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Picogram per Liter Quantification of Pyrethroid and Organophosphate Insecticides in Surface Waters: a Result of Large Enrichment with Liquid-Liquid Extraction and Gas Chromatography Coupled to Mass Spectrometry using Atmospheric Pressure Chemical Ionization

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Abstract

Insecticides such as pyrethroids and organophosphates are extensively used globally. Once released into surface water bodies, they can pose a major threat to aquatic ecosystems already at trace concentrations. Therefore, selected pyrethroids and organophosphates are listed as priority substances within the European Water Framework Directive with chronic quality criteria in the pg L^{-1} range. Previously applied analytical methods were unable to detect pyrethroids and organophosphates at ecotoxicological relevant concentrations, thereby hindering the assessment of surface water quality. In this work, we developed an ultra-sensitive method for the analysis of 12 pyrethroid and two organophosphate insecticides in surface waters. This method is based on the liquid-liquid extraction of surface water samples with *n*-hexane to achieve large enrichment factors (4000×) and subsequent chemical analysis by gas chromatography coupled to tandem mass spectrometry using atmospheric pressure chemical ionization, a soft ionization technique. Quality control parameters including the method limits of quantification (12.5-125 pg L^{-1}), intra-day precision (1-22%), intra-day accuracy (84-133%) and absolute recoveries covering liquid-liquid extraction (67-114%) showed that the method is sensitive and robust and therefore suitable for the analysis of pyrethroids and organophosphates in surface waters. The developed method was applied to Swiss surface water samples and detected pyrethroids and organophosphates below the ecotoxicological relevant concentrations, exemplifying the suitability of the proposed method for aquatic monitoring.

Keywords: Insecticides, Atmospheric pressure chemical ionization, Gas chromatography coupled to mass spectrometry, Surface water, pg L^{-1} quantification, Liquid-liquid extraction

1. Introduction

In areas with mainly agricultural land use, pesticides can enter surface waters, thereby adversely affecting aquatic ecosystems. Pesticides, notably insecticides, pose a high ecotoxicological risk to aquatic organisms [1, 2]. One of the most toxic insecticide classes towards aquatic organisms are pyrethroids and non-polar organophosphates. Even in the low ng L^{-1} range, these compounds can negatively affect crustaceans with amphipods being one of the most sensitive taxa [3-5]. Pyrethroids can exhibit one, two or three chiral centers leading to two, four or eight stereoisomers, respectively [6]. The toxicity and the environmental behavior such as degradation of stereoisomers can vary, and the toxic potential of pyrethroids often only refers to single isomers [7]. As an example, the toxicity of the pyrethroid cypermethrin (see Figure 1a) only stems from two (1R-cis-αS and 1R-trans-αS) out of eight stereoisomers, whereas the remaining stereoisomers are much less active [8, 9]. Since these differing insecticidal activities are known for some pyrethroid isomers, isomer-enriched products have been manufactured (e.g., α- and ζ-cypermethrin) which often only include the most potent isomers [10]. However, toxicity testing of pyrethroids is predominantly based on isomer mixtures that is why available environmental quality standards (EQSs) mostly refer to the mixture of all stereoisomers. Therefore, within the European Water Framework Directive (WFD), the pyrethroid cypermethrin (as a mixture of all stereoisomers) is listed as a priority substance with a chronic quality criterion (AA-EQS: annual average environmental quality standard) in surface waters of 80 pg L^{-1} based on the total water concentration (see Table 2) [11]. In Switzerland, the Swiss Center for Applied Ecotoxicology proposed EQSs for selected pyrethroids and organophosphates in the pg L^{-1} range (see Table 2) that are to be included into the water protection ordinance in the future [12]. Thus, to assess the water quality with respect to long-term ecotoxicological effects, a highly sensitive and selective analytical method is required to detect pyrethroid and organophosphate insecticides below their respective AA-EQSs (in the pg L^{-1} range).

EQSs for the selected insecticides are based on the total water concentration that comprises the dissolved fraction and the particle/dissolved organic matter (DOM)

bound fraction. Since pyrethroids are highly hydrophobic (log Kow ~ 3 – 7, see Table 2) they tend to sorb strongly to particles or to DOM and thus exhibit low water solubilities (few µg L^{-1}) [13, 14]. Liu et al. [15] have shown that only 0.4% to 1% of selected pyrethroids are present in the freely dissolved phase in streams. Therefore, many analytical methods focus on the detection of pyrethroids in sediments [16]. However, pyrethroid toxicity towards epibenthic or pelagic aquatic organisms such as filter feeders is not only dependent on the bioavailable dissolved fraction since particle/DOM associated pyrethroids can be taken up, thereby contributing to the overall toxicity. Accordingly, to determine the total fraction of dissolved and particle/DOM bound pyrethroids, the analysis of unfiltered water samples is required. Most common extraction and enrichment methods of pyrethroids from unfiltered water samples include liquid-liquid extraction (LLE) and solid-phase extraction (see reviews by Albaseer et al. [17] and Feo et al. [16]).

Although some methods using liquid chromatography (LC) [18, 19] were developed for the analysis of pyrethroids in water samples, gas chromatography (GC) coupled to electron capture detection, to single mass spectrometry (MS) or to tandem MS has been predominantly applied for the detection of pyrethroids in water samples [16]. Using conventional GC columns with materials such as fused silica, diastereomeric selectivity of pyrethroids can be achieved, whereas enantiomeric pairs are usually not separated [7, 20]. Enantiomeric separation can be obtained with chiral GC columns but because of the lack of single enantiomer reference standards, a distinct assignment of all stereoisomers is often not possible. However, in the context of EQSs that predominately refer to isomer mixtures, a distinction of single stereoisomers is not mandatory. GC/MS using the hard ionization technique of electron ionization (EI) leads to a strong fragmentation of pyrethroids. The obtained fragments are often unspecific and of low intensity, thereby compromising sensitivity and selectivity [16]. When using GC tandem MS (triple quadrupole) in combination with soft ionization techniques such as chemical ionization (CI) [21, 22] or atmospheric pressure chemical ionization (APCI) [23, 24], the sensitivity and selectivity can be highly improved. Soft ionization techniques reduce fragmentation and often produce molecular ions in

high abundance.

Method limits of quantification (MLOQs) of published studies analyzing total concentrations of pyrethroids in water samples are in the ng L^{-1} range for selected analytes (from several up to several hundred ng L^{-1}), thereby not reaching the required AA-EQSs (see Table 2) for ecotoxicological risk assessment of pyrethroids [25, 19, 26, 27, 18, 28]. These methods are lacking adequate sensitivities either because of hard ionization techniques such as EI [28], the choice of the detector (electron capture detector or high resolution MS versus triple quadrupole MS) [25, 27, 26] or the choice of the chromatographic system (LC versus GC) [19, 18]. Only one study reports MLOQs for a limited number of pyrethroids in unfiltered surface water samples in the sub-ng L^{-1} range (~0.2 ng L^{-1}) using ultrasound-assisted emulsification-extraction and detection by GC-negative CI-MS/MS [22].

Moschet et al. [29] developed a GC-EI-MS/MS method for the analysis of pyrethroids and organophosphates in surface water that reaches MLOQs in the pg L^{-1} range (20-400 pg L^{-1}). However, this method uses passive sampling and thereby only considers the dissolved fraction. Consequently, the developed method does not fulfill the requirements of EQSs given within the WFD based on total water concentrations. In addition, the lack of substance-specific sampling rates hinders a reliable quantification leading to highly uncertain concentration estimates. Hence, an analytical method for detecting the total fraction of pyrethroids and organophosphates in water samples at ecotoxicological relevant concentrations is missing, thereby enabling the assessment of existing AA-EQSs.

Therefore, the goal of this study was to develop an ultra-sensitive, time and cost efficient method for the direct extraction of pyrethroids and organophosphates from unfiltered surface water samples with MLOQs below their corresponding AA-EQSs. The analytical strategy was to apply LLE with large enrichment factors and sensitive analysis by GC-APCI-MS/MS following the APCI optimization by Portoles et al. [23]. The method should allow for the detection and quantification of 12 relevant and frequently used apolar pyrethroids (see log K_{ow} in Table 2) (acrinathrin, bifenthrin, α-cypermethrin, deltamethrin, empenthrin, esfenvalerate, etofenprox, τ-fluvalinate,

λ-cyhalothrin, permethrin, phenothrin, tefluthrin) and two organophosphates (chlorpyrifos, chlorpyrifos-methyl) in the low pg L^{-1} range in surface water samples. Three more polar pyrethroids (see log K_{ow} in Table 2) (allethrin, imiprothrin, tetramethrin) were included into the analysis to evaluate the limitations of the method regarding more polar analytes. Additionally, a focus was given to the fact that many pyrethroids exist and are applied as a mixture of stereoisomers. Finally, the developed method was validated and applied to Swiss surface water samples.

2. Materials and Methods

2.1. Chemicals and Solutions

Single analyte standard solutions and isotopically labelled internal standard (ISTD) solutions were prepared in ethanol. Analyte standard mix and ISTD mix solutions were prepared in acetonitrile to ensure a proper mixing of the water sample, spiked analytes and ISTDs. Detailed information about all chemicals and solutions used during sample preparation and instrumental analysis are provided in the Electronic Supplementary Material (ESM) A.

2.2. Sampling Site and Sampling of Surface Water

Time-proportional 3.5-day composite surface water samples were taken from a small stream (stream order of 1 according to Strahler [30]) located in the Swiss Plateau. The catchment size was 2.8 km^2, and the land use in the catchment was divided into arable land (54%), forest (33%), urban area (6%), grass land (5%), orchards (0.3%), and vegetables (0.2%). Samples were collected between March and October 2017. The samples were accumulated by a permanently installed automatic sampling device (Maxx portable sampler TP5 C, cooled on-site at 4 °C). The composite samples consisted of 112 subsamples of 35 mL each that were automatically taken every 45 min and stored in 4 L glass bottles.

2.3. Sample Preparation of Surface Water by Liquid-Liquid Extraction

Surface water samples were stored in glass bottles at -20 °C. After unfreezing, bottles were thoroughly shaken for 2 min. In total, 200 mL sample aliquots of unfiltered surface water were transferred into 200 mL glass volumetric flasks and 50 µL ISTD mix solution composed of bifenthrine-d5, chlopyrifos-methyl-d6, trans-cypermethrin-d6, etofenprox-d5, fenvalerate-d7 (each 0.005 mg L^{-1}), deltamethrine-d5, and atrazine-d5 (each 0.05 mg L^{-1}) was added. Water samples were stirred for 15 min with 5 mL of *n*-hexane on a magnetic stirrer plate (990 rpm) using glass-coated magnetic stirrers. The organic phases including the emulsion between the two phases, were transferred into 10 mL glass centrifuge vials. Water samples were

stirred again with 5 mL of *n*-hexane for 15 min and the organic phases were merged. Subsequently, samples were centrifuged for 4 min (4300 rcf) and the organic phases were transferred to new 10 mL glass centrifuge vials. Samples were evaporated under a gentle nitrogen stream down to 50 µL, were transferred to measuring vials with 250 µL glass inserts, and stored at -20 °C until chemical analysis. All glassware was annealed before use (7.1 °C/min to 500 °C (4.5 h)).

2.4. Chemical Analysis using GC-APCI-MS/MS

Samples were measured on a GC-APCI-MS/MS instrument (Agilent gas chromatograph 7890B coupled to an Agilent triple quadrupole mass spectrometer 6495 using APCI). For chromatographic separation a fused silica Rxi guard column (5 m, 0.25 mm i.d., Restek) and a fused silica Rtx-5MS column (30 m, 0.25 µm film thickness, 0.25 mm i.d., Restek) were used. The oven temperature gradient was as follows: 100 °C (1 min), 25 °C/min to 150 °C, 10 °C/min to 300 °C (3 min). Each run was performed in backflush mode which lowers the maintenance interval. Splitless injections of 5 µL using a deactivated liner (borosilicate glass, 4 mm i.d., Restek) were performed at 250 °C. Helium (99.999%, Carbagas, Switzerland) was used as carrier gas with a flow rate of 3 mL min^{-1}. The transfer capillary to the APCI interface was constantly maintained at 280 °C, and N_2 (~99%, N_2 generator) was used as source gas (11 L min^{-1}). The source gas temperature was set to 150 °C, the APCI corona discharge current to 1 µA, the capillary voltage to 1000 V and the ion funnels to 100/40 V for high pressure/low pressure RF (radio frequency).

Ionization was performed in positive ionization mode using water as a modifier to promote the formation of protonated molecular ions $[M+H]^+$ (see Portoles et al. [23]). Therefore, nanopure water was pumped with a flow rate of 50 µL min^{-1} into a small uncapped vial placed in the ionization source. The mass spectrometer was operated in dynamic multiple reaction monitoring (MRM) mode with a cycle time of 250 ms and the mass resolution was set to 1.2 Da (quadrupole 1 and 3, wide isolation mode). N_2 (99.999%, Carbagas, Switzerland) was used as collision gas. For all analytes and ISTDs at least two transitions were measured, from which the most sensitive

196 transition was used as quantifier and the remaining transition(s) as qualifier(s). Data
197 acquisition was performed without time filtering (smoothing). The raw data are
198 slightly smoothed afterwards in the Masshunter Qualitative or Quantitative Analysis
199 software B.07.00, Agilent (Gaussian, function width = 15, Gaussian width = 2).

200 **2.5. Quantification**

201 To construct a calibration curve, 200 mL of tap water was transferred into 200 mL
202 glass volumetric flasks and ISTD mix solution was added. Subsequently, analyte
203 standard mix solution was added at different concentrations (1.25, 12.5, 25, 125, 250,
204 1250, 2500, 12500 and 25000 pg L^{-1}), and samples were extracted and analyzed as
205 described in section 2.3. and 2.4.
206 Quantification was based on internal standard calibration (MassHunter Quantitative
207 Analysis software B.07.00, Agilent). Peak area ratios were formed between the area of
208 the analyte and the area of its corresponding ISTD and were compared to those in the
209 respective calibration curve. When diastereomers were chromatographically separated
210 for individual analytes, all peaks were integrated and summed. Matching ISTDs were
211 available for bifenthrin (bifenthrin-d5), chlorpyrifos-methyl (chlorpyrifos-methyl-d6),
212 α-cypermethrin (trans-cypermethrin-d6), deltamethrin (deltamethrin-d5),
213 esfenvalerate (fenvalerate-d7), and etofenprox (etofenprox-d5). For the remaining
214 analytes relative recoveries (accuracies) were calculated based on all available ISTDs.
215 The ISTD which resulted in the best relative recovery was selected for the final
216 quantification (see ESM.B). Calibration curves were acquired at the beginning and at
217 the end of each measuring sequence and were obtained using a linear least square
218 regression with a weighting factor of 1/x. Calibration curves were linear for all
219 analytes in the used calibration range ($R^2 > 0.98$) except for allethrin, imiprothrin and
220 tetramethrin (see section 3.1.).

221 **2.6. Method Validation**

222 The optimized method was validated by determining relative recoveries (intra-day and
223 inter-day accuracies), absolute recoveries of nearly the entire sample preparation
224 covering LLE and phase separation (absolute $recoveries_{LLE}$), precisions (intra-day and

inter-day), instrumental LOQs (ILOQs) and MLOQs. In order to determine inter-day relative recoveries and inter-day precisions, additional recovery experiments were carried out after several months.

Recoveries and Precisions

To determine relative recoveries, 200 mL surface water was spiked with analyte standard mix solution to a concentration of 500 pg L^{-1} and extracted. Additionally, 200 mL unspiked surface water was extracted to determine background contamination of the used surface water. For both sample types ISTD mix solution was added before extraction and samples were analysed and quantified according to the methods described in sections 2.4. and 2.5. Absolute $recoveries_{LLE}$ were determined to track analyte losses that might occur during LLE and phase separation. Therefore, 200 mL surface water was spiked with analyte mix solution to a concentration of 500 pg L^{-1} (final extract concentration of 2 ng mL^{-1}) and ISTD mix solution was added after the extraction but before evaporation with nitrogen. Additionally, unspiked surface water was extracted and spiked with analyte mix solution (final extract concentration of 2 ng mL^{-1}) and ISTD mix solution after the extraction but before evaporation with nitrogen. Background contamination was determined by analysing unspiked surface water and adding ISTD mix solution after the extraction but before evaporation with nitrogen. Samples were analysed and the peak area ratios of each analyte in the surface water sample (analyte standard mix solution spiked before extraction, ISTD mix solution spiked after extraction) were compared to those, where analyte standard mix solution and ISTD mix solution were spiked after extraction taking background contamination into account. All samples were prepared in triplicate to determine method precision.

Method and Instrumental Limits of Quantification

MLOQs were estimated from calibration standards that were prepared in tap water and went through the entire sample preparation (see section 2.3.). MLOQs were defined by the concentration of the calibration standard that yielded analyte peaks with signal-to-noise (S/N) ratios of at least 10 and 3 for the quantifier and qualifier

ion, respectively. ILOQs were determined by directly injecting analyte standards in pure solvent and the resulting peaks had to fulfill the same S/N criteria. Analyte Protection Mix (see ESM.C) composed of sugar and sugar derivatives was added to calibration standards prepared in pure solvent that were directly injected into the GC-system without previous sample preparation. Calibration standards in pure solvent contained less matrix components compared to the extracts from surface water or tap water. Therefore, Analyte Protection Mix was used to block active sites in the GC system, thereby improving the chromatographic peak shape.

Average qualifier ion to quantifier ion ratios (qualifier ratios) based on peak areas were calculated for each analyte using the qualifier ratios of all calibration standards. The qualifier ratio of each analyte in all environmental samples was compared to the average qualifier ratio and had to fall in a specified range according to the EC Directive 2002/657/EC [31].

Blank samples without ISTD (only tap water) and with ISTD (tap water and ISTD) were prepared simultaneously with environmental samples and went through the whole sample preparation. When low signals of analytes were found in blank samples, the highest peak area detected in the blank samples was doubled and compared to the analyte peak areas detected in the calibration standards. MLOQs for analytes present in the blank samples were then defined by the concentration of a calibration standard that exhibited the closest higher peak area compared to those in the blank samples.

2.7. Stability of Pyrethroids and Organophosphates during Storage of Surface Water Samples

To investigate the stability of pyrethroids in surface water samples during storage, six times 500 mL of surface water was spiked to a concentration of 500 pg L^{-1} with analyte standard mix solution in 1 L glass vessels and samples were either stored at +4 °C or at -20 °C in the dark. After one week of storage, vessels were thoroughly shaken for 2 min, and 200 mL of water sample were transferred to 200 mL glass volumetric flasks and ISTD mix solution was added. Initial pyrethroid concentrations in the spiked surface water samples were determined and used for comparison. All

samples were prepared in triplicate, and samples were extracted and analyzed as described in section 2.3. and 2.4.

2.8. Ecotoxicological Risk Assessment

To assess the acute risk to aquatic organisms, the measured environmental concentrations of each detected substance of the 3.5-day composite samples were compared to the corresponding acute quality criteria (MAC-EQS: maximum acceptable concentration environmental quality standard). To evaluate the risk to aquatic organisms towards long-term pollution, time-weighted average concentrations were calculated. Therefore, the concentrations of each detected substance of four subsequent 3.5-day composite samples were averaged resulting in 14-day composite samples and were compared to the corresponding chronic quality criteria (AA-EQS: annual average environmental quality standard). According to Spycher et al. [32], “moving averages” of four 3.5-day composite samples were formed by averaging the concentrations of equal time windows after the initial formed average of the first four samples. In this way, the effect of different 14-day time intervals was investigated.

3. Results and Discussion

3.1. Liquid-Liquid Extraction

Based on existing solvent-based extraction methods of pyrethroids from water samples using a water-immiscible organic solvent (see reviews by Albaseer et al. [17] and Feo et al. [16]), LLE (enrichment factor of 4000×) with *n*-hexane was tested in two cycles. Absolute recoveries$_{LLE}$ were between 67 and 114% (see Table 2) showing sufficient extraction efficiencies.

The more polar pyrethroids allethrin, imiprothrin and tetramethrin could not be quantitatively determined because no linear calibration curves were obtained. These pyrethroids differ in their structures and have lower log K_{ow} values when compared to the remaining pyrethroids (see log K_{ow} values in Table 2). According to Hladik, Kuivila [33], allethrin and tetramethrin are mainly present in the dissolved fraction and do not sorb to sediments. However, the extraction efficiency of allethtrin and tetramethrin from the water phase to the organic *n*-hexane phase does not seem to be hindered, since absolute recoveries$_{LLE}$ of these compounds are ~ 80% (see Table 2). No absolute recoveries$_{LLE}$ could be determined for imiprothrin because at a concentration of 500 pg L^{-1} no chromatographic peaks were visible in the MRM chromatograms of the surface water samples. Reasons can be divers, such as an impaired extraction efficiency and/or suboptimal GC conditions. In general, especially for the more polar pyrethroids, the peak shapes clearly improved with increasing matrix load in the final extracts. Furthermore, no isotopically labelled ISTDs were available for the more polar pyrethroids, which would have added to the linearity of the calibration curves.

However, the focus of this method was on pyrethroids that are applied as plant protection products. Therefore, the substance spectrum of the developed method was regarded as satisfactory since in Switzerland the more polar pyrethroids allethrin, tetramethrin and imiprothrin are only in use as biocides (see Table 2).

3.2. Optimization of APCI-MS/MS Parameters

The effects of different source parameters such as gas temperatures (80, 150 and 290 °C), APCI corona discharge currents (1 and 2 µA) and capillary voltages (500, 1000, 2000, 3000 and 4000 V) were evaluated regarding peak intensities. Highest peak intensities were achieved using a source gas temperature of 150 °C, an APCI corona discharge current of 1 µA and a capillary voltage of 1000 V. In addition, different parameters for the ion funnels (170/80, 150/60 and 100/40 V positive high pressure RF/positive low pressure RF) were tested but they did not significantly change the signal intensities; therefore, the final measurements were performed with 100/40 V (for further details on the different source parameters refer to ESM.D). Furthermore, the positioning of the ion transfer capillary with respect to the mass spectrometer inlet and the corona discharge needle is critical with regard to signal intensities and requires an accurate adjustment (see picture in ESM.E).

In a further step, the influence of water on the formation of $[M+H]^+$ during ionization was tested. Therefore, according to Portoles et al. [23], water was placed as a modifier in the ionization source which clearly favored the formation of $[M+H]^+$ over M^+ during ionization (see ESM.F). Only for bifenthrin, etofenprox and permethrin fragment ions were used as precursor ions because of the lack of $[M+H]^+$, which has been previously observed by Portoles et al. [23] for bifenthrin and permethrin. Furthermore, analyte peak intensities of precursor ions were compared in the presence and absence of water. Peak intensities were higher using water as a modifier with the exceptions of bifenthrin, tefluthrin and esvenfalerate (see ESM.F). Consequently, the use of water increased the sensitivity and the selectivity for most analytes by promoting the formation of $[M+H]^+$. Additionally, when using water as a modifier, ionization is independent of variations in humidity in the lab and remains constants during each instrument run.

Next, product ions and collision energies were optimized for all analytes and ISTDs in initial experiments by injecting individual analytes and ISTDs in *n*-hexane. Therefore, product ion scans of precursor ions were run with different collision energies (10, 20,

30, 40 and 50 eV) to select the most intense product ions depending on the applied collision energies. Each analyte and ISTD was injected individually and measured with the final MRM method. Subsequently, the MRM chromatograms of the individually injected analytes and ISTDs were evaluated with regard to signals occurring on the MRM chromatograms of the other analytes and ISTDs included in the method. In this way, it was ensured that no interfering signals from non-target substances appeared in the MRM chromatograms of each analyte and ISTD. Finally, at least two transitions were measured for each analyte and ISTD, from which the most sensitive transition was selected as quantifier and the remaining transition(s) as qualifier(s) and the qualifier ratio had to fall in the specified range. Table 1 summarizes the optimized conditions used for GC-APCI-MS/MS analysis.

Table 1: Optimized GC-APCI-MS/MS conditions for all target analytes and ISTDs.

Compound	**retention time [min] [i)]**	**precursor ion $[M+H]^+$ [m/z] [ii)]**	**product ions [m/z] [iii)] (collision energy [eV])**	**dwell time [ms]**
Analytes				
Empenthrin	6.6 / 6.7	275.2	**173.0 (20)**, 229.0 (10)	120
Tefluthrin	7.6	419.1	**177.0 (40)**, 325.0 (10)	79
Chlorpyrifos-methyl	8.3	321.9	**125.0 (30)**, 289.9 (20)	42
Chlorpyrifos	9.2	349.9	**198.0 (20)**, 97.0 (40), 294.0 (10)	31
Bifenthrin	13.2	*181.0* [#)]	**165.0 (30)**, 115.0 (40)	26
Phenothrin	13.5 / 13.6	351.2	**183.0 (20)**, 129.0 (50)	29
λ-Cyhalothrin	14.0 / 14.2	450.1	**225.0 (10)**, 141.0 (40)	37
Acrinathrin	14.1 / 14.3	542.1	**181.0 (40)**, 289.0 (20), 93.0 (50)	38
Permethrin	14.9 / 15.0	*355.0* [#)]	**261.0 (20)**, 319.0 (10)	
		391.0	355.0 (10)	42
α-Cypermethrin	15.7 / 15.9	416.1	**191.0 (10)**, 127.0 (20)	25
Etofenprox	16.0	*359.2* [#)]	**183.0 (20)**, 189.0 (20)	32
Esfenvalerate	16.6 / 16.8	420.1	**167.0 (10)**, 125.0 (50)	28
τ-Fluvalinate	16.8 / 16.9	503.1	**208.0 (10)**, 181.0 (20)	28
Deltamethrin	17.1 / 17.3	504.0	**278.9 (10)**, 172.0 (30)	34
Internal Standards (ISTDs)				
Chlorpyrifos-methyl-d6	8.3	327.9	**131.0 (30)**, 293.0 (20)	42
Bifenthrin-d5	13.3	*186.0* [#)]	**170.0 (40)**, 118.0 (50)	26
trans-Cypermethrin-d6	15.8 / 16.0	422.1	**96.0 (40)**, 133.0 (40)	25
Etofenprox-d5	16.1	*364.0* [#)]	**183.0 (30)**, 194.0 (10)	32
Fenvalerate-d7	16.7 / 16.9	427.2	**174.0 (20)**, 127.0 (50)	28

Compound	retention time [min] [i)]	precursor ion $[M+H]^+$ [m/z] [ii)]	product ions [m/z] [iii)] (collision energy [eV])	dwell time [ms]
Deltamethrin-d5	17.2 / 17.4	509.0	**279.0 (30)**, 172.0 (10)	34

[i)] When several retention times are listed, they refer to the separation of stereoisomers.

[ii)] Fragment precursor ions are italicized and are marked with a [#].

[iii)] Product ions that are part of the quantifier transition are marked in bold.

3.3. Stereoisomers of Pyrethroids – Consequences for Identification and Quantification

In total, 12 relevant and frequently used apolar pyrethroids were analyzed. Some of the deployed pyrethroid reference standards (λ-cyhalothrin, esfenvalerate, α-cypermethrin, τ-fluvalinate and deltamethrin) only included a reduced number of possible stereoisomers because of commercial availability or because only selected stereoisomers serve as active ingredients in plant protection products or biocides that are approved in Switzerland. This use of isomer-enriched reference standards can lead to different challenges:

(I) a unique peak assignment has to be ensured even when the following occurs:

(i) different isomerization processes occur in the environment compared to the lab,

(ii) interfering peaks occur in environmental samples,

(iii) additional stereoisomers are approved as active ingredients in plant protection products and/or biocides leading to additional stereoisomer peaks in the MRM chromatograms of environmental samples.

(II) the instrumental response needs to be similar for all stereoisomers of one pyrethroid. Thus, a reliable quantification can be ensured in case additional stereoisomer peaks occur in the MRM chromatograms of environmental samples.

Therefore, it was first tested if all stereoisomers of one pyrethroid exhibit a similar instrumental response. This has been confirmed by quantifying cypermethrin (all stereoisomers) in a certified reference standard using α-cypermethrin, which resulted in a recovery of 110%. Additionally, peak areas of similar concentrations of esfenvalerate (1 stereoisomer) and fenvalerate (4 stereoisomers) as well as λ-cyhalothrin (2 stereoisomers) and cyhalothrin (8 stereoisomers) were compared and peak areas varied by less than 20%. Thus, no issues occurred in quantification even when the reference standards were composed of a reduced number of stereoisomers.

Second, all separated stereoisomer peaks of one pyrethroid had to be unequivocally assigned to each pyrethroid, which is necessary for a reliable peak integration and consequently for a proper quantification. For example, cypermethrin displayed an

additional peak in the MRM chromatogram of the surface water samples when compared to the α-cypermethrin reference standard (see Figure 2). To assign all cypermethrin peaks clearly, different isomer-selective cypermethrin standards and a reference standard of cypermethrin containing all stereoisomers were measured. Figure 1b-g shows the MRM chromatograms of α-, β-, θ- and ζ-cypermethrin and of cypermethrin that contains all stereoisomers. Cypermethrin has three chiral centers and thus comprises eight stereoisomers (see Figure 1a). The different cypermethrin stereoisomers are classified into different subgroups, i.e., α-cypermethrin (2 cis isomers: 1R-cis-αS, 1S-cis-αR), β-cypermethrin (2 cis isomers: 1R-cis-αS, 1S-cis-αR and 2 trans isomers: 1R-trans-αR, 1R-trans-αS), θ-cypermethrin (2 trans isomers: 1R-trans-αS, 1S-trans-αR) and ζ-cypermethrin (4 αS isomers: 1R-cis-αS, 1R-trans-αS, 1S-cis-αS, 1S-trans-αS). The separation of the four diastereomers (enantiomeric pairs) of cypermethrin is displayed in Figure 1g with the elution order cis, trans, cis, trans, which has been previously observed in other studies using similar apolar GC columns [20, 34]. By separately injecting the isomer-selective reference standards of α-, β-, θ- and ζ-cypermethrin (see Figure 1b-f), all peaks in the MRM chromatogram of cypermethrin that comprises all stereoisomers could be assigned to individual stereoisomers (Figure 1g). No baseline separation was achieved for the cis and trans diastereomers eluting after 16.45 and 16.49 minutes. Consequently, the additional cypermethrin peak observed in the MRM chromatogram of the surface water samples with a retention time of 15.8 minutes (see Figure 2) belongs to cypermethrin and is composed of the stereoisomers 1R-trans-αR and 1S-trans-αS. For final identity confirmation, the qualifier ratio of each baseline separated peak of cypermethrin in the surface water samples was compared separately to the corresponding peak in the MRM chromatogram of a reference standard of cypermethrin containing all stereoisomers. The qualifier ratio of each cypermethrin peak in the surface water samples stayed within the allowed range (± 30% for analytes, i.e., cypermethrin with qualifier ratios >10 to 20%) according to the EC Directive 2002/657/EC [31], thereby confirming that each cypermethrin peak in the MRM chromatogram of a surface water sample actually belongs to cypermethrin.

Figure 1b illustrates the isomerization of α-cypermethrin when stored in ethanol. An additional peak occurred at a retention time of 16.28 minutes when compared to α-cypermethrin prepared in *n*-hexane (see Figure 1c). The comparison of the retention time of the additionally observed peak of α-cypermethrin in ethanol with the elution pattern of the peaks present for cypermethrin (all stereoisomers) showed that the isomerization of α-cypermethrin took place at the α-carbon position containing the cyano group and that the chiral centers at the cycloproyl ring were unaffected (see Figure 1a-b, g). Thus, in ethanol, the two stereoisomers 1R-cis-αS and 1S-cis-αR of α-cypermethrin were converted to 1R-cis-αR and 1S-cis-αS, resulting in four instead of the initial two stereoisomers. Isomerization in ethanol at the α-carbon position was also observed for deltamethrin, λ-cyhalothrin and esfenvalerate (see Figure 2). Therefore, especially for pyrethroids carrying the α-carbon, isomerization is critical and polar protic solvents such as water and alcohols should be avoided, whereas stereoisomers remained stable in aprotic solvents, such as in *n*-hexane or ethyl acetate [35-40].

Overall, the use of pyrethroid reference standards composed of all stereoisomers is recommended as a calibration method. Thereby, a unique peak assignment in environmental samples can be guaranteed and, with the prerequisite of comparable instrumental responses of all stereoisomers of one pyrethroid, a reliable quantification can be performed. When special interest is given to the identification and quantification of single stereoisomers in environmental samples, care should be taken not to use protic solvents for the preparation of reference standards. However, assessing the initial isomer composition in environmental samples is in any case difficult because of naturally occurring isomerization processes after entry into the environment.

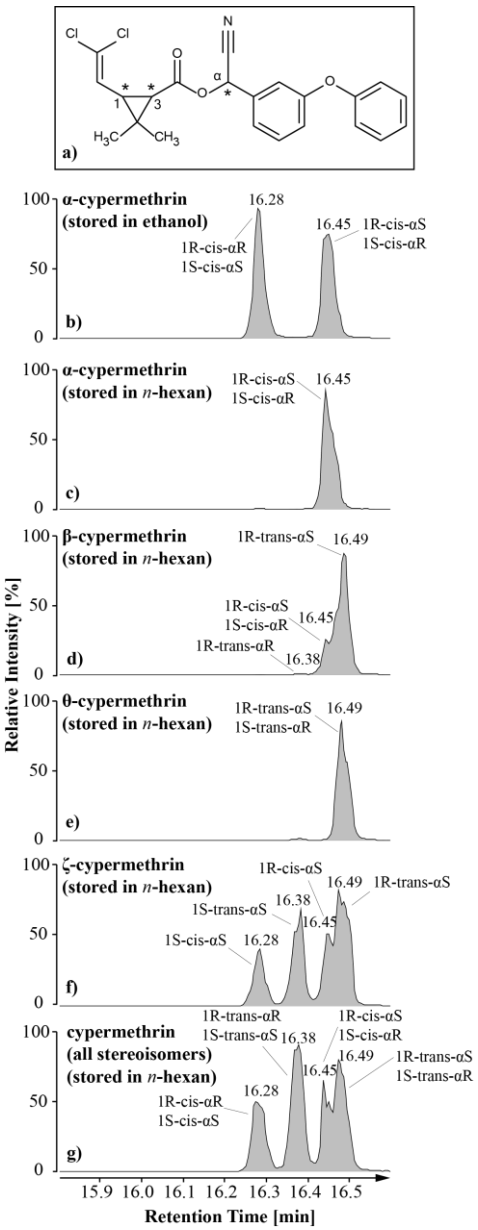

Figure 1: MRM chromatograms of α-cypermethrin (b, c), β-cypermethrin (d), θ-cypermethrin (e), ζ-cypermethrin (f) and of cypermethrin including all stereoisomers (g) using an apolar fused silica Rtx-5MS GC column. All separated peaks are labeled with the underlying stereoisomers. Note that a different GC column of the same type was used for the measurement of the environmental samples and calibration standards displayed in Figure 2, explaining why the retention times of the cypermethrin peaks are slightly shifted.

3.4. Method Validation

Quality control parameters (MLOQs, ILOQs, precisions (intra-day and inter-day), absolute recoveries$_{LLE}$ and relative recoveries (intra-day and inter-day)) were determined for the optimized method.

In general, MLOQs were very low and varied between 12.5 pg L^{-1} and 25 pg L^{-1} for most analytes which is a prerequisite for the assessment of ecotoxicological relevant concentrations (see MLOQs and EQSs in Table 2). Chlorpyrifos and permethrin exhibited MLOQs of 125 pg L^{-1} resulting from low-level contamination in the blank samples (see calculation of MLOQs in section 2.6. for the case of low signals in blank samples). Especially MLOQs resulting from small blank contaminations can vary for each day of sample preparation and the actual instrumental sensitivity can be much lower. For 8 out of 14 analytes, low signals were found in the blank samples (see Table 2 and ESM.G). During later sample preparations, signals in blank samples were strongly decreased (MLOQ for all substances between 12.5 and 25 pg L^{-1}) and were only present for 5 out of the 14 analytes. To test for possible sources of contamination, annealed glass vessels were extracted with *n*-hexane, and the extracts were analyzed. Chlorpyrifos, chlorpyrifos-methyl and permethrin were detected with concentrations of approximately 10 pg L^{-1}, although annealing should lead to residue-free glass vessels. In addition, blank samples composed of nano-pure water were prepared to rule out an initial contamination of the used tap water. Similar blank contaminations were observed compared to those in blank samples prepared in tap water. Blank samples with ISTD were compared with those without ISTD. Bifenthrin and esfenvalerate were only detected in blank samples with ISTD, indicating that the low-level blank contamination is a result of slightly contaminated matching ISTDs with the respective analyte. Based on the excellent instrumental sensitivity (see ILOQ in Table 2) of the analyzed substances, achieving completely clean blank samples remains challenging. Therefore, when dealing with ultra-sensitive methods such as GC-APCI-MS/MS, a thorough cleaning of lab equipment and instrumentation as well as the use of highly pure solvents and standards are required for achieving optimal

494 MLOQs.

495 Differing environmental matrices between calibration standards (tap water) and
496 environmental samples (surface water) potentially influenced the measurements. This
497 possible bias was checked by comparing the S/N ratio and the peak areas in spiked
498 surface water samples (spike level: 500 pg L^{-1}) with those in similar concentrated
499 calibration standards prepared in tap water. Both sample types (tap water and surface
500 water) were processed using the entire sample preparation method. Peak areas varied
501 on average by ~40% between calibration standards and spiked surface water samples.
502 This variation includes the volumetric uncertainty of the final sample volume of
503 50 µL. Furthermore, the possible matrix effects were evaluated by checking surface
504 water samples in which analytes were detected at concentrations around their
505 corresponding MLOQ. No signal suppression was observed in surface water samples
506 for the positively detected pyrethroids and organophosphates (see Figure 2). This
507 result indicates that the ionization of analytes in the GC-APCI interface is not
508 significantly influenced by matrix constituents under the selected conditions.

509 Intra-day precision was on average 8%, intra-day relative recoveries were between 84
510 and 133%, and analyte losses during the entire sample preparation method (absolute
511 $recoveries_{LLE}$) were minimal resulting in absolute $recoveries_{LLE}$ between 67 and 114%
512 (see Table 2). Inter-day precision was on average 12% and inter-day relative
513 recoveries varied between 85 and 108% (for details refer to ESM.H). Additionally,
514 surface water samples were spiked in duplicate to a concentration of 25 pg L^{-1} to
515 determine relative recoveries and precisions in the range of the MLOQ. Resulting
516 relative recoveries were between 85 and 118% and precision was on average 10% (for
517 details refer to ESM.I). However, for chlorpyrifos and permethrin no recoveries at the
518 MLOQ could be determined as chlorpyrifos was already present in the surface water
519 samples with a concentration of 420 pg L^{-1} (the spike level was too low) and for
520 permethrin the spike level was < MLOQ (see Table 2).

521 These quality control parameters of the optimized method using LLE with a large
522 enrichment factor of 4000× in combination with GC-APCI-MS/MS show that the
523 method is ultra-sensitive and robust and is therefore suitable for the analysis of

524 pyrethroids and organophosphates at ecotoxicological relevant concentrations.
525

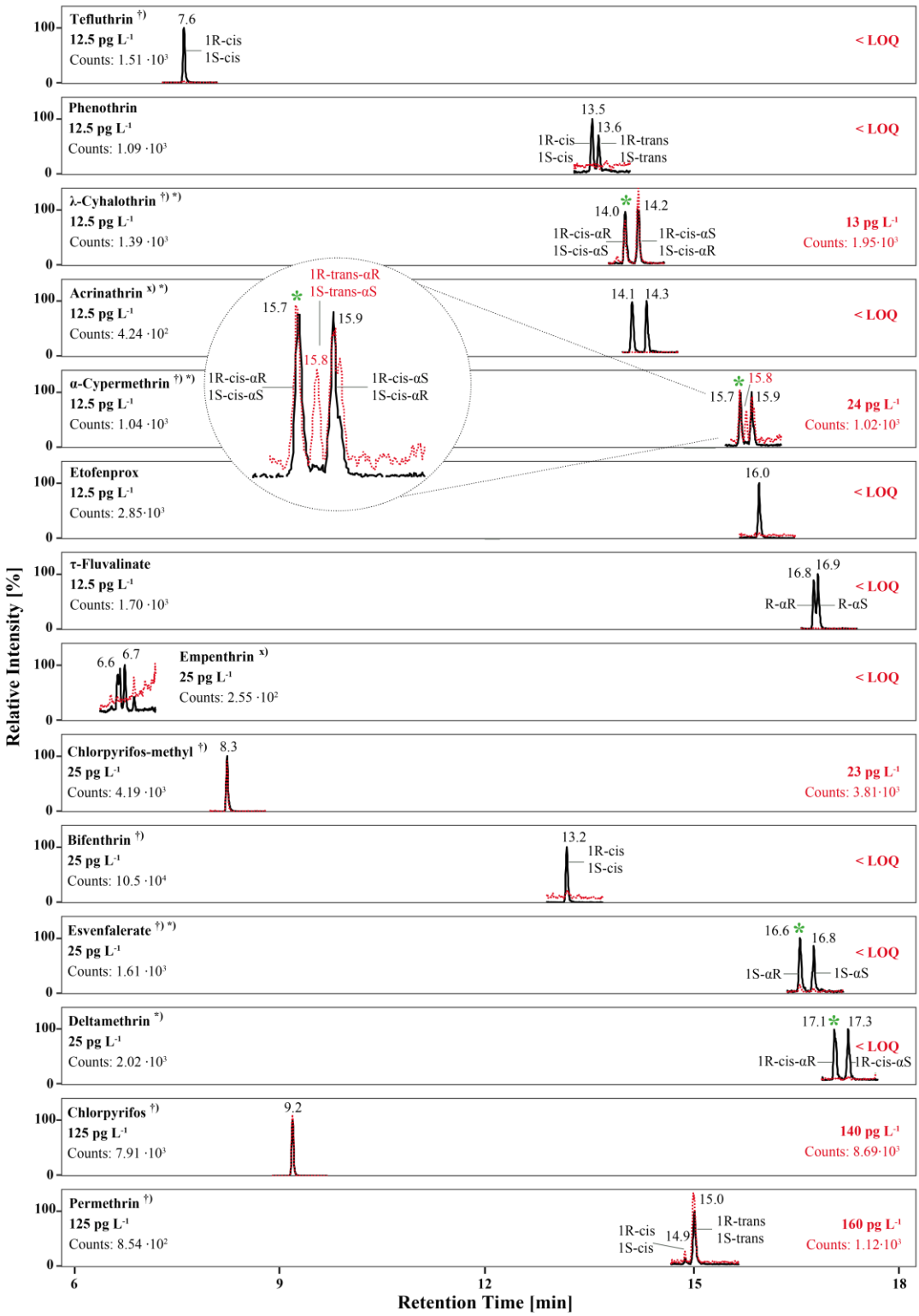

Figure 2: MRM chromatograms showing the quantifier transitions of all analytes in calibration standards at the MLOQ (black, continous) and in surface water samples (red, dashed). Calibration standards and surface water samples went through the entire sample preparation method. For analytes that were detected in the surface water samples, a surface water sample was selected that exhibited analyte concentrations around the corresponding MLOQ, otherwise a surface water sample without positive detection is shown. Analytes are sorted with increasing

MLOQ, and within one concentration by increasing retention time. Note that for analytes marked with a [†)], the MLOQ is a result of low-level blank contamination and the actual instrumental sensitivity can be much lower (see ILOQ in Table 2).

Signals in the calibration standards are displayed as relative intensities (normalized to 100%), and signals in the surface water samples are displayed relative to the highest intensity in the corresponding calibration standard.

Where possible, all separated diastereomers are labelled per pyrethroid based on own or literature data [20, 34, 8, 35]. For acrinathrin and empenthrin that are marked with a [x)], the single isomer peaks could not be assigned because of the high number of 8 stereoisomers per substance. Pyrethroid peaks that are a result of isomerization in ethanol at the α-carbon position are marked with a green asterisk, and all pyrethroids carrying the α-carbon are labeled with an [*)]. Note that a different GC column of the same Rtx-5MS type was used for the measurement of the isomer-selective cypermethrin reference standards displayed in Figure 1, explaining why the retention times of the cypermethrin peaks are slightly shifted.

549 **Table 2: Physico-chemical properties, quality control parameters, limits of quantification in surface water (MLOQ), environmental quality standards (EQSs) and**
550 **environmental concentrations measured in Swiss surface waters.**

Compound [i]	**number of stereoisomers** [ii]	**log K_{ow}** [iii]	**intra-day relative recovery** [iv] **[%]**	**absolute recovery$_{LLE}$** [iv] **[%]**	**ILOQ [pg Inj^{-1}]**	**MLOQ** [v] **[pg L^{-1}]**	**AA-EQS** [vi] **[pg L^{-1}]**	**MAC-EQS** [vi] **[pg L^{-1}]**	**surface water concentration [pg L^{-1}]**	**approval in Switzerland** [vii]
λ-Cyhalothrin [viii]	2 | 2 | 4	6.8	91 ± 5	94 ± 12	0.025	12.5 [ix]	22 [12]	190 [12]	< LOQ – 31000	B, P
α-Cypermethrin [viii]	2 | 2 | 4	6.6	93 ± 13	89 ± 1	0.25	12.5 [ix]	30 [12] */80 [11]*	440 [12] / *600 [11]*	< LOQ - 980	B, P
Tefluthrin	4 | 2	6.5	99 ± 2	96 ± 9	0.25	12.5 [ix]	16 [41]	-	< LOQ	P
Phenothrin	4 |4	6.0	107 ± 11	84± 9	0.25	12.5	1 [41] [x]	-	< LOQ	B
Acrinathrin [viii]	8 | 8	6.5	84 ± 15	89 ± 7	0.25	12.5	-	-	< LOQ	-
Etofenprox	1 | 1	7.1	110 ± 8	96 ± 12	0.025	12.5	5400 [42]	-	< LOQ	B, P
τ-Fluvalinate	2 | 2	6.9	93 ± 8	86 ± 8	0.025	12.5	-	-	< LOQ	-
Empenthrin	8 | 8	5.6	109 ± 5	112 ± 11	0.25	25	-	-	< LOQ	B
Bifenthrin	4 | 2	6.6	133 ± 3	67 ± 5	0.25	25 [ix]	20 [43] [x]	10000 [43]	< LOQ	B, P
Chlorpyrifos-methyl	-	4.3	100 ± 5	103 ± 3	0.025	25 [ix]	1000 [12]	7300 [12]	< LOQ - 140	P
Esfenvalerate [viii]	1 | 1 | 2	6.2	100 ± 2	90 ± 7	0.025	25 [ix]	100 [44]	850 [44]	< LOQ	B
Deltamethrin [viii]	8 | 1 | 2	6.2	100 ± 2	104 ± 3	0.25	25	3 [45] [x]	310 [45]	< LOQ	B, P, VD
Chlorpyrifos	-	5.0	90 ± 22	114 ± 14	0.025	125 [ix]	460 [12] / *30000 [11]*	4400 [12] / *100000 [11]*	< LOQ - 10000	P
Permethrin	4 | 4	6.5	100 ± 11	80 ± 7	0.25	125 [ix]	470 [46]	10000 [46] [xi]	< LOQ - 650	B, VD
Allethrin	8 | 8	4.8	-	84 ± 11	0.25	-	-	-	-	B
Tetramethrin	4 | 4	4.7	-	77 ± 15	0.25	-	290 [41]	-	-	B
Imiprothrin	4 | 4	2.9	-	-	2.5	-	-	-	-	B

551

[i)] Analytes with corresponding ISTD are italized.

[ii)] Theoretical number of stereoisomers | number of stereoisomers in the technical product | number of stereoisomers due to isomerization in ethanol at α-carbon position containing the cyano group.

[iii)] Experimental log K_{ow} values were taken from the Chemistry Dashboard, U.S. Environmental Protection Agency (https://comptox.epa.gov/dashboard [47]). Only for acrinathrin, τ-fluvalinate, and bifenthrin, log K_{ow} values were predicted with MarvinSketch version 14.10.20.0 (http://www.chemaxon.com/ [48]).

[iv)] **S**pike level: 500 pg L^{-1}, number of replicates n=3.

[v)] For the determination of MLOQ refer to section 2.6.

[vi)] For cypermethrin (mixture of all stereoisomers) and chlorpyrifos additional EQSs (marked in italic) are listed that are included in the WFD.

[vii)] Status July 2017: P, plant protection product; B, biocide; VD, veterinary drug.

[viii)] Pyrethroids with a chiral center at the α-carbon position containing the cyano group. Isomerization in ethanol at the α-carbon position was observed for λ-cyhalothrin, α-cypermethrin, esfenvalerate and deltamethrin. For acrinathrin isomerization at the α-carbon position most likely also occurred but it could not be chromatographically shown since acrinathrin already contained the mixture of all possible stereoisomers.

[ix)] MLOQ was determined via blank samples because of low-level blank contaminations.

[x)] AA-EQS is below MLOQ.

[xi)] Ad hoc MAC-EQS based on acute data compiled in Loos et al. [46].

3.5. Storage Stability

Storage stability experiments showed that the storage of spiked surface water at +4 °C over a period of one week in the dark (as it was the case during sampling in the on-site cooled sampling device and during cooled shipping to the laboratory) can lead to analyte losses of on average 40%. Nearly no decrease in concentration was observed for the organophosphates, whereas empenthrin concentrations decreased up to 70%. Moreover, storage stability at -20 °C (storage temperature of surface water samples in the laboratory before analysis) was tested by freezing spiked surface water samples for one week in the dark. Storage at -20 °C showed analyte losses of on average 20%. Analyte concentrations remained constant for the organophosphates and tefluthrin, and the strongest decrease was observed for bifenthrin (~45%). Consequently, freezing seems to be the preferred method for almost all analytes during storage of aqueous samples. For further details on the storage stability of the single analytes refer to ESM.J. However, because of the observed analyte losses during storage at +4 °C and -20 °C, the ecotoxicological risk in the investigated surface water might be even higher.

The reasons for these concentration decreases during storage at +4/-20 °C in the surface water samples can be diverse, such as sorption to sample vessels. However, sorption has been shown to be a reversible process [33, 49] and in this study all glass vessels containing water samples were shaken thoroughly before extraction. Additionally, sorption to glass vessels increases with decreasing dissolved organic carbon (DOC) and is therefore dependent on the DOC content in the water sample [33, 49]. The DOC content in the surface water samples used for the stability experiment in this study was ~2.2 mg L^{-1} and Hladik, Kuivila [33] have observed that ~16-27% of selected pyrethroids were associated with the glass bottle walls in waters containing 1 mg L^{-1} DOC. Moreover, agitation of water samples before extraction leads to a resuspension of particles. Without resuspension of analytes that are associated to particles, recoveries have been shown to decrease strongly since particle bound analytes are excluded from the aqueous phase [49]. Microbial degradation of analytes

seems to be only important when stored at 4 °C, since at -20 °C microbial activity is limited and for some analytes the observed losses at -20 °C are only slightly higher than the method precision (see ESM.J). Further studies are needed to distinguish between possible processes responsible for the observed analyte losses, especially at 4 °C.

3.6. Application to Environmental Surface Water Samples

Out of the 14 analyzed substances, three pyrethroids (cypermethrin, λ-cyhalothrin and permethrin) and both organophosphates (chlorpyrifos and chlorpyrifos-methyl) were detected in the investigated Swiss surface water samples taken between March and October 2017 (see Table 2 and Figure 3). Chlorpyrifos was detected in nearly all samples (57/58), followed by λ-cyhalothrin (43/58), cypermethrin (19/58), chlorpyrifos-methyl (10/58), and permethrin (5/58). Peak concentrations were found between April and June for λ-cyhalothrin (31000 pg L^{-1}), chlorpyrifos (10000 pg L^{-1}) and cypermethrin (980 pg L^{-1}) which is in line with the main period of plant protection product application. By contrast, chlorpyrifos-methyl showed a different concentration pattern over time. Highest concentrations were reached in the middle of March (140 pg L^{-1}), decreased until the beginning of May and disappeared afterwards, likely following the differing application times of this plant protection product in the catchment. Permethrin showed single detects between March and June. Besides permethrin, which is approved as a biocide and veterinary drug, all detected insecticides are permitted as plant protection products in Switzerland (see Table 2) and are registered for the application on vegetables (chlorpyrifos, cypermethrin and λ-cyhalothrin), cereals (chlorpyrifos), orchards (chlorpyrifos, chlorpyrifos-methyl and cypermethrin) and rapeseed (chlorpyrifos, chlorpyrifos-methyl, cypermethrin and λ-cyhalothrin).

The measured concentrations in the original samples (3.5-day composite samples) were compared to the acute quality criteria. MAC-EQS exceedances were present for λ-cyhalothrin, chlorpyrifos and cypermethrin in 10/58, 6/58 and 2/58 samples, respectively. Although the time of exceedance was rather short, MAC-EQSs were

highly exceeded (see Figure 3) indicating an acute risk for aquatic organisms during this time. To assess the risk of aquatic organisms towards long-term pollution, the averaged 14-day composite samples were compared to the chronic quality criteria. Since the averaged 14-day composite samples refer to calculated concentrations, they can exhibit concentrations below the corresponding MLOQ as observed for permethrin and chlorpyrifos-methyl (see Figure 3). AA-EQSs were exceeded for all detected substances except for chlorpyrifos-methyl and permethrin (see Figure 3). Notably for chlorpyrifos and λ-cyhalothrin, AA-EQS exceedances were present for 11/15 and 12/15 averaged 14-day composite samples, respectively, thereby exceeding chronic quality criteria during nearly the entire sampling period. AA-EQS exceedances were not significantly influenced by the differently composed 14-day time intervals ("moving averages") used to calculate averaged 14-day composite samples (overall percentage of AA-EQS exceedance with fixed starting value of 33% and of 35% with moving time intervals).

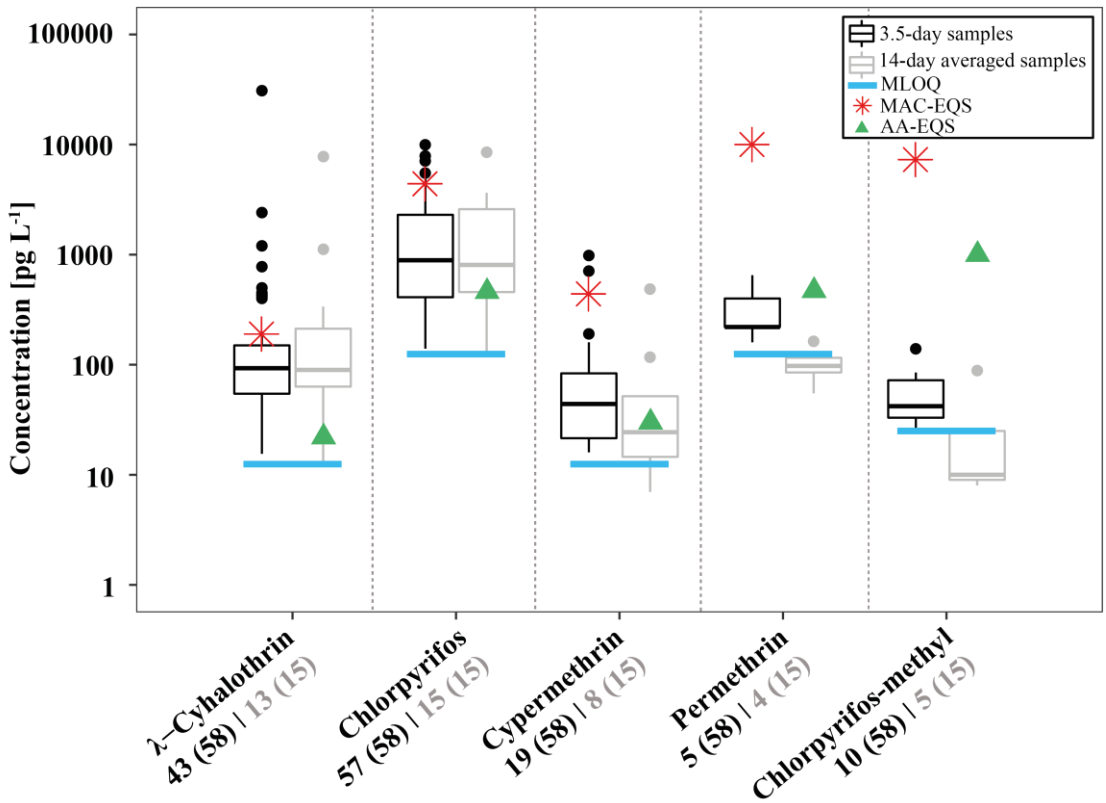

Figure 3: Boxplots of the concentrations of the positively detected pyrethroids and organophosphates in the original 3.5-day composite samples (black) and in the averaged 14-day

composite samples (grey) in comparison with their method limit of quantification (MLOQ) as well as acute (MAC-EQS) and chronic (AA-EQS) quality criteria. The meaning of the numbers below the analyte names: number of detections in the 3.5-day composite samples (total number of analyzed 3.5-day composite samples) | number of detections in the 14-day averaged samples (total number of calculated 14-day averaged samples).

Our study shows the significance of utilizing ultra-sensitive analytical methods to detect pyrethroids and organophosphates at ecotoxicological relevant concentrations. Comprehensive pesticide screenings in surface waters and sediments have shown that pyrethroids and organophosphates are often the main drivers of the overall risk, thereby highlighting the importance including them into ecotoxicological risk assessment [50, 51].

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Schulz R. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: A review. Journal of Environmental Quality. 2004;33(2):419-48. doi:10.2134/jeq2004.0419.

2. Stehle S, Schulz R. Agricultural insecticides threaten surface waters at the global scale. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(18):5750-5. doi:10.1073/pnas.1500232112.

3. Werner I, Moran K. Effects of Pyrethroid Insecticides on Aquatic Organisms. In: Gan J, Spurlock F, Hendley P, Weston DP, editors. Synthetic Pyrethroids: Occurrence and Behavior in Aquatic Environments. ACS Symposium Series, 2008. p. 310-34.

4. Coats JR, Symonik DM, Bradbury SP, Dyer SD, Timson LK, Atchison GJ. Toxicology of synthetic pyrethroids in aquatic organisms: An overview. Environ Toxicol Chem. 1989;8(8):671-9. doi:10.1002/etc.5620080805.

5. Vanwijngaarden R, Leeuwangh P, Lucassen WGH, Romijn K, Ronday R, Vandervelde R et al. Acute toxicity of chlorpyrifos to fish, a newt, and aquatic invertebrates. Bull Environ Contam Toxicol. 1993;51(5):716-23. doi:10.1007/bf00201650.

6. Ali I, Gupta VK, Aboul-Enein HY. Chirality: A challenge for the environmental scientists. Current Science. 2003;84(2):152-6.

7. Perez-Fernandez V, Angeles Garcia M, Luisa Marina M. Characteristics and enantiomeric analysis of chiral pyrethroids. J Chromatogr A. 2010;1217(7):968-89. doi:10.1016/j.chroma.2009.10.069.

8. Liu WP, Gan JJ, Lee SJ, Werner I. Isomer selectivity in aquatic toxicity and biodegradation of cypermethrin. J Agric Food Chem. 2004;52(20):6233-8. doi:10.1021/jf0490910.

9. Liu WP, Gan JJ, Qin S. Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. Chirality. 2005;17:S127-S33. doi:10.1002/chir.20122.

10. Spurlock F, Lee M. Synthetic Pyrethroid Use Patterns, Properties, and Environmental Effects. In: Gan J, Spurlock F, Hendley P, Weston DP, editors. Synthetic Pyrethroids. Acs Symposium Series, 2008. p. 3-25.

11. European Commission, Common Implementation Strategy for the Water Framework Directive (2000/60/EC), (2011).

12. Swiss Center for Applied Ecotoxicology. Proposals for Acute and Chronic Quality Standards, http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards/ last accessed November 2018.

13. IUPAC. International Union of Pure and Applied Chemistry. Pesticide Properties Database. http://sitem.herts.ac.uk/aeru/iupac/.

14. Laskowski DA. Physical and chemical properties of pyrethroids. Reviews of environmental contamination and toxicology. 2002;174:49-170.

15. Liu WP, Gan JJ, Lee S, Kabashima JN. Phase distribution of synthetic pyrethroids in runoff and stream water. Environ Toxicol Chem. 2004;23(1):7-11. doi:10.1897/03-183.

16. Feo ML, Eljarrat E, Barcelo D. Determination of pyrethroid insecticides in

environmental samples. Trac-Trends in Analytical Chemistry. 2010;29(7):692-705. doi:10.1016/j.trac.2010.03.011.
17. Albaseer SS, Rao RN, Swamy YV, Mukkanti K. An overview of sample preparation and extraction of synthetic pyrethroids from water, sediment and soil. J Chromatogr A. 2010;1217(35):5537-54. doi:10.1016/j.chroma.2010.06.058.
18. Gil-Garcia MD, Barranco-Martinez D, Martinez-Galera M, Parrilla-Vazquez P. Simple, rapid solid-phase extraction procedure for the determination of ultra-trace levels of pyrethroids in ground and sea water by liquid chromatography/electrospray ionization mass spectroscopy. Rapid Commun Mass Spectrom. 2006;20(16):2395-403. doi:10.1002/rcm.2600.
19. Ccanccapa-Cartagena A, Masia A, Pico Y. Simultaneous determination of pyrethroids and pyrethrins by dispersive liquid-liquid microextraction and liquid chromatography triple quadrupole mass spectrometry in environmental samples. Analytical and Bioanalytical Chemistry. 2017;409(20):4787-99. doi:10.1007/s00216-017-0422-7.
20. Kutter JP, Class TJ. Diastereoselective and enantioselective chromatography of the pyrethroid insecticides allethrin and cypermethrin. Chromatographia. 1992;33(3-4):103-12. doi:10.1007/bf02275888.
21. Feo ML, Eljarrat E, Barcelo D. A rapid and sensitive analytical method for the determination of 14 pyrethroids in water samples. J Chromatogr A. 2010;1217(15):2248-53. doi:10.1016/j.chroma.2010.02.018.
22. Feo ML, Eljarrat E, Barceló D. Performance of gas chromatography/tandem mass spectrometry in the analysis of pyrethroid insecticides in environmental and food samples. Rapid Commun Mass Spectrom. 2011;25(7):869-76. doi:10.1002/rcm.4936.
23. Portoles T, Mol JGJ, Sancho JV, Hernandez F. Advantages of Atmospheric Pressure Chemical Ionization in Gas Chromatography Tandem Mass Spectrometry: Pyrethroid Insecticides as a Case Study. Anal Chem. 2012;84(22):9802-10. doi:10.1021/ac301699c.
24. Pintado-Herrera MG, Gonzalez-Mazo E, Lara-Martin PA. Atmospheric pressure gas chromatography-time-of-flight-mass spectrometry (APGC-ToF-MS) for the determination of regulated and emerging contaminants in aqueous samples after stir bar sorptive extraction (SBSE). Anal Chim Acta. 2014;851:1-13. doi:10.1016/j.aca.2014.05.030.
25. Mekebri A, Crane DB, Blondina GJ, Oros DR, Rocca JL. Extraction and analysis methods for the determination of pyrethroid insecticides in surface water, sediments and biological tissues at environmentally relevant concentrations. Bull Environ Contam Toxicol. 2008;80(5):455-60. doi:10.1007/s00128-008-9382-0.
26. Deanovic LA, Stillway M, Hammock BG, Fong S, Werner I. Tracking pyrethroid toxicity in surface water samples: Exposure dynamics and toxicity identification tools for laboratory tests with Hyalella azteca (Amphipoda). Environ Toxicol Chem. 2018;37(2):462-72. doi:10.1002/etc.3979.
27. Wang D, Weston DP, Lydy MJ. Method development for the analysis of organophosphate and pyrethroid insecticides at low parts per trillion levels in water. Talanta. 2009;78(4-5):1345-51. doi:10.1016/j.talanta.2009.02.012.

757 28. Serôdio P, Nogueira JMF. Development of a stir-bar-sorptive extraction–liquid
758 desorption–large-volume injection capillary gas chromatographic–mass spectrometric
759 method for pyrethroid pesticides in water samples. Analytical and Bioanalytical
760 Chemistry. 2005;382(4):1141-51. doi:10.1007/s00216-005-3210-8.
761 29. Moschet C, Vermeirssen ELM, Seiz R, Pfefferli H, Hollender J. Picogram per liter
762 detections of pyrethroids and organophosphates in surface waters using passive
763 sampling. Water Res. 2014;66:411-22. doi:10.1016/j.watres.2014.08.032.
764 30. Strahler AN. Hypsometric (area-altitude) analysis of erosional topography.
765 Geological Society of America Bulletin. 1952;63(11):1117-32.
766 doi:10.1130/0016-7606(1952)63[1117:haaoet]2.0.co;2.
767 31. 2002/657/EC: Commission Decision of 12 August 2002 implementing Council
768 Directive 96/23/EC concerning the performance of analytical methods and the
769 interpretation of results (Text with EEA relevance) (notified under document number
770 C(2002) 3044) (2002).
771 32. Spycher S, Mangold S, Doppler T, Junghans M, Wittmer I, Stamm C et al.
772 Pesticide Risks in Small Streams-How to Get as Close as Possible to the Stress
773 Imposed on Aquatic Organisms. Environmental science & technology. 2018.
774 doi:10.1021/acs.est.8b00077.
775 33. Hladik ML, Kuivila KM. Assessing the Occurrence and Distribution of
776 Pyrethroids in Water and Suspended Sediments. J Agric Food Chem.
777 2009;57(19):9079-85. doi:10.1021/jf9020448.
778 34. Liu WP, Gan JJ. Separation and analysis of diastereomers and enantiomers of
779 cypermethrin and cyfluthrin by gas chromatography. J Agric Food Chem.
780 2004;52(4):755-61. doi:10.1021/jf035179m.
781 35. You J, Lydy MJ. A solution for isomerization of pyrethroid insecticides in gas
782 chromatography. J Chromatogr A. 2007;1166(1-2):181-90.
783 doi:10.1016/j.chroma.2007.08.014.
784 36. Nillos MG, Qin S, Larive C, Schlenk D, Gan J. Epimerization of Cypermethrin
785 Stereoisomers in Alcohols. J Agric Food Chem. 2009;57(15):6938-43.
786 doi:10.1021/jf900921g.
787 37. Qin SJ, Gan JY. Abiotic enantiomerization of permethrin and cypermethrin:
788 Effects of organic solvents. J Agric Food Chem. 2007;55(14):5734-9.
789 doi:10.1021/jf0708894.
790 38. Liu WP, Qin SJ, Gan JY. Chiral stability of synthetic pyrethrold insecticides. J
791 Agric Food Chem. 2005;53(10):3814-20. doi:10.1021/jf048425i.
792 39. Perschke H, Hussain M. Chemical isomerization of deltamethrin in alcohols. J
793 Agric Food Chem. 1992;40(4):686-90. doi:10.1021/jf00016a033.
794 40. Leicht W, Fuchs R, Londershausen M. Stability and biological activity of
795 cyfluthrin isomers. Pesticide Science. 1996;48(4):325-32.
796 41. Smit CE. RIVM: indicatieve normen bestrijdingsmiddelen 2014.
797 42. Directive 98/8/EC concerning the placing of biocidal products on the market,
798 Inclusion of active substances in Annex I or IA to Directive 98/8/EC, Assessment
799 Report, Etofenprox, (2007).
800 43. INERIS. Normes de Qualité Environnementale, Bifenthrin - N° CAS 82657-04-3,

DRC-11-118981-13678A2011.
44. van Vlaardingen PLA, Vonk JW, de Jong FMW. Environmental risk limits for esfenvalerate: RIVM Letter report 601716017/2008 2008.
45. de Knecht JA, van Herwijnen R. Environmental risk limits for deltamethrin: RIVM Letter report 601716015/2008 2008.
46. Loos R, Marinov D, Sanseverino I, Napierska D, Lettieri T. Review of the 1st Watch List under the Water Framework Directive and recommendations for the 2nd Watch List, EUR 29173 EN, Publications Office of the European Union, Luxembourg, 2018, ISBN 978-92-79-81839-4, doi:10.2760/614367, JRC111198 2018.
47. U.S. Environmental Protection Agency. Chemistry Dashboard. https://comptox.epa.gov/dashboard.
48. MarvinSketch v14.10.20.0 from ChemAxon. Available at https://www.chemaxon.com/products/marvin/marvinsketch/.
49. Lee S, Gan JY, Kabashima J. Recovery of synthetic pyrethroids in water samples during storage and extraction. J Agric Food Chem. 2002;50(25):7194-8. doi:10.1021/jf0258353.
50. Massei R, Busch W, Wolschke H, Schinkel L, Bitsch M, Schulze T et al. Screening of Pesticide and Biocide Patterns As Risk Drivers in Sediments of Major European River Mouths: Ubiquitous or River Basin-Specific Contamination? Environmental Science & Technology. 2018;52(4):2251-60. doi:10.1021/acs.est.7b04355.
51. Moschet C, Wittmer I, Stamm C, Singer H, Hollender J. Insektizide und Fungizide in Fliessgewässern. Wichtig zur Beurteilung der Gewässerqualität. Aqua & Gas. 2015;95(4):54-65.