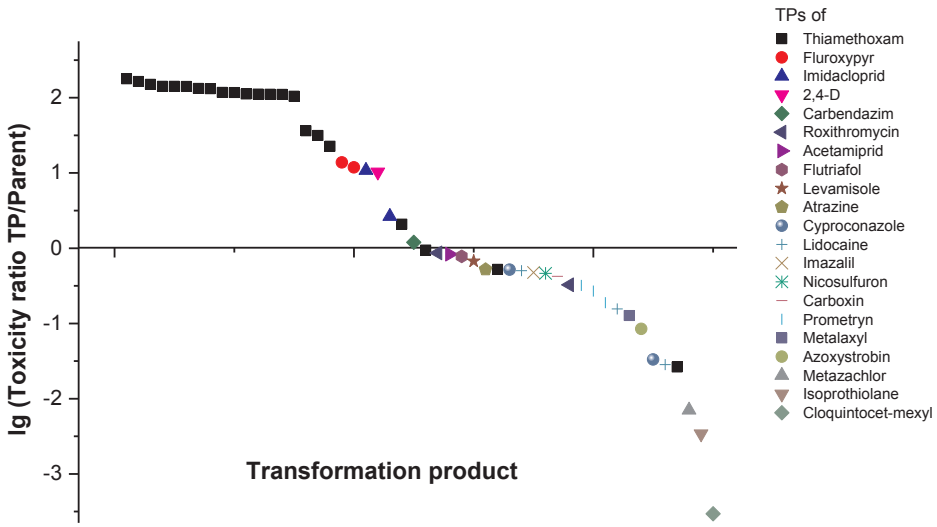


Fig. 6. Toxicity ratio of 50 tentatively identified transformation products (TPs) to their parents.

D, and carbendazim. Nineteen TPs of thiamethoxam had higher toxicity than thiamethoxam, with toxicity ratio ranging from 2 to 179. Among 13 quantified TPs, two TP, 1-(2-chlorothiazol-5-yl)-N-methylmethanamine (TP of thiamethoxam), 4-Amino-3,5-dichloro-6-fluoro-2-pyridone (TP of fluroxypyr) had RQ > 1, while their parent had RQ < 1. Also, no study is found on their occurrence in environmental samples or toxicity of these two TPs. Our study demonstrates the importance of TP analysis in assessing the environmental impact of wastewater.

5. Supporting information

Instrument methods, screening results, MS/MS spectra, quantification and risk assessment results were given in Appendix A (doc) and Appendix A (xls). Figure S1-S60 is given in doc, Table S1-S5 given in xls.

CRediT authorship contribution statement

Xuebing Wang: Writing - original draft, Investigation, Formal analysis, Methodology. **Nanyang Yu:** Supervision, Writing - review & editing. **Jingping Yang:** Supervision, Data curation. **Ling Jin:** Writing - review & editing, Formal analysis. **Huiwei Guo:** Methodology, Visualization. **Wei Shi:** Validation, Software. **Xiaowei Zhang:** Supervision, Conceptualization. **Liuyan Yang:** Writing - review & editing. **Hongxia Yu:** Resources, Supervision. **Si Wei:** Funding acquisition, Project administration, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105599.

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